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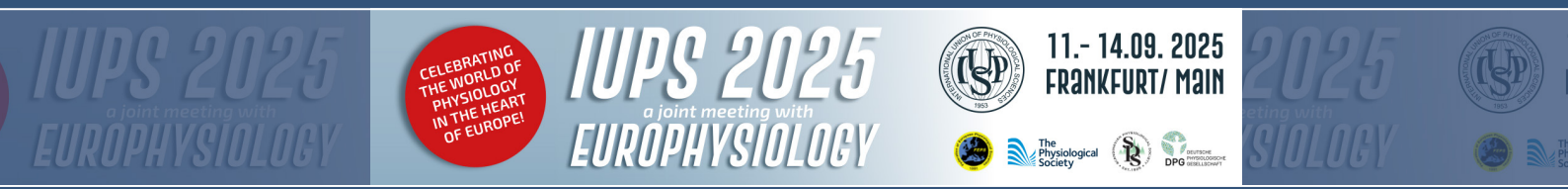

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performed with aortic VSMC isolated from mice carrying two floxed alleles of the *Lpp* gene. AAV9-mediated expression of Cre recombinase resulted in complete inactivation of the *Lpp* gene in these cells within 24 hours. Acute loss of *Lpp* produced similar effects in the VSMC than chronic *Lpp* deficiency with cells exhibiting increased proliferation and migration but decreased contractile and relaxant capacity. Bulk RNA-sequencing analysis of *Lpp*-KO VSMCs exposed to cyclic stretch demonstrated a prominent pro-inflammatory gene expression profile as well as changes in the expression of genes encoding proteins involved in extracellular matrix homeostasis.

Conclusions

In a nutshell, LPP plays a fundamental role in VSMC phenotype control and may protect the vessel wall from hypertension-induced arterial remodelling.

POSTER SESSION A

A 01 | BLOOD, REGENERATION AND DEVELOPMENT

A 01-01 Association of Serum iron deficiency with lower motor development in Santal children of West Bengal, India

S. Dutta Chowdhury

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The author has objected to a publication of the abstract.

A 01-02 Potential role of Prebiotics as Fetal haemoglobin Inducing Agent in Sickle Cell Anaemia Paediatric Patients : A randomized, double-blind, placebo-controlled Phase III trial

L. A. Kaddam[1,2], D. Abdelmaged[2], R. Babiker[6], H. Abdelrazig[5], I. Fadl-Elmula[3], A. M. Saeed[4]

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Aim

[Sickle cell Disease (SCD) is genetic disorder with hemolytic anemia is the cardinal pathogenesis of the disease. Dietary interventions proved its efficacy in modulating SCD. Prebiotics favourably modulate gut microbiota which is found to be altered in SCD for multiple causes. Gum Arabic (GA) is Acacia tree exudates, with proven prebiotics properties. GA ingestion increases short chain fatty acids (SCFA) in the blood. The latter in particular butyrate is known fetal hemoglobin inducing agent. The aim of this study to investigate the effect of GA ingestion on fetal hemoglobin level among paediatric sickle patients.

Methods & Results

Randomized, double-blind, placebo-controlled Phase III trial conducted among forty-

six Hb SS paediatric patients. Twenty- one received 30g of GA daily for 12 weeks. Another Twenty- one patient received placebo (Pectin). Levels of HbF, Hb S and complete blood count were measured before and after intervention. Fetal and sickle hemoglobin were measured by automated haemoglobin electrophoresis and CBC complete blood count test.

Mean age of Placebo arm was 10.76 ± 3.49 (Mean±SD), while in GA arm was 8.62 ± 3.57(Mean±SD). Pre and post analysis showed significant increase in Hb F by 48.8% (95% CI: -12.94 to -3.51; P<0.0001) and Hb A by 9.7% (95% CI: -1.21 to -0.0012; P<0.04) following regular intake of GA for 12 weeks. Significant reduction in Hb S 9.2% (95% CI: 2.2673 to 14.0755; P<0.002) among GA group.

Conclusions

Oral GA ingestion significantly increased fetal hemoglobin level and decrease sickle haemoglobin. No effect was noticed in the placebo group.

A 01-03 Effects of Quercetin on Erythrocyte Function in Normotensive and Hypertensive Rats

T. Jasenovec[1], M. Bartekova[2], U. Dulova[2], D. Radosinska[3], J. Janko[4], N. Vrbjar[2], J. Radosinska[1]

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Aim

Quercetin is a flavonoid widely recognized for its antioxidant properties. As erythrocyte function is essential for maintaining efficient microcirculation and tissue oxygenation, quercetin may help preserve erythrocyte integrity and functionality by mitigating oxidative damage, thereby supporting its reported vascular benefits of quercetin administration. Therefore, this study aimed to evaluate the effects of quercetin on selected erythrocyte parameters in Wistar and spontaneously hypertensive rats (SHR).

Methods & Results

Twelve-week-old Wistar and SHR rats were treated with quercetin (20 mg/kg/day) for six weeks. Following thiopental anesthesia (50 mg/kg), blood was collected from the aorta into heparinized tubes. Erythrocyte deformability was assessed using ektacytometry. Osmotic resistance and Na,K-ATPase kinetic parameters were measured spectrophotometrically. Nitric oxide (NO) production and intracellular free radicals were analyzed by flow cytometry.

SHRs exhibited increased erythrocyte count, hematocrit, mean cell volume, and red cell distribution width compared with Wistar. Erythrocytes from SHRs also demonstrated greater resistance to hypotonic stress, impaired deformability, and reduced Na,K-ATPase functionality, reflected by lower maximal enzyme velocity (Vmax). Free radical levels were also elevated in SHR erythrocytes compared with those from Wistar rats. Quercetin treatment didn't significantly alter erythrocyte parameters in Wistar nor in SHRs, except for a modest increase in Na,K-ATPase Vmax when compared with non-treated animals. Erythrocyte NO production remained unchanged.

Conclusions

Hypertension significantly affected basic hematological parameters and functional erythrocyte properties, including its deformability. However, under the conditions of this study, quercetin treatment did not significantly improve erythrocyte function or alleviate oxidative stress.

Acknowledgement: Study was supported by: APVV-22-0154 and APVV-21-0194.

A 01-04 Thromboxane A2 or activated platelets slightly lower Fgf23 expression in vitro

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Aim

Fibroblast growth factor 23 (FGF23) has emerged as an important endocrine regulator of renal phosphate and vitamin D metabolism and as a factor implicated in pathophysiological processes in further organs including heart. In myocardial

infarction, elevations of plasma FGF23 can be observed that may be related to left ventricular hypertrophy or fibrosis. A critical event in the development of myocardial infarction and thrombosis is platelet aggregation due to thromboxane A2 (TxA2) formation. We studied whether TxA2 is a regulator of FGF23.

Methods: Experiments were performed in rat UMR-106 osteoblast-like cells and mouse MC3T3-E1 upon exposure to TxA2, pharmacological manipulation of TxA2 signaling, or co-incubation with platelets isolated from healthy volunteers. Fgf23 transcripts were analyzed by qRT-PCR and FGF23 protein by ELISA

Results: As a result, TxA2 or stable TxA2 receptor agonists I-BOP or U46619 significantly suppressed Fgf23 gene expression, an effect abrogated by TxA2 receptor antagonist SQ29548. TxA2 signaling also down-regulated FGF23 protein concentration in the cell culture supernatant. Co-incubation of UMR-106 cells with freshly isolated human thrombocytes activated with thrombin, but not with non-activated platelets or thrombin alone significantly lowered Fgf23 gene expression in UMR-106 cells.

Conclusions

Taken together, TxA2 signaling suppresses FGF23 production in UMR-106 and MC3T3-E1 bone cells. TxA2-dependent regulation of FGF23 synthesis may be particularly relevant for common diseases associated with enhanced platelet aggregation.

A 01-05 Acute thrombocyte-dependent clearance of *Escherichia coli* requires D-mannose-sites for bacterial adherence

E. H. Lambertsen, N. Johnsen, H. Prætorius, T. Corydon

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Uropathogenic *E. coli* (UPEC) is the leading cause of urinary tract infections (UTIs). UPEC causing severe disease express various virulence factors that allow the UPEC to colonise the bladder, ascend to the kidney and disseminate to urosepsis. Our preliminary data show that thrombocytes are important for acute clearing of UPEC from the blood in a murine model of urosepsis. UPEC are known to adhere to epithelia via type 1 fimbriae, recognising D-mannose-rich structures. We investigate if thrombocyte-UPEC complex formation is prevented by pre-incubating UPEC with D-Mannose or preincubating thrombocytes with concanavalin A, a soluble lectin that binds D-mannose.

Methods We use an *in vitro* assay of GFP-expressing UPEC ($165 \cdot 10^6$ ml^{-1}) in blood samples or isolated thrombocytes from humane volunteers. Complex formation between UPEC and thrombocytes was determined at various time points by flow cytometry. Data are analysed using one-way ANOVA and given as mean±SEM.).

ResultsD-mannose reduced UPEC-thrombocyte complex formation from $6.71 \cdot 10^6 \pm 0.78 \cdot 10^6$ counts/ml to $2.69 \cdot 10^6 \pm 0.56 \cdot 10^6$ counts/ml equal to ~60% decrease (n=8, p=0.023). Concanavalin-A reduced complex formation from $3.21 \cdot 10^5 \pm 0.34 \cdot 10^5$ counts/ml to $1.57 \cdot 10^5 \pm 0.91 \cdot 10^5$ counts/ml equal to a ~51% decrease (n=7, p=0.004). Opposingly, thrombocyte-UPEC complex formation was not prevented by blocking TLR4 with antagonist or TLR4-specific antibody or LPS. Similarly, complex formation was not prevented by blocking either DC41 or CD42.

Conclusions We show that pre-treatment of UPEC with D-mannose, and pre-treatment of thrombocytes with Concanavalin A reduced complex formation *in vitro*. These data suggest that type 1 fimbriae are important for UPEC-thrombocyte interaction. Currently, we are gaining further evidence for this notion.

A 01-06 A Metabolic Profile of Cardiorespiratory Fitness in 327 Robust and Prefrail Older Adults

D. Houtkamp[1,2], R. Wüst[2], B. C. Bongers[1,3], M. Pijnappels[2], S. Chettouf[1], A. van de Wiel[1,4], I. Bautmans[1,5], A. L. Pool-Goudzwaard[1,2]

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Aim

Cardiorespiratory fitness is a strong predictor of morbidity and mortality during aging. Metabolic blood biomarkers may help elucidate biological mechanisms underlying

variation in cardiorespiratory fitness, particularly in individuals who are prone to become frail.

Methods & Results

This cross-sectional study utilized data from the AMCOHF cohort study in 337 adults (55–75 years). Cardiorespiratory fitness was measured via incremental cardiopulmonary exercise testing, measuring peak oxygen uptake, oxygen uptake at the first ventilatory threshold, peak work rate, and the oxygen uptake efficiency slope. Frailty was determined using Fried's Phenotype and the Rockwood Frailty Index. A panel of 250 metabolic biomarkers was quantified by nuclear magnetic resonance (NMR) spectroscopy. After normalization, principal component analysis (PCA) was performed on standardized biomarker data. Top principal components (PCs) were entered into multivariable linear regression models predicting cardiorespiratory fitness outcomes and receiver operating characteristic (ROC) curve analyses evaluated biomarker prediction accuracy for low and high cardiorespiratory fitness profiles. A similar approach to stratify these associations was conducted in a nested subgroup among prefrail individuals. 69 participants were classified as prefrail. Triglycerides in small and medium low-density lipoprotein and in very low-density lipoprotein contributed most strongly to PC1. The first three PCs explained 74% of total variance in $\dot{V}O_{2peak}$, independent of age and sex. Further data analysis is still ongoing.

Conclusions

This study identifies distinct metabolic profiles associated with cardiorespiratory fitness in older adults, supporting biomarker-driven strategies to identify individuals at risk of developing adverse health outcomes and support healthy aging.

A 01-07 Effect of Reduced Energy Intake on Blood and Erythrocyte Parameters in a Rodent Model of Metabolic Syndrome

J. Radosinska[1], R. Drabek[1], T. Jasenovec[1], I. Szadvari[1], D. Radosinska[2], R. Vazan[1], P. Balis[3], M. Kvandova[3]

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Aim

Oxidative stress and proinflammatory state are typical features of metabolic syndrome (MetS). Under such conditions, erythrocyte properties may deteriorate. Therapeutic strategies for managing MetS include lifestyle modification, including reduced caloric intake. We aimed to check whether the MetS induced by a high-fat diet (HFD) affects blood parameters and erythrocyte deformability and to evaluate the effect of fasting.

Methods & Results

We divided Wistar rats into the following groups: control, HFD (10-week-HFD) and HFD+fasting (6-week-HFD; 2 weeks - standard diet; 35% daily calorie intake reduction for 2 weeks). We registered a complete blood count, while ektacytometry was used for the erythrocyte deformability. We did not observe differences in the leucocyte count in general, however, monocyte count was greater in HFD while reduced after fasting. The percentage of lymphocytes was increased in HFD and was not affected by fasting. Contrariwise, fasting resulted in a decrease in the portion of monocytes and neutrophils. Erythrocyte count, hemoglobin concentration and hematocrit were lower in HFD and normalized after fasting. Erythrocyte deformability was better in fasting animals than HFD in higher shear stress values. The osmolality for optimum erythrocyte deformability was shifted towards hyperosmolality in HFD rats compared with controls.

Conclusions

Although a complete blood count is not used to diagnose MetS, certain parameters can provide valuable insights into the inflammatory and hematological changes

associated with MetS. This can aid in early detection, monitoring, and risk assessment. Non-pharmacological intervention (fasting) showed normalization of several blood and erythrocyte parameters. Supported by: APVV-22-0154, APVV-21-0194, VEGA2/0123/24 and SASPRO1368/03/02.

A 01-08 Precursor B-cell acute lymphoblastic leukemia induced autophagic cell death by novel compound.

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Aim

Precursor B-cell acute lymphoblastic leukemia is the most prevalent pediatric cancer, but its pathogenic mechanism is not known and conventional therapeutic agents have problems in terms of safety and efficacy. Thus, we are exploring natural products to find seed compounds with fewer side effects. In this study, we aimed to clarify the biological effects of flavacitropone A, a uniquely structured isolate from *Glycosmis citrifolia* (Willd.) Lindl (Rutaceae), in a human pre-B cell leukemia cell line (NALM6).

Methods & Results

In NALM6 cells treated with flavacitropone A, we were investigated cell death (MTT assay, annexin-V expression, mitochondria membrane potential, and caspase 3/7 activity) and comprehensively analyzed its mechanism (e.g., microarray, protein array, and proteome analysis by LC-MS). Flavacitropone A suppressed the growth of NALM6 cells but not that of peripheral blood mononuclear cells, and it time-dependently increased the number of annexin V-positive cells. Additionally, caspase 3/7 activity was higher in NALM6 cells treated with flavacitropone A than in non-treated cells. Microarray analysis revealed that flavacitropone A dramatically upregulated DDIT3 and ATF3 genes, and functional enrichment analysis using GSEA software predicted that this biological activity of flavacitropone A was involved in MYC and FOXO3 signaling. DDIT3, ATF3, and p21 mRNAs were upregulated and cMyc mRNA was downregulated, consistent with the microarray results. In the protein expression of signaling molecules, CHOP, ATF3, p21 and LC3A/B were upregulated, while phosphorylated FoxO1/3 and cMyc were downregulated.

Conclusions

In conclusion, flavacitropone A appears to induce autophagic cell death via upregulation of p53 expression in NALM6.

A 01-09 Enhancing potassium channel function impairs proliferation, differentiation and synaptic wiring of adult-born GABAergic interneurons.

D. Dodon[1,2], M. Polozhentseva[1,2], Y. Kovalchuk[1], V. Shvadchak[2], J. Gorzolka[1], K. Li[1], **O. Garaschuk**[1]

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Gain-of-function mutations of K^+ channels are implicated in neurodevelopmental disorders, neural network hyperexcitability, and severe drug-resistant epilepsy, but the underlying mechanisms remain unclear. Here, using the mouse olfactory bulb (OB) as a model system, we analyzed how increased activity of Kv1.2 or Kir2.1 K^+ channels impacts proliferation, differentiation, and integration (synaptic wiring) of adult-born OB granule cells (GCs) into the pre-existing neural network.

Neural stem cells, generated in the subventricular zone of the lateral ventricles, were virally modified in the rostral migratory stream on their way to OB to overexpress genetic constructs encoding either voltage-gated Kv1.2- or inwardly rectifying Kir2.1-channels together with the Ca^{2+} indicator Twitch-2B. Analyses of adult-born GC morphology and transcriptome using immunohistochemistry, FACS-sorting, and bulk RNA sequencing revealed retardation in cell growth and morphogenesis, in line with a profound downregulation of genetic pathways encoding proteins involved in central neural system development, neurogenesis, neuronal migration, and dendritic (as well as axonal) morphogenesis. Moreover, we have observed significant downregulation of pathways related to synapse assembly, including pre-, trans- and postsynaptic elements (e.g., synaptic vesicle exo- and endocytosis; regulation of trans-synaptic signaling, glutamate- and GABAergic synaptic transmission,

postsynaptic membrane potential, etc.). Surprisingly, however, this developmental retardation did not influence the timely loss of the immature cell marker doublecortin. Taken together, our data reveal a key role of K^+ channels in the development and synaptic integration of GABAergic neurons and provide an unexpected mechanistic explanation of how gain-of-function K^+ channel mutations might cause Developmental and Epileptic Encephalopathy.

A 01-10 Elastogenesis in the pregnant uterus as a paradigm to reinstate elasticity in organs

A. Schneider, T. Warwick, J. Hefendehl, R. Gilsbach, R. P. Brandes

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Introduction:
Elastolysis, the loss of elastic fibers, contributes to severe diseases like aortic aneurysms. The uterus is the only known adult mammalian tissue capable of reactivating elastogenesis, producing elastic fibers during pregnancy-induced expansion. Understanding the molecular mechanism governing this process could permit the development of therapeutic approaches to tackle elastolytic diseases.
Methods:
Time-resolved single-nuclear RNA-sequencing was used to analyze elastogenesis-related gene regulatory networks in the pregnant uterus and compare them to cardiac tissue. Isolated fibroblasts were stimulated with pregnancy hormones to explore mechanisms driving elastic gene expression.
Results:
Elastogenesis-associated genes (*Eln, Fbn1, Fbn2*) show a rapid increase in uterine tissue during pregnancy. At single-nuclear level, cell type-specific expression patterns identify uterine fibroblasts and SMC's as key players, with both cell types upregulating *Eln* and *Fbn1*. Time-resolved gene regulatory network analysis in the heart and uterus suggests that unique transcription factor cascades terminating in elastogenesis are present in the uterus, which may be controlled by *Foxo1*, *Esrrg* and *Dux*. *In vitro* experiments with isolated fibroblasts demonstrated that estrogen and progesterone provoke antagonistic effects on elastogenesis-related gene expression, with estrogen upregulating of *Eln* and *Fbn1*. These results hint that hormonal changes upon pregnancy may stimulate elastogenesis in a tissue-specific manner, as determined by receptor expression.
Conclusion:
In conclusion, elastogenesis in the pregnant uterus is driven by hormonal activation of gene regulatory networks targeting key elastogenic genes. While this network is dormant in the adult cardiovascular system, identifying critical molecular junctions could reactivate elastogenesis, offering novel therapeutic avenues for diseases like aortic aneurysms.

A 01-11 Comprehensive analyses of dorsal forebrain organoids

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In recent years, significant advances in neuroscience research have been made by novel and pioneering in vitro 3D cultures, now referred to as neural organoids, derived from induced human pluripotent stem cells (hiPS cells). These organoids closely mimic key aspects of human brain structure and function, exhibiting sophisticated cellular organisation, layer formation and mature gene expression profiles. This innovative technology is vital for studying human neural development, mechanisms of neurodegenerative diseases and, for example, drug responses. In addition, patient-specific organoids could serve as invaluable in vitro models for exploring therapeutic interventions.
A variety of different assays are utilised within our laboratories to facilitate a comprehensive investigation of dorsal forebrain organoids at various developmental stages. Patch-clamp electrophysiological recordings are used to measure intrinsic firing properties and synaptic activity. To explore the heterogeneity of neuronal populations and to decipher synaptic protein composition, we use immunostaining and advanced microscopy techniques. Our overall goal is to track the emergence

and maturation of active, functional neuronal networks and to assess their long-term viability. To this end, we plan to generate cortical assembloids by fusing different organoids to create more complex neural systems. These efforts will be crucial for understanding how neurons develop synaptic connections in healthy three-dimensional models and in common neuropsychiatric disorders such as depression, autism and Alzheimer's disease.

A 01-12 Small Open Reading Frames: Emerging regulators in embryonic development.

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Small open reading frames (sORFs), encoding peptides under 100 amino acids, represent an understudied class of genes in the mammalian genome. With approximately 7,264 cataloged human sORFs, most are evolutionarily recent, emerging in primates or humans.These microproteins play crucial regulatory roles across multiple biological domains. The microprotein "toddler," previously annotated as lncRNA, drives cell internalization during zebrafish embryogenesis. "Bouncer," expressed in oocytes, mediates species-specific fertilization and can enable cross-species fertilization between zebrafish and medaka. The conserved microprotein BRAWNIN is essential for respiratory chain complex III assembly in human mitochondria.Many microproteins localize to mitochondria and regulate cellular metabolism. This connection is particularly significant in cardiomyocytes, which have exceptional metabolic requirements to maintain lifelong contractility. During development, cardiomyocytes exit the cell cycle and undergo metabolic maturation, forming complex mitochondrial networks and switching to fatty acid oxidation.Hundreds of microproteins are enriched in cardiomyocyte mitochondria and become dysregulated in cardiac disorders like hypertrophic cardiomyopathy. Despite indications of their importance in cardiac development and function, most remain uncharacterized.This study aims to predict, identify, categorize, and catalog sORFs expressed during human cardiac development, followed by molecular and functional characterization of microproteins essential for metabolic programming in human cardiomyocytes.

A 02 | CARDIAC PROTECTION AND TREATMENT

A 02-01 An SGLT2 inhibitor and SGLT1/2 inhibitor confer similar benefit on diastolic function male and female diabetic mice

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Aim

Sodium-glucose cotransporter (SGLT)-2 inhibitors were originally developed to lower blood glucose in diabetic patients and later showed efficacy in reducing cardiac risk. Mechanisms underlying this cardiac benefit remain unclear. The heart predominantly expresses SGLT1 which may provide a more direct cardiac target for intervention. This study aimed to track diabetic cardiomyopathy progression in male and female mice and compare cardiac efficacy of SGLT2 vs SGLT1/2 inhibitors.

Methods & Results

Male and female C57BL/6J mice were fed a high-fat high-sugar diet (HFSD) to induce diabetes (n=9-10/grp). After 16 weeks of diet, mice received either SGLT2 inhibitor (empagliflozin 20mg/kg), SGLT1/2 dual inhibitor (sotagliflozin, 20mg/kg) or vehicle (20mg/kg methylcellulose in saline) by daily oral gavage for 4 weeks. Left ventricular cardiac function was assessed via echocardiography under anaesthesia (1-5% isoflurane). Data were analysed using 1- or 2-way ANOVA with Bonferroni post-hoc tests and are reported as mean ± SEM.
The HFSD diet induced obesity, hyperglycaemia, glucose intolerance and diastolic dysfunction in both sexes. In males, SGLT2i and SGLT1/2i partially reduced weight gain (−8.0±1.3% and −6.8±1.6% respectively; $p<0.05$). In females, SGLT1/2i showed a trend toward weight loss (−13.1±1.9%, $p=0.068$), while SGLT2i had no

significant effect. Diastolic dysfunction (increased E/e' ratio) was normalised by SGLT2i and SGLT1/2i in HFSD male and HFSD female mice. Further molecular work is underway.

Conclusions

These preliminary findings provide the first evidence that inhibition of SGLT1 confers similar cardiac benefit to SGLT2 inhibition in both sexes. Sex-specific changes in weight loss suggests potential differences in the systemic metabolic response.

A 02-02 Brown Adipose Tissue and its Emerging Cardiovascular Protection.

T. Egan Beňová[1], M. Sýkora[1], K. Ondreják Andelová[1], M. Lepáček[2], M. Šoltésová Prnová[2], P. Babál[3], V. Farkašová[1], D. Janko[1], N. Andelová[1], M. Ferko[1], B. Szeiffová Bačová[1]

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Aim: Brown adipose tissue (BAT) may protect against obese type 2 diabetes (T2D), but the underlying mechanisms are not fully understood. This study investigates whether connexin 43 (Cx43), a key gap junction protein involved in cardiac and BAT function, might be a link between BAT activity and cardiometabolic health. The antioxidant Cemtirestat was also tested for therapeutic potential.

Methods: Male obese Zucker Diabetic Fatty (ZDF) rats and lean ZDF controls were treated with Cemtirestat (2.5 mg/kg/day, drinking water, six months) and compared with untreated. Biometric and biochemical parameters were assessed in rats anesthetized with chloral hydrate (20 mg/100 g, i.p.). BAT was analyzed for Cx43 expression, its topology and relevant modulating signaling markers. Early signs of diabetic cardiomyopathy were evaluated using echocardiography.

Results: Obese T2D (ZDF) rats showed increased body/heart weight, white AT, BAT, blood glucose, insulin, cholesterol and triglycerides. BAT whitening was accompanied by reduced Cx43 and a shift to unilocular adipocytes. In the BAT of obese T2D rats, PKCε was decreased and PKCδ increased, unaffected by Cemtirestat. Thermogenic/metabolic markers (UCP1, UCP3, FGF21, GDF15, PPARγ) were downregulated, while IL-6, IL-10, and TNF-α were elevated. Cemtirestat increased Cx43 in lean ZDF rats; normalized IL-6, TNF-α, and attenuated diastolic dysfunction in obese T2D rats.

Conclusions: Downregulation of Cx43 associated with BAT whitening in obese T2D rats suggests BAT dysfunction contributing to disorders of thermogenesis and BAT-related protective effects. Further research is needed to elucidate the molecular mechanisms of Cx43 upregulation by Cemtirestat.

Supported by VEGA 2/0133/24, APVV 21-0410, APVV-20-0411, APVV-22-0264, VV-MVP-24-0278 and SAS Return Project Scheme.

A 02-03 Versican knockout limits adverse cardiac remodeling after myocardial infarction

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Acute myocardial infarction (AMI) is a life-threatening event that majorly contributes to morbidity and mortality even after survival of the initial event. Versican as a large chondroitin sulfate proteoglycan binds to hyaluronan (HA) in extracellular matrix (ECM) and is dramatically upregulated within hours after AMI. While the role of versican in wound healing and cardiac remodeling after AMI remains to be fully understood, it is well established that fibrotic responses, including those in the heart, are linked to pathologically elevated levels of ER stress and UPR signaling. Here, we investigate versican's function after AMI, focusing on its role in UPR signaling. Mice with ubiquitous or fibroblast-specific conditional knockout (KO) of versican underwent 45 minutes of closed-chest ischemia/reperfusion (I/R) injury to induce AMI (anesthesia: ketamine/xylazine (100/10mg/kg), isoflurane (1.5-3%), analgesia: buprenorphine (0.05-0.01 mg/kg)). Single-cell RNA sequencing revealed that ER

stress- and UPR-related gene expression (e.g. *Hspa5*, *Manf* and *Xbp1(s)*) is diminished in versican-deficient fibroblasts. Mechanistically, versican deficiency diminishes the unfolded protein response as observed by reduced expression of UPR target genes in cardiac fibroblasts, which is known to limit fibrotic remodeling. Ubiquitous versican KO improved systolic pump function. Consistently, also fibroblast-specific KO of versican improved ejection fraction and limited ventricular dilation 3 weeks post I/R. Overall, both global and fibroblast-specific knockout of versican in mice result in a protective phenotype due to limitation of adverse fibrotic remodeling after AMI guided by reduced ER stress and UPR signaling.

A 02-04 Inhibition of the endothelial clearance of natriuretic peptides has blood pressure-independent cardioprotective effects

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Left ventricular (LV) hypertrophy is a powerful predictor of cardiovascular events, independent of blood pressure and other risk factors. Its prevalence increases with age and prevention or even reversal represents a major therapeutic goal for the treatment of hypertensive patients. The natriuretic peptides ANP, BNP and CNP, via their cyclic GMP-synthesizing guanylyl cyclase receptors, exert protective cardiovascular and metabolic actions. The role of their shared receptor-C (NPR-C), which lacks guanylyl cyclase activity, is controversial. Depending on the cell type, NPR-C seems to either internalize the NPs or mediate their beneficial effects. Endothelial cells (ECs) express prominent NPR-C levels. To dissect the impact of endothelial NPR-C on cardiovascular ageing, we generated mice with EC-restricted deletion (KO).

In comparison with their control littermates, such EC NPR-C KO mice showed reduced clearance of ANP from the circulation. Despite, their steady-state NP plasma levels were unaltered. Arterial blood pressure of EC NPR-C KO and control littermate mice similarly raised during ageing. In male and female control mice, this was associated with progressive LV hypertrophy and contractile dysfunction. Notably, KO mice of both sexes were almost fully protected from these changes. This was associated with enhanced myocardial cyclic GMP levels, greater protein kinase G (PKG I)-mediated phosphorylation of titin and molecular signatures of improved cardiomyocyte autophagy.Our data indicate that inhibiting the NPR-C-mediated endothelial clearance of NPs reinforces their local cGMP-mediated cardioprotective actions. This attenuates aging-related LV hypertrophy and dysfunction in blood pressure-independent manner.

A 02-06 Deletion of Endothelial SOX9 Reverses Aging-Induced Cardiac Dysfunction

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Aim

Previously, we reported that the transcription factor SOX9 serves as master regulator of fibrotic remodeling in endothelial cells (ECs) in different heart failure entities. Whether endothelial SOX9 contributes to the development of cardiac

dyfunction during aging, has so far not been investigated, and was hence aim of the study.

Methods & Results

Aged mice (24 months old) displayed endothelial induction of SOX9 alongside a reduction of cardiac function, capillary rarefaction and myocardial fibrosis. Deletion of *Sox9* (*Sox9*$^{EC\text{-}KO}$) in ECs upheld cardiac function and reduced fibrotic lesions throughout 24 months. To investigate the underlying mechanisms, we performed single-cell RNA sequencing. We found increased B cell (BC) and reduced neutrophil (NT) numbers as well as several upregulated immune cell-activating ligand-receptor relations from ECs to BCs in aged *Sox9*$^{EC\text{-}KO}$ vs. *Sox9*$^{fl/fl}$ control mice, and profound anti-inflammatory and pro-apoptotic signaling from BCs to NTs, suggesting cardioprotection by regulatory BCs in aged *Sox9*$^{EC\text{-}KO}$. Additionally, FB stimulating signaling was upregulated in aged control mice, but less in *Sox9*$^{EC\text{-}KO}$ mice, suggesting maturation from resident to rather secretory FB subtypes, which was confirmed by trajectory analysis. Lastly, EC senescence was remarkably reduced in aged *Sox9*$^{EC\text{-}KO}$ vs. control mice, indicating that SOX9 promotes senescence in ECs.

Conclusions

Here, we present at least three mechanisms by which endothelial SOX9 promotes aging-induced cardiac dysfunction in a cell autonomous manner as well as in concert with fibroblasts and immune cells. Inactivation of endothelial *Sox9* might be a promising therapeutic approach against cardiac dysfunction in the elderly.

A 02-07 Treatment with Atglistatin of diet-induced obesity mice after cardiac I/R normalizes body weight but does not improve cardiac function

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Obesity belongs to the main risk factors for cardiovascular diseases such as myocardial infarction (MI). White adipose tissue (WAT) function is severely disturbed in obese subjects. MI stimulates WAT lipolysis acutely but also affects WAT metabolic and secretory function chronically.

In this study, the ATGL-inhibitor Atglistatin was used to characterise the role of WAT function chronically following an ischemia/reperfusion (I/R) injury in the context of obesity in C57BL/6J male mice. Morphology, structure and function of cardiac tissue, subcutaneous (iWAT), visceral (gWAT) WAT and blood were analysed. Induction of cardiac I/R using a closed-chest model (anaesthesia: ketamine/xylazine (100/10mg/kg), isoflurane (1,5-3%), analgesia: buprenorphine (0.05 mg/kg)) was performed after a 9-week high fat/high sucrose diet. Atglistatin-treatment started the day after ischemia. Mice were sacrificed by cervical dislocation or CO_2 24hrs, 7d and 28d after I/R.

In contrast to lean mice, diet-induced obesity (DIO)-mice did not show acute lipolytic activation in response to I/R. Atglistatin treatment induced a significantly reduced body weight after 28d of reperfusion and a reduction in iWAT weight and adipocyte size. On the molecular level ATGL gene expression was upregulated, while leptin and galectin 3 (macrophage marker) were downregulated, most pronounced in gWAT. Cardiac function and scar size were not altered between Atglistatin and vehicle fed animals.

In obese mice the acute lipolytic activation is blunted after I/R. Despite a normalization of body weight and reduction in inflammation and leptin expression in adipose tissue, Atglistatin treatment did not alter cardiac function after I/R.

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A 02-08 Hyperforin enhances calcium transients and fractional shortening but impairs NCX activity in rat cardiomyocytes

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Aim

Non-selective cation currents mediated through TRPC channels play an important

yet elusive role in cardiomyocyte physiology and pathology. We investigated how hyperforin, a TRPC6 activator, modulates calcium cycling and contractility in rat cardiomyocytes.

Methods & Results

Isolated adult rat ventricular and atrial cardiomyocytes were electrically stimulated, treated with 0.3-1µM hyperforin and sarcomere shortening was recorded using an IonOptix system. Calcium transients (CaTs) in cytosol and nucleus were measured via confocal imaging (LSM700) after Fluo-4/AM loading. Sarcoplasmic reticulum (SR) and nuclear envelope (NE) calcium load and SERCA/NCX activity were assessed using caffeine-induced CaTs.

Hyperforin caused a positive-inotropic effect (PIE) in ventricular and atrial cardiomyocytes, followed by contractile failure in a subset of cells. The PIE was blocked by 100nM BI-749327, an inhibitor of TRPC6, which alone had no effect on contractility. The cellular mechanisms underlying the PIE were further investigated in ventricular cardiomyocytes. Here, cytosolic diastolic calcium (+36%) and CaT amplitude (+15%) were increased by hyperforin (n=57, both $P<0.01$) and CaT decay was prolonged (ΔTau: 15ms in Ctrl versus 74ms in Hyperforin, $P<0.01$). Similar observations were made for nuclear CaTs. Moreover, SR/NE calcium load and fractional release were elevated by hyperforin (n=26, $P<0.05$). Finally, NCX activity was reduced by hyperforin ($P<0.01$), while SERCA activity remained unchanged.

Conclusions

Hyperforin enhances contractility and CaTs. The latter is mediated by increased SR calcium load and fractional release brought about – most likely – by sodium and calcium influx via TRPC6 and reduced calcium extrusion via NCX. Elevated nuclear CaTs may alter calcium-dependent transcription.

A 02-09 An Interpretable Machine Learning Platform for Genotype-specific Cardiotoxicity Risk Prediction Using Patient-Derived iPSC-CMs

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The author has objected to a publication of the abstract.

A 02-10 Neuregulin-1/ErbB2 axis-mediated protection of systolic function in the early onset of diabetic cardiomyopathy

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Aim: Diabetic cardiomyopathy patients present with left ventricular (LV) diastolic dysfunction at an early stage, then systolic dysfunction as the disease progresses. This study aimed to elucidate the mechanisms by which diastolic dysfunction precedes systolic dysfunction in the early stage of diabetic cardiomyopathy.

Methods & Results: Animal experiments were approved by the Animal Care and Use Committee of Toho University and performed according to the Guiding Principles for the Care and Use of Laboratory Animals. Type-1 diabetes mellitus (T1DM) model mice were generated by injection of streptozotocin in 8-week-old male C57BL/6J mice. Age-matched control mice were treated with citrate-buffered saline. LV diastolic dysfunction, but not systolic dysfunction, was observed in the T1DM model mice 4 weeks after STZ administration (STZ-4W). Surprisingly, the plasma neuregulin-1 (NRG1) concentration was significantly increased, and NRG1 expression was markedly upregulated in the ventricle, liver, and kidney of STZ-4W mice. In STZ-4W mice, the blockade of the NRG1 receptor ErbB2 with trastuzumab (TRZ) significantly reduced the systolic function without affecting diastolic function and markedly reduced ErbB2/Akt signaling pathway, including Akt-dependent regulation of Ca^{2+}-signaling/E-C coupling machinery proteins, whereas TRZ had no effect in control mice. Database analysis of a dataset of heart failure patients

revealed that the upregulation of the NRG1/ErbB2 axis in the early stage of diabetic cardiomyopathy may be shared with T2DM patients.

Conclusions: These results indicate that the compensatory upregulated NRG1 contributes to maintaining the LV systolic function, which explains why diastolic dysfunction precedes systolic dysfunction at the early stage of diabetic cardiomyopathy.

A 02-11 Targeting Metabolic Pathways to Promote Brown Fat Thermogenesis and Cardioprotection in Obese ZDF Rats

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The global increase in obesity and type 2 diabetes (T2D), key contributors to cardiovascular diseases, is strongly associated with metabolic dysfunctions, including chronic inflammation and cellular stress. A decline in brown adipose tissue (BAT)—essential for thermogenesis—further compromises metabolic homeostasis. We hypothesize that Cemtirestat (CEM), a potent antioxidant, may mitigate these risk factors and promote the beiging of white adipose tissue, thereby offering cardiometabolic protection.

Four-month-old male Zucker diabetic fatty (ZDF) rats were assigned to four groups (n = 10/group): lean controls, CEM-treated lean, untreated ZDF-T2D, and CEM-treated ZDF-T2D. Animals received CEM (2.5 mg/kg/day) for six months. After treatment, rats were euthanized with an overdose of chloral hydrate (20 mg/100 g, i.p.), and BAT and left ventricular (LV) tissue were collected for analysis.

Untreated ZDF-T2D rats exhibited BAT whitening, accompanied by increased body/heart mass, hyperglycemia, and hyperinsulinemia. Protein expression related to stress signaling (FGF21, PPAR-γ), metabolic regulation (DPP4), and cell communication (Cx43, PKCε) was upregulated, while the anti-inflammatory cytokine IL-10 was reduced in the LV. CEM treatment partially reversed these changes, notably decreasing DPP4 expression. Importantly, UCP-1, a key thermogenic protein in BAT, was significantly diminished in ZDF-T2D rats and restored with CEM therapy.

These findings suggest that CEM could improve metabolic and thermogenic function, with potential cardioprotective effects in T2D. However, its moderate efficacy at the administered dose highlights the need for further dose optimization and mechanistic studies.

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A 02-12 Coffee reduces cardiac fibrosis by regulating NCX activity in cardiac fibroblasts

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Aim

Large-scale epidemiological studies demonstrated a positive correlation between moderate daily coffee consumption and improved cardiovascular outcomes. However, the precise mechanisms remain unclear. This study aims to uncover the physiological pathways by which coffee exhibits its cardioprotective effects.

Methods&Results

Coffee and caffeine were administered to healthy and L-NAME-treated rats at a dose mimicking the regular coffee consumption in humans, whereby caffeine plasma concentration reached a low micromolar range. Coffee and caffeine improved cardiac remodeling and function, as assessed by echocardiography, independently of blood pressure regulation. In addition, coffee and caffeine reversed L-NAME-induced interstitial cardiac fibrosis and reduced the proliferation and activation of cardiac fibroblasts. At the molecular level, antibody arrays revealed that coffee and caffeine attenuated the activation of key fibrotic signaling pathways, including NFATc3, CAMKII, and NF-κB, in L-NAME-treated rats. *In* vitro, calcium imaging revealed that low micromolar caffeine concentration activates sodium-calcium

exchanger (NCX), with a transient ORAI1-mediated calcium rise in isolated cardiac fibroblasts. These calcium fluctuations occurred without affecting intracellular sodium levels or endoplasmic reticulum calcium stores. Patch-clamp experiments on these cells further confirmed NCX activation. These events inhibited the calcineurin/NFATc3 pathway, leading to a less fibrotic phenotype in cardiac fibroblasts. Finally, caffeine selectively affected cardiac fibroblasts, with no observed effects on calcium or sodium handling in cardiomyocytes.

Conclusions

This study unveils a new mechanism by which moderate coffee consumption modulates NCX activity in cardiac fibroblasts, with a subsequent reduction in cardiac fibrosis. These findings highlight for the first time coffee's potential as a natural cardioprotective agent.

A 03 | CARDIOTOXICITY AND FAILURE

A 03-02 Cardiovascular effects of bismuth nitrate on chlorpromazine induced cardiotoxicity in young adult male Wistar rats

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Aim

Chlorpromazine is one of the antipsychotic drugs listed as essential drugs by WHO to treat both acute psychosis and chronic psychosis. It has been associated with unhealthy and life threating cardiovascular side effects. Given that Bismuth nitrate has the ability to increase nitric oxide bioavailability, mop up free radicals and induce metallothionines. We sought to ascertain the plausible cardiovascular protective effects of Bismuth nitrate in chlorpromazine induced cardiotoxicity.The aim of the study was thus to ascertain the cardiovascular effects of Bismuth nitrate in Chlorpromazine induced cardiotoxicity.

Methods & Results

All procedures were undertaken in accordance with regulations as set out by the Babcock University Research and Ethical Committee (BUREC). Young adult male Wister rats weighing 100-120 g were randomly divided into four groups (n=8). Animal experimentation lasted for 5 days. Group 1 animals served as control and were untreated. Cardiotoxicity was induced by 5 days chlorpromazine (10 mg/Kg) treatment in groups 2-4. Groups 3 & 4 animals were co-treated with Bismuth nitrate 50 and 200 mg/Kg respectively for 5 days. Cardiovascular, biochemical, histological and molecular parameters were determined at the end of the animal study. Transcriptomic profiling of the left ventricle following Bismuth nitrate administration was also determined.

Conclusions

Bismuth nitrate especially at 50 mg/Kg ameliorated Chlorpromazine induced cardiotoxicity which was associated with the modulation of GSK3B/CNTI signaling pathways.

A 03-03 Distinct disruptions of Ca^{2+}homeostasis and mitochondrial function underlie the direct cardiotoxicity of combustible and heated tobacco products

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Aim

Smoking of combustible cigarettes is a major risk factor for cardiovascular diseases, yet the direct toxic effects of tobacco smoke on cardiomyocytes remain poorly understood. Additionally, the cardiac safety of heated tobacco products (HTPs), increasingly used due to their perceived reduced harm, has not been thoroughly evaluated. This study aimed to assess and compare the direct effects and underlying mechanisms of smoke extracts from combustible and HTP products on cardiomyocyte function.

Methods & Results

Cigarette smoke extracts (CSEs) were prepared from combustible cigarettes (with and without nicotine) and two widely used HTPs (HTP-1: PloomX and HTP-2: IQOS). Primary rat cardiomyocytes and human iPSC-derived cardiomyocytes were

exposed to these extracts. Combustible cigarette CSE significantly reduced cell viability and contractility in a concentration- and time-dependent manner. These effects were accompanied by abnormal intracellular Ca^{2+} handling, mitochondrial membrane depolarization, increased mitochondrial ROS, and cytochrome c release. Both HTPs also exhibited direct cardiotoxicity, with the degree of toxicity ranked as RF > HTP-2 > HTP-1. All CSEs impaired mitochondrial respiration, reduced ATP production, and disrupted Ca^{2+} homeostasis. Glycolytic compensation was observed with RF and HTP-2 but was absent in HTP-1, suggesting product-specific metabolic disruption.

Conclusions

Combustible cigarettes and HTPs both exert direct toxic effects on cardiomyocytes via abnormal Ca^{2+} signaling and mitochondrial dysfunction, regardless of nicotine content. However, mechanistic differences among products suggest varying levels of cardiac risk. These findings underscore the need for critical re-evaluation of the cardiovascular safety of all tobacco products, including HTPs.

A 03-04 A High-Fructose and High-Salt Diet Combination Promotes Fibrosis and Hypertrophy Through B-Type Natriuretic Peptide (BNP) Inhibition In Rat Hearts

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Aim

[A high-fructose diet increases cardiac fibrosis, but the combined effect of a high-fructose and a high-salt diet on cardiac hypertrophy and fibrosis is unclear.Natriuretic peptides may have anti-hypertrophic and anti-fibrotic properties. This study aimed to investigate the effect of natriuretic peptides on possible fibrotic changes in the heart under high-fructose and high-salt diet conditions.]

Methods & Results

[Twenty-four male Sprague-Dawley rats (8 weeks old) were fed with fructose (F, 60%), salt (S, 4%), fructose+salt (FS) and standart control (C) diet for 6 weeks with free access to water. On the last day of the feeding period the animals were sacrificed by exsanguination under ketamine-xylazine anesthesia (100 mg/kg and 10 mg/kg,). ANP, BNP, and CNP levels were measured by ELISA, cardiomyocyte diameter and fibrosis were evaluated histopathologically in the left ventricle. Statistical comparisons were made using One-way ANOVA and Tukey's test. BNP level was lower in the FS group (5,26±2,03) compared to F (8,98±1,42) and S (8,93±1,25) groups ($p<0.05$ for all) but ANP and CNP levels did not change in the left ventricle. Cardiomyocyte diameter increased in the F, S, and FS groups compared to control ($p<0.05$ for all). The fibrotic area was higher in the FS group than all in the other groups ($p<0.01$ for all).]

Conclusions

[Consuming combined high fructose and high salt diet may further increase the development of cardiac hypertrophy and fibrosis by decreasing BNP levels compared to their separate consumption.]

A 03-05 Acute exposure of Bisphenol S decreases *in-vitro* right atrial contractility in rats involving NO-cGMP independent pathway

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Aim

Bisphenols are widely used in manufacturing of polycarbonate material and epoxy resins. Bisphenol A (BPA) is reported to possess toxicity in various organs. Therefore, BPA is being replaced with safer analogs like BPF, BPG, BPS. Bisphenol S (BPS) is considered to be safe as it is heat and light resistant. However, BPS is reported to produce ventricular arrhythmia on acute exposure. Hence, the present study was undertaken to evaluate the effect of BPS, on spontaneously beating *in vitro* rat right atrial preparations.

Methods & Results

The *in vitro* spontaneous contractions of right atria obtained from adult female rats (200-250 grams, Wistar strain) were recorded. In group 1 (n=7), the atria were exposed to BPS (10^{-6}–10 mM; dissolved in Ethanol), and its effects on atrial

contractions were recorded as a cumulative concentration-response. In group 2(n=6), the tissue was exposed to different volumes of ethanol present in the corresponding concentration of BPS solution (10^{-6}-10mM) and contractility was recorded as mentioned in group 1. In Group 3 (n=15), after obtaining the spontaneous contractility, the atria were exposed to antagonists namely atropine (muscarinic receptor blocker), L-NAME (nitric oxide synthase inhibitor) and methylene blue (guanylyl cyclase inhibitor). BPS decreased the rate and force of spontaneous atrial contractions. Ethanol did not produce any change in contractility. L-NAME blocked the decrease in right atrial contractility produced by BPS, however, atropine and methylene blue could not antagonize the effects of BPS on atria indicating the involvement of NO-cGMP independent pathway.

Conclusion:

BPS significantly decreases right atrial contractility.

A 03-06 Proteomic and phosphoproteomic profiles of human heart failure with preserved versus reduced ejection fraction

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Aim

Low-grade systemic inflammation and endothelial dysfunction may be involved in the pathomechanism of human heart failure with preserved ejection fraction (HFpEF); however, evidence remains limited. Here, we aimed to identify a cardiac proteomic and phosphoproteomic signature that distinguishes HFpEF from heart failure with reduced ejection fraction (HFrEF), compared to nonfailing donor hearts.

Methods & Results

Left ventricular myocardial tissues of explanted or donor human hearts (Medical University Graz, Austria) were classified into nonfailing Control, HFpEF or HFrEF (mean EF (%): 63 (Control), 62 (HFpEF), 24 (HFrEF); mean age 58.1±9.5; mean BMI 26.6±2.7) and analysed by mass spectrometry (N=6-7/group). Gene Ontology (GO) enrichment analysis of significantly regulated proteins in HFpEF versus Control hearts revealed only two biological process terms, "complement activation" and "innate immune response". However, these terms also appeared in the analysis of HFrEF versus Control hearts suggesting low-grade systemic inflammation as a disease factor in both HFpEF and HFrEF hearts. The presence of low-grade systemic inflammation in either heart failure syndrome was confirmed by immunoblot analyses and immunohistochemistry, which showed substantially increased ICAM1 and S100A8 expression, respectively, in failing versus Control hearts. Phosphoproteomic alterations in HFpEF and HFrEF versus Control hearts concerned mainly sarcomeric proteins (e.g., titin, desmin), which were predominantly hyper-phosphorylated.

Conclusions

Low-grade systemic inflammation is present in both HFpEF and HFrEF patient hearts, with no distinct inflammatory proteomic signature observed in HFpEF hearts.However, our data suggest a link between systemic inflammation and sarcomeric protein phosphorylation, which we will further investigate in follow-up studies.

A 03-07 Severe Right Heart Failure Resulting from Inducible Cardiomyocyte-specific Titin Deletion

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Aim

Titin (Ttn) plays an important role in sarcomere assembly and passive/active force generation in the heart. To investigate the effect of cardiac titin deletion in the adult, we used a tamoxifen-inducible, cardiomyocyte-specific, homozygous Ttn knock-out mouse model with termination of translation after exon 3 (Ttn_tm1c/αMHC-MerCreMer: TtnC-KO). As earlier studies have mainly focused on the role of titin in the left ventricle (LV), we concentrated on the right ventricle (RV).

Methods & Results

For hemodynamic analysis, pressure-volume catheter measurements were performed. Morphological changes were assessed in heart sections stained with wheat germ agglutinin and liver sections stained with H&E.

Hemodynamic analysis showed severely impaired RV function, with a reduction in stroke volume and ejection fraction and an increase in end-systolic volume (ESV) compared to controls. In addition, TtnC-KO animals revealed a large increase in RV end-diastolic volume (EDV) and elevated end-diastolic pressure (P_{ed}). Preload independent measurements of end-systolic pressure-volume relationship (ESPVR) and end-diastolic pressure-volume relationship (EDPVR) showed a reduction in both contractility and passive stiffness in the RV of TtnC-KO mice.

Morphometry revealed that titin deletion did not alter cardiomyocyte area/tibia length and RV weight/tibia length in TtnC-KO compared to controls. RV heart failure was accompanied by severe hepatic congestion.

Conclusions

Cardiac titin deletion in the adult leads to RV heart failure, highlighting titin's crucial role in maintaining heart function and integrity.

A 03-08 Impact of DUSP1 on the progression of right ventricular failure in rats

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Aim

Among the genes that are specifically deregulated in the failing RV are dual-specificity phosphatases (DUSPs). Here we investigated the role of DUSP1, which is known to dephosphorylate cardiac ERK, p38 MAPK and JNK.

Methods & Results

Pulmonary artery banding (PAB) was performed in weanling rats to reach a stage of compensatory hypertrophy (at 7 weeks) or RV failure (RVF) 22 weeks after surgery. Injection of AAV9 ($5x10^{12}$GC/kg) 1 week and 15 weeks after surgery resulted in an approximately five-fold increase in DUSP1 expression throughout the study. While no differences in cardiac function were observed 7 weeks after surgery, PAB animals with DUSP1 overexpression revealed an improved RV function (TAPSE, RV FAC) but comparable RV hypertrophy, lower cardiac BNP expression (PCR, Western blot) or release (ELISA) and strongly reduced cardiac inflammation (cytokine array) at the stage of RVF. Sham animals (22 weeks after surgery), however, showed an unexpected deterioration in RV and LV function and impaired mitochondrial morphology in response to chronic DUSP1 overexpression compared to sham animals with GFP overexpression or sham animals without AAV9 treatment.

Conclusions

Chronic DUSP1 overexpression slows down the progression to RVF in PAB rats. However, there appears to be a significant difference in the impact of DUSP1 overexpression in a system of cardiac stress (PAB) or at baseline (sham). Future studies need to focus on the mechanisms underlying these DUSP1 effects, considering also that nuclear DUSP1 is thought to have an important role in the feedback loop of MAPKs nuclear signaling.

A 03-09 Adding insult to injury: stressed rat females at greater risk following myocardial ischemia?

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Aim

Although chronic stress is an important risk factor for cardiometabolic diseases, the

underlying mechanisms remain relatively poorly understood. This study therefore investigated whether chronic stress triggers sex-dependent cardiac dysfunction in isolated rat hearts exposed to regional ischemia.

Methods & Results

10-week-old female Wistar rats underwent chronic restraint stress (CRS) for four weeks (1 hour daily) versus matched controls, followed by euthanasia (sodium pentobarbitone) and heart excision for *ex vivo* functional assessment pre- and post-regional ischemia reperfusion. Snap frozen non-ischemic and ischemia-reperfused female cardiac tissues were further subjected to total and phosphoproteomics analysis *via* LCMS. The female CRS group displayed decreased plasma corticosterone ($p<0.001$), a lowered estradiol-to-progesterone ratio ($p<0.01$), and attenuated interleukin levels (IL-1α/1β/10/6; $p<0.05$) versus controls. However, they also exhibited elevated cardiac troponin T ($p<0.05$), tumor necrosis factor-a ($p<0.01$), and adrenocorticotropic hormone ($p<0.01$) levels versus controls. Although baseline functional parameters remained unchanged, post-ischemic recovery was especially compromised, evidenced by attenuated cardiac work performance ($p<0.05$), stroke volume ($p<0.05$), and cardiac output ($p<0.01$) versus controls. Proteomic analysis unveiled significant alterations in proteins associated with cardiac contraction (e.g. desmin, titin, MYLC), mitochondrial biogenesis (e.g. TAMM41, MDH2, DHTDK1), protein homeostasis (e.g. DRAP1, EIF4ENIF1), cell death signaling pathways (e.g. ANAPC1, PPP1R13L, WSB2), and pro-fibrotic mediators (e.g. ACLY, COMP, TIMP3) between CRS and controls, and across non-ischemic and ischemia-reperfused zones.

Conclusions

These findings suggest that chronic stress induces cardiac dysfunction particularly in females, with this heightened vulnerability due to a blunted hypothalamic-pituitary-adrenal axis response, together with a dysregulated inflammatory response and a unique proteomic signature.

A 03-10 Assessment of myocardial function and stiffness in isolated hearts of diabetic mice with HFpEF phenotype

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Heart failure with preserved ejection fraction (HFpEF) is a heterogeneous, multifactorial syndrome commonly associated with obesity, diabetes and hypertension. Diabetic mice with diet-induced obesity and streptozotocin treatment (DIO-STZ) develop a robust HFpEF phenotype with elevated filling pressures. Here, we investigated cardiac intrinsic alterations of contraction and relaxation as well as tissue stiffness.

Isolated perfused hearts of DIO-STZ or chow fed controls were used to assess pressure-volume-relationship as marker for ventricular stiffness, systolic and diastolic function at baseline and under dobutamine-stimulation as well as vascular function. Pressure-volume relationship was not different between the groups. In line with this, myocardial collagen content showed no differences. Basal systolic and diastolic function was depressed in DIO-STZ hearts as seen by 16% reduction of developed pressure (LVDP), and reduced dP/dt_{min} (LVDP; 72.4 ±5.0 vs. 86.3 ±6.6 mmHg , dP/dt_{min}; 3812 ±466 vs. 3996 ±413 mmHg/s). Dobutamine-response was abolished in DIO-STZ (LVDP; 83 ±5.0 vs. 120 ±6.3 mmHg). Coronary flow was increased in DIO-STZ hearts at baseline (4.3 ±0.6 vs 3.3 ±0.89 ml/min), but no increase was observed in DIO-STZ hearts under dobutamine-stimulation or adenosine treatment suggesting maximal vasodilation already under basal conditions. Repayment flow during reactive hyperemia was comparable between chow and DIO-STZ hearts indicating no endothelial dysfunction.

The systemic metabolic alterations of the diabetic DIO-STZ model induce a myocardial phenotype with disturbed systolic and diastolic function, and reduced coronary reserve due to increased basal flow. The increased filling pressures observed DIO-STZ mice *in vivo* are not caused by structural cardiac remodeling affecting tissue stiffness.

A 03-11 PIEZO1 Variation in Myofibroblasts Obtained at Open Heart Surgery

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Type 2 diabetes (T2D) comprises a clustering of inter-related and co-incident cardiovascular disease (CVD) risk factors, of which cardiac fibrosis is a common hallmark. Fibrosis is characterised by excessive extracellular matrix deposition by cardiac fibroblasts (CF) which, over time, results in stiff matrices that are less compliant to applied forces. Mechanical forces activate CFs and PIEZO1, a mechanosensitive, calcium-permeable ion channel, has emerged as central to maintaining myocardial mechanical properties and efficient pumping through regulating CF. This project aims to characterise PIEZO1 channels in CFs from CVD patients, with or without associated T2D. CF were isolated and cultured from right atrial appendage (RAA) biopsies (N=35) from patients undergoing open heart surgery (IRAS ID 200339). Cell characterization via immunohistochemistry identified a homogenous myofibroblast (myoCF) population. Gene expression analysis outlined collagen and inflammatory markers linked with PIEZO1 signalling in myoCF, which differ with severity and type of disease (N=18). Robust calcium entry is triggered upon application of Yoda1 and KC289 (Yoda2), two specific agonists of PIEZO1 channels (N=33). Preliminary analysis suggests a trend towards heightened PIEZO1 activity in ischaemic heart disease, and in patients with T2D, compared to those without. However, this is complicated by high inter-patient variability potentially due to confounding factors such as sex, age, BMI and global calcium changes. PIEZO1 channels are expressed and functionally active in human primary myoCF obtained at open heart surgery. There is high variability in the channel activity, which is potentially due to differing severities of ischaemic heart disease or T2D.

A 03-12 Localization of ryanodine receptor 2 and mitochondrial calcium uniporter in rat cardiomyocytes during postnatal maturation

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Aim

Cardiomyocyte contraction relies on calcium-stimulated mitochondrial ATP production. Calcium is released from the sarcoplasmic reticulum (SR) through ryanodine receptors (RyR), allowing uptake via mitochondrial calcium uniporters (MCU). Differences in the spatial relationship between the SR and mitochondria among cardiomyocytes from maturing, adult, and failing hearts suggest that mitochondrial calcium uptake mechanisms are affected during postnatal development and in heart failure.

Methods & Results

We address structural interaction between the SR and mitochondria in isolated left ventricular cardiomyocytes from female and male Wistar rats. Fixed cells are double stained to image both RyR2 and MCU proteins using the direct stochastic optical reconstruction microscopy (dSTORM) technique. This approach allows us to report protein clustering tendency and to describe distribution patterns of named clusters with high precision. Our results show a gradual increase in RyR2 density in cardiomyocytes from 16-week-old rats compared to 3-week and 1-week-old rats. We observe dense packing of MCU clusters in the centre of younger cells; these clusters become widespread across the cardiomyocyte during maturation. At the same time, western blotting of whole heart homogenates shows that MCU expression significantly decreases with animals' age.

Conclusions

Previous studies have demonstrated significant changes in the ultrastructure and function of cardiomyocytes in failing hearts, which resembled the characteristics of maturing cardiomyocytes. As such, our insight into the nanoscale organization of RyR2 and MCU proteins during postnatal maturation accommodates for investigating their potential functional interaction as a part of the calcium- stimulated mitochondrial ATP production mechanism.

A 03-13 Differential Expression of Somatostatin Receptors in Healthy and Failing Human Hearts

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The author has objected to a publication of the abstract.

A 04 | COMPARATIVE PHYSIOLOGY

A 04-01 Evolutionary convergence of the heart in Mammals and Cephalopods: shortening of connectin/titin in ventricular myocardium with increased activity

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Aim: Vertebrates and mollusks share an atrio-ventricular heart. During evolution, the elasticity of connectin, a spring molecule that determines the extensibility of cardiomyocytes, has been regulated to adapt to the mechanical environment of the heart. Energy-saving amphibians have spongy ventricles and long elastic regions of connectin, resulting in high ventricular compliance. Energy-consuming mammals have coronary-circulated ventricles with compact myocardium, the smaller atria, and shorter elastic regions of connectin. Here we compare the sessile oyster and the highly motile octopus to investigate whether there is an analogy in the relationship between activity and cardiac connectin, which regulates ventricular compliance at the molecular level, in mollusks.
Methods & Results: Mollusks, the only invertebrates with atria, were analyzed—specifically, oysters and octopuses. All animal procedures followed the university's animal committee regulations. Octopuses were humanely sacrificed by inserting a sharp spike into the brain to instantly disable the central nervous system. Oyster had large atria and spongy ventricular myocardial tissue. In contrast, octopuses had compact ventricles with coronary circulation and small atria, implying limited atrial contribution to ventricular filling. Compared to oysters, the elastic regions of connectin in the octopus ventricles were shortened, similar to their relationship in amphibians and mammals. The compliance of the octopus ventricles, assessed by diastolic pressure-volume relations, was intermediate between those of amphibians and mammals.
Conclusions: To support high activity levels, cephalopod mollusks and mammalian vertebrates may have independently evolved ventricles with compact myocardium perfused by the coronary circulation and reduced compliance to maintain coronary circulation.

A 04-02 Growing up in the heat: effects of post-hatch temperature changes on physiology and morphology in a wild bird

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Global temperatures have increased because of climate change and are predicted to continue to rise in the future. Laboratory studies in birds suggest that changes in temperature during development can affect offspring metabolism and growth. In the wild, resources invested in thermoregulation must be balanced against demands from other competing pressures, such as somatic maturation, repair, and plumage development. This complexity can hardly be addressed in a laboratory environment. Therefore, we investigated the consequences of the thermal environment in early post-hatching life in wild blue tit (*Cyanistes caeruleus*) nestlings, by simulating heatwave conditions during the first week of life when the chicks lack competent thermoregulatory ability. At the end of the heatwave period, we found negative effects of heating on body temperature in the heated compared to unheated control nestlings, which were compensated for by a lower metabolic heat production. Despite the physiological compensation, heated nestlings suffered lower body mass. When we measured the birds again shortly before fledging, after more than

one week in naturally occurring temperatures, no physiological effects of the previous heatwave simulation remained, and body mass was indistinguishable from the control treatment. Thus, physiological and morphological consequences of heat exposure appear flexible in the short term. This study brings new insights into how developmental priming and plasticity allow birds to cope with warmer temperatures, if phenotypic effects are long-lasting and under which circumstances such responses are adaptive or maladaptive.

A 04-03 Convergent Evolution of Mitochondrial Physiology in the World's Highest-Dwelling Mammals

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Aim

Life at extreme elevation must overcome the metabolic challenges of severe hypoxia and cold temperature. We investigated whether convergent changes in mitochondrial function underlie high-altitude adaptation in two small mammals from the high Andes – the Punta de Vacas leaf-eared mouse (*Phyllotis vaccarum*) and the Andean Altiplano mouse (*Abrothrix andina*), whose elevational range limits are the two highest records for mammals (6,739 m and 5,837 m elevation, respectively).

Methods & Results

Mitochondrial respiration, reactive oxygen species (ROS) emission, and enzymes activities were measured in skeletal muscles, left ventricle, and brown adipose tissues. High-altitude populations of *P. vaccarum* exhibited significantly higher mitochondrial respiration capacity and enzyme activities (COX, CS, HOAD) in the gastrocnemius muscle compared to a low-altitude conspecific and a closely related low-altitude congener (*P. darwini*) when all were studied after prolonged lab acclimation. This greater respiration capacity in high-altitude populations was preserved after mice were *bred and raised in captivity,* suggesting that the divergence has a genetic basis. Similar increases in gastrocnemius respiration were also observed in *A. andina* compared to a closely related low-altitude congener (*A. olivaceae*). By contrast, mitochondrial respiration in other tissues was not increased in high-altitude taxa, but there were some other differences in mitochondrial enzyme activities.

Conclusions

These findings suggest that an increased capacity for oxidative phosphorylation in skeletal muscle is a conserved mechanism of high-altitude adaptation across small mammals, likely to support shivering thermogenesis and locomotion in cold hypoxic environments. Supported by NSERC, NSF, and NIH.

A 04-04 Powering Life at the Top: Mitochondrial adaptations in high-altitude deer mice (*Peromyscus maniculatus*)

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Aim

High-altitude environments pose significant metabolic challenge for endotherms, as cold temperatures increase thermogenic demands while low O_2 levels (hypoxia) constrain aerobic ATP supply. We have examined how phenotypic plasticity and evolved changes in mitochondrial physiology contribute to overcoming this challenge in high-altitude deer mice (*Peromyscus maniculatus*).

Methods & Results

Mice from populations native to high and low altitudes were born and raised in captivity, then acclimated to either warm (25°C) normoxia or cold (5°C) hypoxia (~12 kPa O_2) in a full-factorial design. Mitochondrial respiration, ATP production rate, metabolic enzyme activities and reactive oxygen species (ROS) emission were measured in cardiac and skeletal muscles. In the heart, high-altitude mice exhibit greater lactate dehydrogenase activity than low-altitude mice, likely to enhance lactate oxidation. High-altitude mice also exhibit lower mitochondrial ROS emission due at least partly to increased intra-mitochondrial ROS consumption. In some skeletal muscles (diaphragm and gluteus maximus), high-altitude mice have evolved increased ATP production and phosphorylation efficiency, which should support

contraction and/or shivering thermogenesis. In some other skeletal muscles (vastus medialis), exposure to cold hypoxia increases leak respiration and phosphorylation efficiency, which may augment non-shivering thermogenesis. Consistent across skeletal muscles, however, cold hypoxia exposure tends to reduce ROS emission, with more pronounce reductions in high-altitude mice.

Conclusions

These findings demonstrate that many evolved and plastic changes in mitochondrial physiology may help enhance thermogenesis and minimize oxidative stress in deer mice at high altitude. Supported by NSERC.

A 04-05 Comparing the feline Body Mass Index (fBMI) and X-ray absorptiometry body composition in a feline population in Portugal

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Obesity is a risk factor common to multiple comorbidities in human and in domestic feline. However, obesity assessment and severity grading in cats remains unclear and sometimes equivocal. Indirect methods have been used to assess cats' body composition but their accuracy has to be determined. Our study compares body composition results from the feline Body Mass Index (fBMI) with those obtained with the dual-energy X-ray absorptiometry (DEXA) to determine their predictive potential. Forty healthy Portuguese domestic cats were selected to participate in this study after physical examination. All animals were sedated for the procedure (100 µg of medetomidine hydrochloride/kg, IM) prior to the DEXA evaluation. Body mass, lean body mass, adipose mass, and bone mass were measured The fBMI formula was adapted from the human BMI formula, where height was replaced by the length of the vertebral column from C1 to S3. All procedures were conducted in accordance with the current EU animal welfare legislation.

A significant ($p < 0.001$) correlation of $r = 0.77$ was observed between the percentage of body fat (%BF) measured by DEXA and the fBMI. This correlation between the tested fBMI formula and DEXA results indicates that both might be used inveterinary clinical practice. However, DEXA results also suggest that the percentage increase of body fat is followed by the preferential deposition of visceral adipose tissue over subcutaneous adipose tissue.

A 04-06 Salt loading affects gaping and voluntary thermal limits in *Tropidurus catalanensis* lizards

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Aim

We investigated the effects of salt loading - as a proxy for dehydratation - in gaping and voluntary thermal maximum (VTM) thresholds in *Tropidurus catalanensis* lizards (n=31) during summer and winter.

Methods & Results

Animals were assigned to hydration (154 mmol/L isotonic saline) or salt loading (2500 mmol/L hypertonic saline) states. Body temperature (Tb) was monitored using a thermocouple and osmolality confirmed hydration levels (Ethics Committee License n.2249/24). We used ANOVA to test for differences in Tb. Salt loading induced lower temperature thresholds for gaping ($Tb_{SL}=40.0\pm1.1°C$; $Tb_H=40.5\pm1.1°C$; $p=0.031$) and VTM ($Tb_{SL}=38.1\pm1.9°C$; $Tb_H=39\pm1.8°C$; $p=0.037$). Gaping thresholds did not vary between seasons, whereas VTM was higher in summer (38.7±1.9°C) compared to winter (37.8±1.7°C; $p=0.04$). Post-reproductive females showed higher gaping thresholds than males ($Tb_F=40.5\pm1.0°C$; $Tb_M=39.9\pm1.1°C$; $p=0.003$), but no sex differences were found in VTM.

Conclusions

The reduction in both gaping and VTM thresholds indicate a narrower range of tolerable body temperatures when osmotic balance is affected, highlighting the limiting role of water on thermoregulatory capacity. Also, the absence of seasonal variation in gaping suggests a more conserved physiological mechanism, likely triggered only under extreme thermal stress. This stability may reflect a conserved emergency response aimed at protecting critical organs, such as the brain,

regardless of environmental fluctuations. Finally, females' delay in gaping threshold might represent a water conservation strategy due to post-reproductive costs. These findings reveal the complex interaction between hydration state, seasonality, and thermoregulatory strategies in tropical lizards.

A 04-07 Methodological Advancements in the CAM Model: Yolk-Delivered Tracers, Dual-Tracer Autoradiography, Dynamic PET, *in ovo* Proton Irradiation and OCT Vascular Assessment

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Aim

The chorioallantoic membrane (CAM) model is a scalable preclinical model capable of high-throughput tumor-bearing *in vivo* growth of tumor material for oncological research. Our purpose has been to establish the necessary methods for the CAM model to become an attractive platform for advanced nuclear medicine and proton radiation experiments on CAM tumors. The model is based on an extra-embryonal membrane in the eggs of avian and reptilian species.

Methods & Results

Commercial Dekalb White eggs (gallus gallus domesticus) were opened in a square window and grafted with C3H mammary carcinoma or MOC2 mouse oral carcinoma tumor pieces. We established feasibility of *in ovo* proton irradiation and optical coherence tomography (OCT) of tumor vasculature. Radioactive tracer uptake via alternative administration routes was proved and quitified with PET and autoradiography.

Conclusions

Autoradiography was performed on 53 tumors and PET scans of 11 tumors were obtained after using alternative administration routes of various tracers to evaluate intratumoral distribution and pharmacokinetics. Furthermore, we have investigated *in ovo* proton irradiation and following growth on 16 tumors. To evaluate vessel morphology, we have developed a tool using OCT angiography data to investigate key vessel characteristics. The tumor-bearing CAM model is highly relevant as a preclinical model in nuclear medicine and radiation biology. *In ovo* proton irradiation with OCT angiography follow-up allows for high radiation doses and functional, non-invasive post-irradiation vascular evaluation. Autoradiography and PET scans after yolk injection of tracers can be used to evaluate physiological changes of vascularization and tumor micromilleu post-irradiation.

A 04-08 Continuous sampling of cerebrospinal fluid from the 3rd ventricle of the hypothalamus during hibernation

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The author has objected to a publication of the abstract.

A 04-09 Chronic Developmental *in ovo* Hypoxic Exposure Alters Femoral Artery and Vein Contractility in Juvenile Alligators (*Alligator mississippiensis*)

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Aim

Low oxygen experienced during development can influence the juvenile and adult

phenotypes. Studies of American alligators (*Alligator mississippiensis)* have demonstrated that the cardiovascular phenotype exhibits plasticity that persists into juvenile life when embryos are incubated under hypoxia conditions. Here we investigated the phenotypes of juvenile femoral arteries and veins in response to chronic hypoxic incubation to determine if developmental plasticity during the embryonic stages is observed in the vasculature at the juvenile stage.

Methods & Results

Alligator eggs were collected from nests and incubated at 30°C. At approximately 20% of incubation, eggs were randomly assigned to either normoxia (21% O_2) or hypoxia (10% O_2) for the remainder of *in ovo* development. After hatching, animals were raised in normoxic conditions for 4 to 6 years. Developing under low O_2-incubation conditions produced significant effects on juvenile vein and artery function. In response to angiotensin II (Ang II), vein contractile response was significantly blunted by O_2 incubation levels, with veins from hypoxic incubated animals producing significantly less tension when compared with veins from normoxic animals. Normoxic arteries and veins generated higher tensions in response to the thromboxane A mimic U 46619 when compared to vessels from hypoxic incubated animals. Neither arteries nor veins responded to the alpha-adrenergic stimulant phenylephrine.

Conclusions

Our findings suggest chronic developmental hypoxic incubation alters femoral artery and vein contractile phenotypes in juvenile alligators. This may be linked to decreased heart rate and lower mean arterial blood pressure seen in previous studies.

A 04-10 Preserved bile acid signaling in hibernating bear skeletal muscle

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Some mammals are naturally adapted to prevent muscle atrophy during hibernation, under conditions of prolonged starvation and physical inactivity. The existence of humoral factors contributing to the anti-atrophic strategy during hibernation has been reported, but they have not yet been identified. In the present study, we comprehensively analyzed bile acid (BA) levels in serum and feces collected from brown bears (*Ursus arctos*) to investigate their role in skeletal muscle maintenance. The concentration of total BA in serum was reduced during hibernation (1.14 ± 0.35 µM) compared with summer (5.76 ± 1.39 µM). However, the ratio of primary to secondary BA concentration was 3.5 times lower in winter, suggesting that the activity of the intestinal microbiota to synthesize secondary BA remained unchanged. On the other hand, the proportion of glycine conjugated BA was higher in winter. Based on the findings that secondary and conjugated BA can stabilize the binding of BA to its receptors, we analyzed the expression level of BA-related signaling molecules using transcriptomics and proteomics. The expression of TGR5 target genes such as *SIK1* and *NR4A1*, which induce muscle hypertrophy, and of downstream molecules such as *FBXO32* and *TRIM63* were stable across the seasons, suggesting no accelerated protein degradation during hibernation. Meanwhile, the expression of *DIO2* and *SERCA1/2*, which increase basal metabolic rate, was suppressed, suggesting the selective regulation of TGR5 signaling to prevent futile energy expenditure. Further studies should identify the contributing BA

in maintaining TGR5 signaling to prevent excessive protein degradation during hibernation.

A 04-11 Methods for studying the acute physiological and behavioural effects of keel bone (sternal) fracture in the laying hen (*Gallus gallus*).

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Keel bone (sternal) fracture occurs in >80% of egg-laying hens by the end of their commercial lifespan - across strains and production systems - and is a major concern in terms of animal welfare. Laying hens have vast calcium fluxes, with an equivalent of ~10% total body calcium deposited every 24hrs into the eggshell. Hence, physiologically explicit links exist between fracture risk, and both nutrition and endocrine control of mineralization, combined with environmentally-driven external fractures or internally prompted fractures resulting from the passage of large numbers of eggs (up to 500 eggs per hen commercial lifetime goal).

Diagnosis of fracture at scale has been reported via palpation and/or necropsy, with limitations in terms of the sensitivity of palpation and the retrospective nature of necropsy which precludes direct comparison with acute physiology. As an alternative, radiography and CT have been implemented within recent studies. Here, we study the acute physiological effects of fracture via twice weekly lateral radiography over the peak of production and fracture occurrence (n= 50, commercial hybrid- DeKalb housed 17-45 weeks of age) with acquisition of behavioural data in all birds, and i) heart rate, activity, ii) intermittent closed respirometry, iii) thermal and mechanical nociceptive testing, iv) preference for analgesia (meloxicam, conditioned place preference) in subgroups of 8-12 birds.

We present the refinement of the methodology for keel bone radiography, present fracture occurrence and reoccurrence in our population with radiological healing rates, and the positioning of loggers for determining chicken heart rate without interfering with keel bone function.

A 05 | EDUCATION AND TEACHING (1)

A 05-01 Exploring the Effectiveness of ChatGPT in Physiology: Usability, Reliability, and Educational Impact

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Aim

In recent years, use of artificial intelligence technologies in the field of education has gained significant importance. In this context, AI-powered tools like ChatGPT are actively contributing to educational processes, transforming teaching and learning methods. This study's aim was to evaluate the accuracy and value of the responses provided by ChatGPT from professionals in the field of physiology.

Methods & Results

Top 10 most frequently asked questions to ChatGPT-4 about physiology were determined. The questions were then answered by ChatGPT-4. Answers were evaluated by five physiology experts from different universities with seven-point Likert-type reliability and usefulness scales. The study found a moderate inter-rater agreement for usability (0.4 to 0.6) and good reliability (0.6 to 0.8) according to Cronbach's alpha scoring for Likert-scaled responses of the ChatGPT. The highest-rated answers were about homeostasis, scoring 3.4 for both usefulness and reliability. In contrast, the lowest-rated answers focused on the nervous system, with scores of 2.4 for usefulness and 2.2 for reliability. Overall, usefulness scores ranged from 2.2 to 3.5, while reliability scores varied between 1.8 and 3.6. Usefulness and reliability are scored on a scale from 1 to 7,where 1 is the lowest score and 7 is the highest.

Conclusions

The answers provided by ChatGPT to these 10 questions may lack the academic depth and terminology required by medical and health students. However, asking more specific and detailed questions can significantly improve the reliability and usability of AI-generated responses, making it essential for obtaining more accurate and satisfactory answers.

A 05-02 Artificial Intelligence in Healthcare Education: Insights from Undergraduate Health Sciences Students in Karachi, Pakistan

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Background & Objective:

Artificial Intelligence (AI) is rapidly transforming healthcare delivery and medical education. Understanding the perceptions, readiness, and barriers among future healthcare professionals is crucial for effectively integrating AI into undergraduate curricula. This multicenter study explores the knowledge, acceptance, and perceived barriers to AI among undergraduate health sciences students in Karachi, Pakistan.

Methods:

A cross-sectional survey was conducted using a structured questionnaire across multiple health sciences institutions. The tool assessed familiarity with AI applications, perceptions of AI's role in healthcare and education, and institutional support for AI integration. Preliminary findings from partial data collection are presented; complete results will be shared at the congress.

Results & Conclusion:

Preliminary analysis showed that while most students (69%) recognised AI's significance in healthcare, only 24% understood its basic computational principles. A large majority supported integrating AI education, with 75% endorsing AI teaching initiatives and 84% recommending curriculum inclusion. Although 80% believed AI enhances understanding of medical concepts, only 48% considered AI-generated information fully authentic, and just 15% reported receiving formal AI training. Major barriers included lack of awareness, limited access to training, and insufficient technological infrastructure. Institutional gaps were evident, with 80% reporting no formal exposure to AI education. About half of the respondents viewed AI as a tool that could complement or replace healthcare providers. These findings highlight strong acceptance but significant educational gaps, indicating an urgent need for structured AI integration into health sciences curricula. Final results and recommendations will be presented.

A 05-03 Development of a Physiology Learning and Assessment System to Automatically Generate Test Questions Constructed by Deep Learning Model and Aligned with Bloom's Taxonomy

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Aim

The rapid growth of knowledge in physiology makes it challenging for teachers to select important core concepts to assess student learning. Knowledge Graphs help students organise complex cause-and-effect relationships in physiological mechanisms, while well-designed multiple-choice questions (MCQs) support self-assessment and independent learning. Although commercial Generative AI (GenAI) has been used to automate question generation, it often produces low-quality questions with errors in stem and answer, requiring extensive manual verification. Other shortcomings include uneven coverage of topics and poor control of question difficulty. To promote self-directed learning, it is necessary to develop a system that automatically generates in-depth MCQs, allowing students to practice anytime and anywhere.

Methods & Result

We applied the principle of Deep Learning to train a Transformer-based NLP model that extracts content from physiology textbooks to construct Knowledge Graphs and generate test questions. These questions have been modelled on manually generated examples and cover all major physiology topics. In addition, they are aligned with different cognitive levels of Bloom's taxonomy to increase learning depth. Using our automated self-directed learning and assessment system, students can freely choose the physiology topics they want to study and practice on their own, and teachers can track and analyse students' performance based on their answers.

Conclusions

Our findings indicate that the physiology learning and assessment system we developed facilitates a deeper understanding of physiology and improves learning

outcomes compared to traditional MCQs. This physiology learning and assessment system can be used as an effective tool for students' self-directed learning.

A 05-04 Medical Students' Critical Internet Search - An Eye Tracking Study

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Aim

Students increasingly search, evaluate and use online information for their studies, a process defined as 'Critical Online Reasoning' (COR). We investigate students' successful task solving processes during internet search.

Methods & Results

Eye tracking data was collected of 20 first-year medical students solving a clinical case online.

Task performance (maximum score 12) ranged from 2 to 9 (mean 5.13 ± 2.05 SD; no gender difference: p=0.40). The upper performing third (6 high performers (HP), score 6-9) differed significantly (p<0.001) from the lower third (6 low performers (LP), score 2-4). HP spent less time reading the task (p=0.06; Cohen's d=1.24) and visited few more web pages than LP (p=0.12; Cohen's d=-0.97). Surprisingly, HP showed a higher total fixation time on text sections with unnecessary background information (p=0.04; Cohen's d=-1.41) and less total fixation time on task-relevant text (p=0.13; Cohen's d=0.96). Interestingly, HP and LP used websites with similar validity (p=0.25; Cohen's d=0.39), showed similar duration of search phases (vertical reading: p=0.91; Cohen's d=0.40), total fixation times on website orientation (subheadings + sidebar; p=0.37: Cohen's d=0.54) and validation elements (logo + date; p=0.28; Cohen's d=0.66), linear reading phases (information extraction; p=0.48; Cohen's d=0.43) and also similar writing time (p=0.64; Cohen's d=0.28).

Conclusions

Medical students' COR performance was less determined by retrieval and selection of task-relevant valid information. However, HP grasped the task and the task-relevant website areas faster and decided to gather additional deeper information. LP found enough needed information but used it less, possibly due to a lack of understanding.

A 05-05 Evaluation of medical students' academic performance in assignment preparation using ChatGPT: criteria of highly successful prompts

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Writing assignments are an essential component of medical curricula. However, plagiarism is a significant challenge, which becomes more evident with advancements in artificial intelligence.

Aim:

To assess students' performance after using ChatGPT to prepare for their assignments and clarify the criteria for highly successful prompts.

Methodology:

It was conducted with 83 first-year dental students at Gulf Medical University. Students were required to submit a scientific assignment on anemia and were divided into two groups based on their chosen research method: Literature Research Group (A) used textbooks and scientific websites, and ChatGPT Group (B) used ChatGPT as a research tool. After submission, all students took a post-assignment exam consisting of 10 MCQs. Additionally, group B completed a survey to reflect on their experience during this task: the benefits, challenges, and prompts used.

Results:

The ChatGPT group had a slightly lower mean assignment mark (18.26 ± 0.3) compared to the literature research group (18.90 ± 0.25), though the difference was not statistically significant.

Conversely, the ChatGPT group had a slightly higher mean exam score (9.68 ± 0.09 vs. 9.58 ± 0.10), but this difference was also statistically insignificant. Regarding the prompts used, the high achiever students asked ChatGPT for research links on the topic and potential websites for information, explaining the concept, and paraphrasing.

Conclusions:
Integrating ChatGPT in assignment preparation does not significantly change student performance compared to traditional methods. ChatGPT might not improve immediate assignment outcomes, but it could help with better understanding and retention of knowledge, which benefits exam performance.

A 05-06 Leveraging technology for impactful gamification in Physiology education

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Aim
There has been a growing interest in exploring the use of technology platforms in teaching and learning, and how they can be leveraged to enhance student engagement, concentration and performance. One innovative approach that has gained traction in recent years is gamification, which involves incorporating game elements into educational activities to make learning more engaging and interactive. The brain plays a central role in the learning process, with different brain waves being associated with different cognitive functions. By analyzing brain wave patterns, one can gain insights into how students are processing information and tailor teaching methods accordingly. Hence, the purpose of the study was to investigate gamification tools using technology supported strategies in the teaching and assessments of Physiology in a cohort of students in the Bachelor of Clinical Medical Practice (BCMP) programme.
Methods & Results
Students were assigned randomly to a gamification (G) with game-based contect or non-gamification (N-G) group. Concurrently, brain activity was monitored using non-invasive electroencephalogram (EEG) headsets connected to a computer. Students completed questionnaires to determine the leaner experience. The study was approved by the Research Ethics Committee of the University of Pretoria.
Conclusions
Results showed improved engagement and evaluation scores in the gamification group. In addition, distinct differences in brain activity between the gamification and non-gamification groups were observed. Thus, gamification may be a useful tool to improve student performance. Further studies on more aspects of brain function are needed to elucidate the effect of gamification on the teaching and learning of Physiology.

A 05-07 Impact of An Integrated Interdisciplinary Team-Based Learning Session of Medicine and Health sector Programs at Galala University

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Aim: This study aimed at demonstrating the effect of interdisciplinary session on student satisfaction and student-centered learning.
Methods & Results
An interdisciplinary team-based learning (TBL) session was established at Galala university. Five faculties are included namely Medicine, Pharmacy, Physical therapy, Applied health sciences and Nursing. Five teams of students (n=25) of these fields voluntarily shared in this TBL session. The topic was bronchial asthma. A minilecture was taught (Phase-1), individual and team readiness assurance tests were performed followed by a debriefing session (Phase-2), and a group application task (Phase-3). A student satisfaction survey was distributed among the students at the end of the session.
Results: There was an overall increase in t-RATscore compared to i-RAT in the used nine multidisciplinary questions, similarly, the average score was higher in t-RAT in comparison to i-RAT. Most of participants demonstrated gaining benfits of this interdisciplinary session through their responses in the student satisfaction survey.
Conclusions: Applying an interdiscipilnary TBL session has a beneficial role in strengthening gain of knolwedge, information retention, team working and problem solving in health education, ensuring active student-centered learning. It could be

recommeneded to use this strategy to enhance the outcomes of programs/faculties of health promotors.

A 05-08 Cap-Biospace a biology/physiology teaching program for human exploration of space and extreme environments.

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Human space exploration beyond low-Earth orbit will require new knowledge to ensure the survival of humans. The core of human exploration of space, but also of extreme terrestrial environments, requires an understanding of the interactions of the living world with its environment, and the physiological and biological responses to these interactions that support adaptations, fitting with the “one health” concept. It also demands the development of (bio)technologies to enable life support and preserve health in space and after return to Earth. To achieve this, it is essential to train a new generation of students, from undergraduate to PhD, and provide cross-disciplinary knowledge to space and biotech professionals. Cap-Biospace concerns 1/ health including physical and cognitive performances, 2/ biology to ensure the construction of a life support system enabling qualitative food production in a closed circuit and 3/ biotechnology for and in space (Pharma-in-Space). A consortium of 16 French universities, research organizations and companies have set up the Cap-Biospace program to structure a multi-disciplinary program to train multi-skilled technicians through to engineers and researchers. The partners are developing innovative, interdisciplinary courses, incorporating experimental methodologies, in-company placements and collaborative projects; they will enhance the attractiveness of professions linked to space exploration and biotechnologies to young people and professionals undergoing retraining. The partners are engaged in international cooperations to meet the training needs of the companies that will be developing the resources for the human space exploration. Cap-Bioscience has received squpport from France2030 and ANR (2025-2030).

A 05-09 Education Workshop at the University of Health Sciences Laos through the IUPS International Mentoring Program in Asia

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The IUPS International Mentoring Program was established to enhance global collaboration in physiological sciences and to inspire the next generation of physiologists. The program in Asia started in 2023 chaired by Prof. Yoshihiro Kubo, IUPS 2nd Vice President. Eight members of The Physiological Society of Japan volunteered to be a mentor. Eleven mentees joined from 5 countries in Asia. Prof. Noriyuki Koibuchi was assigned to a mentor of Dr. Dalounny Phatthananouluck from the University of Health Sciences, Laos, who has a strong desire to develop skills in teaching physiology. After extensive discussion, we decided to hold an education workshop in Laos to share the experience with colleagues. Then a two-day workshop was held in February 2025. The first day featured four sessions: conducting research in Laos, scientific writing, crafting multiple-choice questions, and team-based learning (TBL). Approximately 30 teachers from basic and clinical departments attended. The discussions were engaging, extending the duration of each session beyond the schedule. The second day was TBL model lectures with 50 medical students. Using clinical cases, students discussed topics such as hormone action, glucose metabolism, and blood pressure regulation. It was their first experience attending a bidirectional-style class. In the post-session questionnaire, students expressed great enthusiasm, stating that they thoroughly enjoyed the classes. The majority believed TBL was more effective than traditional lectures, enhancing their understanding. They also felt that they need more training opportunity to improve communication skills. We hope this workshop has contributed to achieving the objectives of the IUPS Mentoring Program.

A 05-10 Brand Building:Students'InsightsUsing the Touchpoints Wheel Model at Galala University, Egypt

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Background: University branding significantly influences student satisfaction and institutional reputation. It is valuable to measure the impact of pre-admission expectations, in-course experiences, and post-passing engagement on brand perception to enhance student retention, loyalty, and overall institutional branding strategies at Galala University (GU). **Aim: to** explore and to analyze students' experience of GU brand by using the Touchpoints Wheel Model, which addresses the various interactions (touchpoints) between students and the university throughout their study duration at the university.**Methods**: A cross-sectional descriptive design was followed, with randomly chosen students from academic programs at GU. Data on pre-admission perception, during-course experience, and post-passing engagement were collected through a structured questionnaire. Reliability was achieved with Cronbach's alpha values. Analysis of data included descriptive statistics, Pearson correlation, and multiple linear regression using SPSS SPSS.30.**Results**: There was a right-skewed distribution for all brand perception stages, with the highest scores in influencing touchpoints and overall brand perception. Pearson correlation analysis showed significant positive correlations between all brand touchpoints, with very strong correlations between Pre-Admission and During-Course experiences, and During-Course and Overall Brand Perception. A multiple regression analysis revealed that all three phases contributed significantly to brand perception, with During-Course experience being the most dominant predictor, followed by Post-Passing interaction and Pre-Admission expectations, influencing touchpoints.**Conclusion**: The study highlights the holistic nature of brand experience, with all three touchpoints playing a significant role in the university experiences of students. The During-Course phase was the most influential factor, highlighting the need for academic excellence, industry engagement, and co-curricular development to drive institutional reputation.

A 05-11 M$_{(1)}$ündlich – S(t)imulation of Competency-Oriented Learning

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Aim

Medical students often enter oral exams with insufficient preparation, especially in articulating knowledge under stress. To improve communication and structured answering, we developed a peer-teaching format simulating oral exams.

Methods & Results

190 third-semester medical students participated in the study. In groups of five, they either acted as examiner or examinee. They simulated two oral exams under tutor (trained medical students of higher semesters) supervision with a time interval of three months. Each session included extensive structured feedback. The Ethics Committee of the Medical Faculty of the University of Duisburg-Essen had approved the study. Participation was voluntary and based on informed consent.

After participation students reported greater confidence and a stronger sense of preparedness. More than 57% perceived an improvement in their exam performance. Self-assessment scores shifted positively, with more students rating themselves higher and fewer reporting poor performance. Stress levels decreased in the second round, with more students experiencing no or only mild stress. Around 90% of the students considered the feedback helpful, and students became more aware of their own performance and challenges in verbal expression. Most students appreciated the peer-group format being effective and planned to include it in their prospective preparation for oral exams.

Tutors also noted a marked improvement after the second round, especially in students' articulation and presentation quality

Conclusions

Collegial simulation of oral exams effectively enhances communication skills and exam awareness while reducing stress. It offers a valuable component to the traditional medical curriculum and is easily adaptable to other disciplines.

A 05-12 Muscle Physiology Escape Box – An interactive Teaching Resource

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The author has objected to a publication of the abstract.

A 05-13 Association Between Chronotype and Learning Motivation in Medical Students in Several Countries

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Aim

Chronotypes are typically categorized as morning, intermediate, or evening types, and have been associated with differences in cognitive performance, mental health, and daily functioning. Learning motivation is a critical factor in academic success, influencing not only study habits and performance but also students' well-being and future engagement in medical research. This study aimed to investigate the relationship between chronotype and learning motivation in medical students from several countries.

Methods & Results

A total of 540 first- and second-year medical students from Russia, Poland, Japan, and Australia participated in the study. Chronotype was assessed using the reduced Morningness–Eveningness Questionnaire (rMEQ), and learning motivation was evaluated using the Motivated Strategies for Learning Questionnaire (MSLQ). The MSLQ includes six subscales: intrinsic goal orientation, extrinsic goal orientation, self-efficacy for learning and performance, control of learning beliefs, task value, and test anxiety.

Based on rMEQ scores, students were classified into morning (26.7%), intermediate (60.5%), or evening (12.7%) types. Evening-type students showed significantly lower scores in intrinsic and extrinsic goal orientation, task value, and self-efficacy compared to morning- and intermediate-type students ($p < 0.05$).

Conclusions

Evening chronotype was associated with reduced learning motivation among medical students. These findings suggest that considering students' circadian preferences in curriculum planning—such as offering core classes in the afternoon or providing flexible schedules—may support the motivation and academic success of evening-type learners. This study highlights the relevance of chronobiology in optimizing educational strategies and promoting student well-being in medical education.

A 05-14 The Impact of Social Skills on Grade Point Average (GPA) Level in Medical Students

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The author has objected to a publication of the abstract.

A 05-15 Integrating Research into Teaching: Designing Effective Research-Oriented Learning Sessions

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Aim This session aims to explore strategies for designing research-oriented teaching sessions that enhance student engagement, critical thinking, and research skills. It will provide practical approaches to integrating research methodologies into teaching to foster an inquiry-based learning environment.

Methods & Results

- The session will take an interactive approach by integrating presentations, case studies, and group discussions.
- The main strategies include: Describing effective frameworks for teaching that is focused on research.
- Showcasing best practices through practical examples.
- Involving participants in collaborative activities to create their own lesson plans that incorporate research.

Conclusions

Integrating research into teaching enhances student learning by promoting active inquiry and evidence-based reasoning.

This session will equip educators with practical tools to design and implement research-oriented teaching strategies, ultimately fostering a more research-engaged learning environment.

A 06 | EXERCISE: THERMOREGULATION AND TRAINING

A 06-01 Cool-seeking behaviour in older adults and in people with multiple sclerosis during simulated heatwave conditions

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Aim

Understanding cool-seeking behaviours during heatwaves in vulnerable groups—e.g., older adults (ELDERLY) and young people with multiple sclerosis (pwMS)—can inform better person-centred heat resilience strategies. Hence, we investigated cool-seeking behaviours in ELDERLY and pwMS during simulated heatwave conditions.

Methods & Results

A cohort of healthy ELDERLY (≥65y) and young pwMS (≤45y) underwent a 1-h exposure in a climatic chamber set at 37.2(±0.3)°C and 40(±4)% RH, during which they performed: (*i*) 25-min rest; (*ii*) 10-min low-intensity cycling; (*iii*) 25-min rest with access to cool-seeking behaviours consisting of self-fanning and water-mist spraying. We continuously recorded core (T_{core}) and mean skin (T_{sk}) temperature, thermal discomfort, mental and physical fatigue, and characterised cool-seeking behaviours (i.e., fanning duration and water volume used). At the time of writing, 7 ELDERLY (69±2 years) and 4 pwMS (39±3 years) completed the trial (target N=10 per group). Heat exposure similarly increased T_{core} (+0.37±0.23°C) and T_{sk} (+4.22±0.67°C) in ELDERLY and pwMS. More pwMS engaged with self-fanning (4/4) and water-mist spraying (3/4) than ELDERLY (fanning: 1/7; water-spraying: 3/7). PwMS also fanned for longer (13m01s±1m55s vs 5m04s; p=0.039), yet they used the same water volume as ELDERLY

(~2.97±1.84ml). Thermal discomfort increased in both groups (from comfortable to slightly uncomfortable), yet only pwMS experienced increases in mental and physical fatigue (p<0.05).

Conclusions

Preliminary observations indicate that pwMS engage in cool-seeking behaviours to a greater extent than ELDERLY, and experience greater mental and physical fatigue, during heat exposure. This highlights a complex interaction of age and clinical status in driving heat vulnerability.

A 06-02 Thermoregulatory response during a heat tolerant test: implications in exertional heatstroke

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Aim

Exertional heatstroke (EHS) is the most serious heat illness, defined by core temperature above 40°C and central nervous system dysfunction. Most EHS cases are related to circumstantial factors, while others can't be explained by circumstances, and are consequently qualified as atypical. The present study focuses on determining whether biological and physiological signatures are present in theses atypical EHS.

Methods & Results

Overall, 56 soldiers -18 healthy controls, 17 atypical and 21 circumstantial EHS- performed a heat tolerant test (HTT). Heat intolerance was declared if core temperature exceeded 38.5°C after two hours of treadmill walk at 5 km/h in a chamber set to 40°C and 40% of relative humidity. Physiological and anthropometric data relative to thermoregulation and biological samples for thermal stress biomarkers were collected before, during, after HTT and in recovery. Atypical group had the highest intolerance rate and significantly higher final core temperature (65%, 38.81°C±0.63), than circumstantial (33%, 38.38°C±0.52) and control (39%, 38.34°C±0.42) participants. Physiological and anthropometric data were not different between groups, whereas considering whole cohort, intolerance was associated to higher body weight and fat percentage, and lower body surface area/weight ratio and sweat rate. Intolerant group showed greater heat shock response and energy substrates mobilization, with respect to thermal stress level. However, none of these biological variables distinguished atypical group from the others.

Conclusions

To conclude, intolerance appears to be associated with anthropometric factors and biomarkers. Further research is needed to understand physiological and biological mechanisms behind atypical EHS, including the potential involvement of genetic polymorphisms.

A 06-03 Exercise regulates the influence of local temperature on microvascular functions in humans

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Aim

Reactive hyperemia (RH) assesses peripheral microvascular functions and has been associated with obesity and all-cause mortality. Temperature regulates vascular tone and oxygen availability but its effect on RH with and without exercise remains unclear. This study aimed to investigate how muscle temperature modulation influences RH responses before and after an exercise bout.

Methods & Results

Seven healthy participants completed three 3-minutes all-out exercise bouts on a cycle ergometer following thigh muscle heating (+3℃), cooling (-6℃) and control. RH assessment was conducted with a rapid pressure cuffing system and near-infrared spectroscopy (NIRS) and measures of max tissue saturation index (Max TSI), and reperfusion slope were collected. Non-parametric (Friedman) tests were performed to compare pre- and post-exercise conditions. Muscle temperature was significantly different between conditions before ($p < 0.005$), and after exercise (control: 35.59 ± 0.63°C, Hot: 37.39 ± 0.38°C, Cold: 33.84 ± 1.63°C; $p < 0.005$). Max TSI was significantly higher in the hot condition (82.03 ± 3.51%) compared to the cold condition (79.94 ± 3.29%; $p < 0.001$) and control (81.27 ± 3.19%; $p < 0.001$) after exercise only. Reperfusion slope was also significantly lower in the cold condition (79.59 ± 3.24%) compared to control (80.84 ± 3.17%; $p = 0.008$) and hot conditions (81.54 ± 3.44%; $p = 0.021$).

Conclusions

Muscle temperature has limited influences on microvascular function at rest, but exercise-induced metabolic stress increased vascular sensitivity to temperature, suggesting enhanced vascular function responses from tissue heating. These results may be relevant for those suffering from vascular dysfunctions.

A 06-04 Activation of Hypothalamic Q neurons During Exercise: A Possible Role in Defending Against Exercise-Induced Hyperthermia

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The author has objected to a publication of the abstract.

A 06-05 A role of central glucose-sensing in thermoregulation

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Glucose serves as the primary energy substrate for numerous physiological processes, including thermogenesis. Although body temperature is generally well-maintained under normal conditions, it can become dysregulated during metabolic challenges such as prolonged fasting in mice. This indicates that energy deficits—particularly hypoglycemia—may impair thermal homeostasis. In fact, hypothermia is a known complication in diabetic individuals with low blood glucose levels. Pharmacological inhibition of glycolysis using 2-deoxyglucose (2DG) induces hypothermia not only in rodent models but also in humans, further implicating glucose availability as a key regulator of body temperature. Therefore, we hypothesized that central glucose-sensing mechanisms contribute to thermoregulation by monitoring and responding to systemic energy status. Our study revealed a significant correlation between blood glucose levels and core body temperature. Moreover, central administration of alternative energy substrates, such as lactate or pyruvate, mitigated 2DG-induced hypothermia, suggesting a compensatory metabolic effect. Notably, we observed elevated expression of c-fos—a marker of neuronal activity—in the preoptic area (POA) of the hypothalamus during 2DG-induced hypothermia. Functional inhibition of POA neurons via chemogenetic silencing (DREADD) attenuated the hypothermic response to 2DG. This points to a critical role for the POA as a neural hub integrating metabolic signals and coordinating thermoregulatory responses. These findings highlight the importance of brain glucose-sensing neurons in the regulation of body temperature.

A 06-06 The maturation of regional sweating patterns during pubertal development in adolescent girls

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The regional distribution of local sweat rates (LSR) in pre-pubertal girls (~8 years old) does not resemble the pattern seen in adult women, with the highest LSRs seen over the extremities (hands/feet) in the former and over the upper torso in the latter. Yet, it is unknown when this maturation occurs. This study investigated changes in LSR throughout puberty in girls. We hypothesised that LSRs across the torso would increase to a greater extent with aging than the periphery, to align with sweating patterns seen in adulthood.

Twenty-eight healthy 8- to 25-year-old females, aligning to 5 Tanner puberty stages: T1 (n=5), T2 (n=4), T3 (n=4), T4 (n=8), T5 (n=7) were recruited. Participants exercised on a cycle ergometer for 41 minutes at a fixed metabolic heat production (Hprod) relative to body surface area (155±10 W/m2), in a climatic chamber (36"℃" , 50% RH). LSR was measured at 7 body sites during the final 5 minutes of exercise. Correlation analysis was performed between age and LSR. LSR at the bra triangle ($r=0.858$, $p<0.001$), abdomen ($r=0.602$, $p<0.001$), upper back ($r=0.481$, $p=0.010$), and lower back ($r=0.519$, $p=0.005$) significantly and positively correlated with age. LSR at the hand ($r=-0.295$, $p=0.127$), thigh ($r=0.242$, $p=0.215$), and shin ($r=0.242$, $p=0.214$) did not significantly correlate with age. Our findings indicate that pubertal development in young women brings about significantly increased LSR over the torso (including the bra triangle), while LSR at the hands and at the lower limb sites are unchanged during pubertal development.

A 06-07 Sweating and cutaneous vasomotor function in para-athletes with various sweating disorders

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Aim

Para-athletes with spinal cord injuries tend to have reduced heat tolerance due to impaired autonomic thermoregulatory functions such as sweating and vasomotion at the injury site. This study investigated sweat gland and cutaneous vasomotor function in para-athletes with various sweating disorders.

Methods & Results

Participants were 6 para-athletes with sweating disorders. Sweat gland function was evaluated by filling a sweat capsule with 10% acetylcholine and allowing the 10% acetylcholine to penetrate the skin by iontophoresis. Iontophoresis was performed at two locations: a non-sweating area (disabled skin area) during daily exercise and a non-disabled skin area. Cutaneous vasomotor function was evaluated using lower leg immersion in carbon dioxide-rich water (1000 ppm) at a water temperature of 34°C.

The sweat gland function in the non-disabled skin area was within the normal range in all para-athletes, and the sweating reaction was confirmed by palpation. Additionally, cutaneous vasodilation was observed in all para-athletes at the immersion area in the carbon dioxide-rich water. Meanwhile, sweating was observed at the disabled skin area in para-athletes whose injury occurred relatively recently, but no sweating response was observed in para-athletes whose injury occurred more than 20 years.

Conclusions

The results of this study suggest that the decline in sweat gland function caused by various injuries may be related to how long ago the injury occurred.

A 06-08 Thermoregulatory response during a heat tolerant test: implications in exertional heatstroke

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Aim

Exertional heatstroke (EHS) is the most serious heat illness, defined by core temperature above 40°C and central nervous system dysfunction. Most EHS cases are related to **circumstantial** factors, while others can't be explained by circumstances, and are consequently qualified as **atypical**. The present study focuses on determining whether biological and physiological signatures are present in theses atypical EHS.

Methods & Results

Overall, 56 soldiers -18 healthy controls, 17 atypical and 21 circumstantial EHS- performed a heat tolerant test (HTT). Heat intolerance was declared if core temperature exceeded 38.5°C after two hours of treadmill walk at 5 km/h in a chamber set to 40°C and 40% of relative humidity. Physiological and anthropometric data relative to thermoregulation and biological samples for thermal stress biomarkers were collected before, during, after HTT and in recovery.

Atypical group had the highest intolerance rate and significantly higher final core temperature (65%, 38.81°C±0.63), than circumstantial (33%, 38.38°C±0.52) and control (39%, 38.34°C±0.42) participants. Physiological and anthropometric data were not different between groups, whereas considering whole cohort, intolerance was associated to higher body weight and fat percentage, and lower body surface area/weight ratio and sweat rate. Intolerant group showed greater heat shock response and energy substrates mobilization, with respect to thermal stress level. However, none of these biological variables distinguished atypical group from the others.

Conclusions

To conclude, intolerance appears to be associated with anthropometric factors and biomarkers. Further research is needed to understand physiological and biological mechanisms behind atypical EHS, including the potential involvement of genetic polymorphisms.

A 06-09 Mechanism involved in cold water immersion after exercise: Blood redistribution and increase in energy expenditure during rewarming.

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Thermogenesis is well understood, but the relationships between cold water immersion (CWI), post-CWI rewarming and associated physiological changes are not. This study investigated muscle and systemic oxygenation, cardiorespiratory and haemodynamic responses, and gastrointestinal temperature during and after CWI. 21 healthy men completed randomly 2 protocols. They consisted of a 48 minutes heating cycling exercise followed by 3 recovery periods (R1-R3). R1 lasted 20 minutes in a passive semi-seated position on a physiotherapy table at ambient room temperature (AMB). Depending on the protocol, R2 lasted 15 minutes either at room temperature (R2_AMB) or in a water at 10°C up to the iliac crest (R2_CWI). R3 lasted 40 minutes at AMB while favouring rewarming after R2_CWI. This was followed by 10 minutes of cycling. Compared to R2_AMB, R2_CWI ended at higher $\dot{V}O_2$ in the non-immersed body part due to thermogenesis (7.16(2.15) vs. 4.83(1.62) $ml.min^{-1}.kg^{-1}$) and lower femoral artery blood flow (475(165) vs. 704(257) $ml.min^{-1}$) ($p < 0.001$). Only after CWI, R3 showed a progressive decrease in vastus and gastrocnemius medialis O_2 saturation, significant after 34 minutes ($p < 0.001$). As blood flow did not differ from the AMB protocol, this indicated local thermogenesis in the immersed part of the body. After CWI, a lower gastrointestinal temperature on resumption of cycling compared to AMB (36.31(0.45) vs. 37.30(0.49) °C, $p < 0.001$) indicated incomplete muscle thermogenesis. In conclusion, the rewarming period

after CWI was non-linear and metabolically costly. Immersion and rewarming should be considered as a continuum rather than separate events.

A 06-10 The impact of stratum corneum hydration on underarm wetness perception during contact with fluids varying in thermal conductivity

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Introduction

Enhanced design and comfort of antiperspirant deodorants requires an improved understanding of skin physiology and wetness perception in relation to users' hygiene habits. This study investigated how showering-induced changes in stratum corneum hydration impact wetness perception during application of fluids varying in thermal conductivity at the underarm.

Method and Results

Twenty women (25±5 yrs) continuously reported their wetness perceptions (WP) at the underarm on a digital 100-mm visual analogue scale (i.e. dry to extremely wet) during 60-s contact with cotton patches (25 cm^2) secured to a temperature-controlled probe (23°C). The patches were saturated with 1 mL of either water (0.60 W/m·K) or polyethene glycol (PEG; 0.19 W/m·K), prior to and following stratum corneum overhydration (~50 % increase) via a 5-min exposure to running warm water (34 ± 1 °C). Area under the curve (AUC) for WP for each stimulus was calculated and analysed for the effects of hydration status and fluid type using a two-way ANOVA.

Fluid type ($p < 0.001$), but not hydration status (-16.1 mm^2, 95% CI: -217, +185; $p = 0.867$), had an effect on WP (Fig. 1), such that greater AUC occurred during contact with water than contact with PEG (+430 mm^2, 95% CI: +119, +741; $p < 0.05$).

Conclusion

Given the same contact and environmental conditions, application of fluids with greater thermal conductivity results in greater underarm WP, irrespective of skin hydration status. This knowledge may impact future APDO fluid formulations to reduce WP during application.

A 06-11 Investigating heart rate regulation in ultra-endurance cyclists: A pilot study

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Aim

Extreme endurance racing induces temporary changes in heart rate response to stimuli. We hypothesise this is due to modulation of the autonomic nervous system's response to exercise, which may be altered by ageing. We sought to ascertain the responsiveness of the cardiovascular system in ultra-endurance cyclists to postural and exercise stimuli.

Methods & Results

This study was approved by the University of Westminster REC and follows the principles of the Declaration of Helsinki. Heart rates of 12 ultra-endurance cyclists (nine males, three females; six aged 18–35 years, six aged 45–70 years) were measured in a supine position, then standing, followed by a maximal exercise test to exhaustion to determine maximal heart rate (HRmax) values. Heart rate recovery (HRR) was assessed post-exercise. Age groups were compared using the Mann-Whitney test for probability of differences.

In line with the normal population, HRmax was higher in younger (185±9 bpm) than older (170±8 bpm, p=0.015) participants. Heart rate reserve was also higher in younger (130±9 bpm) than older (113±8 bpm, p=0.015) participants. Heart rate responses to postural change and onset of exercise were similar in younger versus older participants. HRR was 0.35±0.05 Hz in younger versus 0.31±0.02 Hz in older participants (Cohen's d 1.3, p =0.08).

Conclusions

Ultra-endurance cyclists of all ages exhibited maximum heart rates and chronotropic indexes in line with normal population values. Parasympathetic tone, inferred by

heart rate recovery response, may be affected by age but needs confirming with adequately powered studies. Our preliminary data indicate 16/group is required.

A 06-12 Endurance training increases brain's prefrontal cortex oxygenation indices at submaximal exercise

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Aim

Sufficient blood and oxygen supply to the brain is crucial for optimal performance and exercise tolerance. The purpose of this study was to test the effect of endurance training on brain oxygenation hypothesizing that increased fitness would cause enhanced oxygenation.

Methods & Results

Sixteen male distance runners (age: 34.1±5.1 yrs, weight: 70.8±4.1 kg) participated in the study. Training consisted of 2 high-intensity interval sessions (90-100% VO2max) and 3 continuous sessions (70-80% VO2max) per week for 2 months. Pre and post training measurements of VO2max in treadmill and cycling were conducted to assess aerobic capacity. Then, submaximal cycling exercise was executed for 10 minutes at 130 Watt corresponding to 5% under the anaerobic threshold during which brain's left and right prefrontal cortex and vastus lateralis muscle's oxygenation were measured using functional multichannel near-infrared spectroscopy.

Training increased VO2max both in running ($p<0.01$) PRE: 55.2±1.2 vs POST: 58.8±1.8 ml/kg/min and cycling ($p<0.01$) PRE: 51.1±2 vs. POST: 54.3±2.2 ml/kg/min. During submaximal cycling increases were observed throughout the 10-minute exercise ($p<0.01$) in oxyhemoglobin (O2Hb), deoxyhemoglobin (HHb) and total hemoglobin (THb) for both left and right prefrontal cortex. Moreover, muscle O2Hb and THb decreased ($p<0.05$), while HHb did not change. End-tidal partial pressure for carbon monoxide (PETCO2) remained similar after training.

Conclusions

Endurance training and increased fitness seem to facilitate redistribution of blood volume from the muscle to the brain augmenting brain oxygenation at a given submaximal exercise intensity.

A 07 | EXERCISE AND CARDIOVASCULAR SYSTEM

A 07-01 Local Negative Pressure Enhances the Venoarteriolar Reflex Activity in Healthy Humans

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Aim

The venoarteriolar reflex (VAR) is a local vasoconstrictor response of arterioles to increased venous transmural pressure, serving to prevent edema formation. Recently, a consensual contralateral vasoconstrictor response to VAR has also been demonstrated. Experimentally, the VAR can be elicited by placing a limb in a dependent position or by applying proximal venous occlusion. Cupping therapy is known to induce transient vascular congestion through the application of local negative pressure (LNP). We therefore hypothesized that prior exposure to LNP would modulate the magnitude of the VAR.

Methods & Results

Ten healthy subjects (21.4 ± 1.8 years) underwent two standardized VAR assessments, performed before and after LNP. Each VAR protocol consisted of a 3-minute baseline, a 3-minute passive arm dependency phase, and a 3-minute recovery. Between the two VAR tests, a 3-minute LNP stimulus (–525 mmHg) was applied to the volar forearm using a vacuum cup connected to a manual pump. Skin perfusion was continuously recorded during each VAR test using photoplethysmography (PPG) on both forearms. Nonparametric statistics were used for analysis ($p < 0.05$). Following LNP, the VAR elicited a significantly greater reduction in perfusion during the dependency phase compared to pre-LNP. These

results suggest that LNP enhances VAR potency, potentially through increased venular capacitance or augmented arteriolar myogenic tone. No significant difference was observed in the magnitude of the contralateral perfusion response between the two VAR tests.

ConclusionsThis pilot study supports the use of LNP as a physiological stimulus to probe vascular reactivity and reflex integrity in vivo.

A 07-02 Multimodal Optical Assessment of Vascular Dynamics During Post-Occlusive Reactive Hyperemia

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Aim

Post-occlusive reactive hyperemia (PORH) represents a transient microvascular overcompensation following ischemia, widely studied through photoplethysmography (PPG). However, the integration of alternative low-cost optical tools such as videocapillaroscopy (VC) and near-infrared imaging (NIRI) remains underutilized in this context. We investigated whether combining these techniques can enhance the quantification of PORH responses in healthy individuals.

Methods & Results

Fourteen healthy subjects (21.5 ± 4.2 years) underwent suprasystolic occlusion of one upper limb, with synchronous recordings of PPG (finger pulp), VC (nailfold), and NIRI (dorsal hand veins). The protocol comprised a 5 min baseline, 3 min occlusion at 200 mmHg, and 5 min recovery. PPG-derived perfusion, a hemoglobin index (CHb) from VC, and venous diameter from NIRI were analyzed using nonparametric statistics. Occlusion elicited pronounced drops in PPG perfusion (–95.3%, $p<0.001$) and CHb (–8.3%, $p=0.007$), along with venous dilation ($p=0.008$). All parameters returned toward baseline post-occlusion. VC and NIRI reproduced key hemodynamic trends, albeit with lower sensitivity than PPG. Interestingly, venous expansion correlated with the rate of perfusion decline.

Conclusions

These findings support the complementary role of VC and NIRI in vascular assessment and highlight the feasibility of multimodal optical approaches in both research and clinical vascular physiology.

A 07-03 Near-Infrared Imaging of Superficial Vein Morphology During Transient Venous Congestion

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Aim

Superficial veins are essential for vascular access and systemic hemodynamics, yet their structural assessment is often overlooked due to limitations of conventional imaging. Near-infrared imaging (NIRI) devices, commonly used to guide venipuncture, may offer novel insights into venous morphology and compliance under physiological stress. We evaluated the feasibility of using NIRI to monitor structural changes in dorsal hand veins during a standardized venous congestion protocol.

Methods & Results

Fourteen healthy subjects (21.5 ± 4.2 years) underwent upper-limb occlusion (200 mmHg, 3 min). Real-time NIRI recordings captured morphological parameters from metacarpal veins and tributaries, including vein width, branching angles, asymmetry, junctional exponent deviation, and optimality ratio. Nonparametric statistics were applied ($p<0.05$). A significant dilation of trunk and branch veins during occlusion was observed ($p < 0.001$), while geometric parameters remained stable. Notably, junctional metrics remained consistent with Murray's law, suggesting preserved vascular architecture despite hemodynamic load.

Conclusions

These findings underscore the potential of NIRI-based vein imaging for dynamic, non-invasive assessment of venous compliance and geometry. The method's low cost, portability, and compatibility with automated analysis workflows highlight its promise for clinical and perioperative vascular monitoring.

A 07-04 Texture Analysis of Superficial Veins During Post-Occlusive Reactive Hyperemia

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Aim

The venous network plays a key role in microvascular physiology, yet functional imaging of its dynamic behavior remains limited. Near-infrared reflectance imaging (NIRI), widely employed for vein localization, may also provide functional insights when paired with texture analysis (TA). In this study, we examined whether TA applied to NIRI images can detect subtle venous changes during post-occlusive reactive hyperemia (PORH).

Methods & Results

Fourteen healthy subjects (21.5 ± 4.2 years) underwent a standardized upper-limb occlusion protocol (200 mmHg, 3 min), during which real-time vein finder images of the dorsal hand were collected. Nonparametric statistics were applied ($p<0.05$). Regions of interest were manually defined, and both morphological (vein width) and classical texture parameters (contrast, correlation, entropy, energy, homogeneity, fractal dimension, lacunarity) were extracted. Composite indices combining texture and geometry were also computed. While occlusion significantly increased vein width ($p < 0.001$), most texture parameters remained stable. Notable exceptions included increased correlation during occlusion ($p = 0.023$) and decreased lacunarity during recovery ($p = 0.024$). Composite metrics were more sensitive: entropy-to-width and correlation-to-width ratios decreased during occlusion ($p < 0.001$), and total entropic content rose ($p < 0.001$), suggesting augmented signal complexity. A modest increase in the correlation-to-entropy ratio during recovery ($p = 0.026$) may reflect delayed structural reorganization.

Conclusions

These findings highlight the value of texture-based features and integrative indices for uncovering functional venous adaptations not captured by morphology alone. This approach could inform the development of affordable, non-invasive tools for vascular assessment at the bedside.

A 07-05 Stronger vasoconstrictive response to hypovolemic stimuli in males compared to females

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Aim

While it is generally accepted that women exhibit a lower tolerance to orthostatic stress than men, the issue of sex-differences in the vasoconstrictive capacity is controversial.

This study aims to assess the extent of sympathetic vasoconstriction in terms of reduced tissue oxygenation detected by near infrared spectroscopy in males and females during mild hypovolemic stimuli induced by lower body negative pressure (LBNP). A 2-WAY mixed-ANOVA was performed to evaluate sex- and pressure-dependence of TOI and HHb slopes.

Methods & Results

Twenty-eight subjects (age 23.8±7.3) were subjected to a randomized sequence of LBNP stimuli (10-40 mmHg, 90-s duration) while monitoring the slopes of the tissue oxygenation index (TOI), and concentration changes in deoxygenated haemoglobin (HHb) in the right thigh and forearm, indicative of the ongoing sympathetic vasoconstriction and blood flow reduction.

Analysis of time courses revealed initial transients related to fluid shifts, followed by linear reduction in TOI and increase in HHb, with a significant dependence on sex ($p<0.05$) and LBNP ($p<0.01$) in both arm and leg. At -40 mmHg, in males vs.

females, respectively, TOI slope was -4.99±2.33 vs. -2.20±1.24 %/min in forearm, and -3.96±2.92 vs. -1.58±1.06 %/min in thigh, while HHb slope was +63.9±35.9 and +26.6±15.6 μM*cm/min in forearm, and +79.1±36.4 and +40.0±14.6 μM*cm/min in thigh.

Conclusions

The results consistently evidenced a stronger vasoconstrictive sympathetic response in males than females in both upper and lower limbs and suggest that this may constitute a relevant mechanism behind the male's stronger tolerance to hypovolemic stimuli.

A 07-06 Investigating the effect of transcutaneous vagal nerve stimulation on cardiorespiratory parameters in healthy adults

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Aim

Transcutaneous vagus nerve stimulation (tVNS) uses non-invasive stimulation of vagal fibres. While previous research suggests that tVNS may influence heart rate variability (HRV), thermoregulation and respiratory data, findings remain inconsistent. This study investigated the effects of tVNS on HRV, breathing rate, surface temperature, and SpO_2 in healthy adults.

Methods & Results

Thirty participants underwent two randomised five-minute tVNS interventions: at the tragus and earlobe, with corresponding rest phases before and after interventions. tVNS stimulation was 25% above their sensory threshold. Ethical approval was given by the University of Plymouth Research Ethics and Integrity Committee. Physiological data was recorded throughout using Equivital wireless monitoring equipment and LabChart software. Repeated measures ANOVA was used for analysis.

Results indicated only surface temperature was significantly higher with testing ($p < 0.005$). No significant changes were observed in HRV, breathing rate, or SpO_2. Some significant changes in breathing rates were found in the first three minutes of testing.

Conclusions

These findings differ with previous research on tVNS effectiveness. Variability in tVNS electrode placement, stimulation intensity/duration, and age of participants may explain these differences. Surface temperature effects were likely due to initial adaptation or environmental factors rather than genuine vagal modulation. Despite limitations, the study supports the potential of tVNS in modulating autonomic function and provides valuable insights into its effects on HRV in healthy individuals and possible clinical applications. Future research should explore varied electrode placement, longer-term effects, and optimise stimulation parameters.

A 07-08 Fitness parameters as a marker of cardiac autonomic neuropathy in newly diagnosed diabetes mellitus.

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Aim

Cardiac autonomic neuropathy (CAN) is one of the frequent complications of diabetes mellitus, which may contribute to morbidity as well as mortality in diabetes mellitus patients. CAN is affected by glycaemic control. Fitness level may play a role in achieving glycemic control. Thus, fitness level may contribute to CAN development in diabetes mellitus patients. We aimed to assess the role of various fitness parameters in the development of cardiac autonomic neuropathy in newly diagnosed cases of diabetes mellitus.

Methods & Results

This cross-sectional study was done on newly diagnosed diabetes mellitus patients (n=110) in the Physiology Department, Medicine and Endocrinology Department, AIIMS, Bhopal. A fitness assessment was done using fitness protocol guidelines. The parameters measured were flexibility, muscular strength, muscular endurance, balance, and cardiovascular endurance. Cardiac autonomic function test and heart rate variability were assessed via 16 Channel Polygraph system (Gentech).

Autonomic neuropathy was confirmed using Ewing's battery of autonomic reactivity tests.

A statistical analysis: simple linear regression analysis, Spearmon correlation analysis, and unpaired t-test.

Result: A significant difference was observed for cardiovascular endurance (p=0.02) early to normal involvement of cardiac autonomic neuropathy vs. definite to severe involvement of cardiac autonomic neuropathy. However, no significant correlation was observed between fitness parameters, autonomic function involvement score, and heart rate variability parameters.

Conclusions

Cardiovascular endurance may act as an early marker of cardiac autonomic neuropathy in newly diagnosed cases of diabetes mellitus; thus, fitness assessment may be recommended for diabetes mellitus patients. However, longitudinal studies are recommended in this regard.

A 07-09 Macro- and micro-vascular, renal, and liver function in transgender women

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Aim

While estrogen is known to confer cardioprotective benefits in cisgender women, transgender women on gender-affirming hormone therapy may experience unique cardiovascular risks. The hormonal milieu is also known to improve or impair liver and renal functions. Despite growing recognition of these potential health concerns, there is very limited information on the vascular, renal, and liver functions of transgender women.

Methods & Results

In this cross-sectional study, two groups of transgender women who had been taking hormone therapy with orchiectomy (30±4 years; n=15) or without it (27±4 years; n=15) were compared with cisgender men (28±5 years; n=15) and cisgender women (29±5 years; n=15) who were matched for age and physical activity level. Transgender women had been on hormone therapy for 11±3 years. Plasma estradiol concentration was lower and testosterone and DHEA concentrations were higher in cisgender men than in the other three groups. Carotid artery intima-media thickness, brachial-ankle pulse wave velocity (a measure of arterial stiffness), brachial artery flow-mediated dilation, and post-occlusive skin reactive hyperemia were not significantly different between any of the groups. Liver enzyme concentrations, including SGOT and SGPT, and kidney function, as assessed by blood urea nitrogen and creatinine, were higher in cisgender men than in the other three groups. There were no significant group differences in blood concentration of nitric oxide (nitrite + nitrate).

Conclusions

Collectively, these results indicate that transgender women, irrespective of orchiectomy, demonstrate similar macro- and microvascular function as well as liver and renal function to cis-gender women.

A 07-10 Interstitial fluid accumulation and alveolar-capillary permeability after strenuous exercise in COPD and healthy controls

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Background: Individuals with chronic obstructive pulmonary disease (COPD) experience severe and persistent dyspnoea after exercise, even upon normalisation

of breathing pattern, but the underlying mechanisms remain unknown. In the present study, we investigated whether this may be caused by sustained interstitial fluid accumulation from abnormal extravasation, and whether this could be related to increased alveolar-capillary permeability in COPD.

Methods: Lung tissue mass (LTM) was measured using low-dose CT-scans at rest and post-submaximal (12 COPD and 12 controls) and maximal-exercise (16 COPD and 15 controls) - three participants measured at both intensities. Additionally, 16 COPD participants and 15 controls had lung clearance of ^{99m}Tc-labeled diethylenetriaminepentaacetic measured at rest and post-maximal-exercise, and underwent assessment of pulmonary capillary blood volume via dual-gas diffusing capacity using carbon monoxide and nitric oxide. A linear mixed effect model was used to investigate rest-to-post-exercise changes.

Results: LTM was unaffected by exercise in COPD, whereas it increased by 44 [9;79] g after maximal exercise in controls (p=0.01). Lung clearance was similar at rest in COPD and controls (p=0.248), and neither group showed any rest-to-post-exercise changes (COPD: -0.01 [-0.11;0.08] %/min, p=0.976; control, -0.06 [-0.16;0.04] %/min, p=0.33). Rest-to-post-exercise change for V_C was only found in controls (p=0.042).

Conclusion: Individuals with COPD do not exhibit evidence for increased alveolar-capillary permeability or abnormal lung fluid extravasation after exercise; thus being unlikely mechanisms of persistent post-exercise dyspnoea. Increased LTM observed in controls can only to a limited extend be explained by V_C changes, and more likely reflect greater extravasation because of higher absolute workloads and cardiac output in this group.

A 07-11 Selective increases in beat volume and blood kinetic energy enhance venous return and cardiac filling during ATP infusion and exercise-induced hyperaemia in humans

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Exercise substantially increases exercising limb and systemic blood flow (cardiac output: *Q*), but the underlying peripheral and central mechanisms are not fully understood. To determine how alterations in peripheral haemodynamic forces affect cardiovascular control, we investigated the regional (leg and head) and systemic haemodynamics and left ventricular (LV) volumes in eight healthy males during (1) incremental one-legged knee-extensor exercise and (2) stepwise intrafemoral artery ATP infusion at rest. Incremental exercise progressive increased exercising leg blood flow (LBF; +3.1±0.3 L·min^{-1}), *Q* (+8.7±2.5 L·min^{-1}), mean arterial pressure (MAP; +28±9 mmHg) and systemic vascular conductance (SVC) without altering head and brain perfusion. The augmented *Q* was associated with a plateau in stroke volume (+24±13 mL) and a graded rise in heart rate (+51±15 beats·min^{-1}). Comparable increases in LBF (+3.3±0.5 L·min^{-1}), *Q* (+5.6±2.0 L·min^{-1}) and SVC were also observed during ATP infusion, but MAP remained stable. ATP infusion augmented *Q* by inducing a larger increase in stroke volume (+61±28 mL) compared to exercise, owing to a marked elevation in LV end-diastolic volume in the face of a much lower rise in heart rate (+11±3 beats·min^{-1}). In both conditions, the enhanced end-diastolic volume was largely related to increases in exercising/infused leg beat volume and blood kinetic energy, evidenced by strong positive relationships between these variables across trials (R^2=0.739, P=0.006 and R^2=0.616, P=0.021, respectively). These findings revealed that selective increases in circulating beat volume and blood kinetic energy largely determine venous flow to the heart and cardiac filling during pharmacological and exercise-induced limb hyperaemia.

A 07-12 Exercise reverses obesity and aging-induced endothelial senescence

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Aim

Previous studies have shown that obesity induces premature cellular senescence in cardiac vasculature, similar to the effects of aging. Importantly, we discovered that exercise training has opposite effects to aging and obesity on the cardiac endothelial cell transcriptome. Given the ubiquitous presence of vasculature in all tissues, we hypothesize that endothelial cells have a unique opportunity to mediate the health benefits of exercise throughout the body.

Methods & Results

We utilized pre-clinical animal models for cardiometabolic disease and aging, combined with single-cell RNA sequencing (scRNAseq) analyses, patient-derived endothelial cells from cardiometabolic disease patients with healthy control cells, and human tissue samples to investigate the effects of exercise on endothelial cell senescence.

Our results show that exercise training significantly reverses obesity-induced changes in cardiac endothelial cells, including cellular senescence. In a cell culture model, treatment of senescent endothelial cells with serum collected after an acute exercise bout markedly improved tube formation compared to serum collected before exercise. Using bioinformatics analyses, the Connectivity Map (CMAP) database, and our endothelial cell RNA sequencing data, we identified small molecules that induce similar effects on the endothelial cell transcriptome as exercise. These molecules are currently being evaluated for their potential as novel therapeutics.

Conclusions

Our findings highlight the potential of exercise training to reverse obesity-induced derangements and cellular senescence in endothelial cells. Additionally, human serum collected after exercise improved the function of senescent endothelial cells, underscoring the therapeutic benefits of exercise on vasculature.

A 07-13 Hemodynamic Effects of Local Negative Pressure Application on the Human Forearm

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Aim

Cupping therapy has been used for centuries in the symptomatic treatment of musculoskeletal, cardiovascular, and dermatological conditions. Despite its popularity, the local and systemic vascular effects of cupping remain poorly characterized. Previous studies have employed various perfusion measurement techniques, but none have combined photoplethysmography (PPG) and videocapillaroscopy (VC), nor targeted the forearm as the site of application. In this study, we aimed to evaluate the hemodynamic response to local negative pressure applied to the forearm in healthy adults.

Methods & Results

Ten healthy subjects (21.4 ± 1.8 years) participated in this study. A vacuum cup was applied and suctioned to –525 mmHg for 3 minutes. PPG was used to assess skin perfusion at the cupping site (pre/post), and continuously at the middle forearm and fingertip of both limbs. Additional metrics included pulse, capillary oxyhemoglobin content (CHb, via VC), and electrodermal activity (EDA), recorded from the contralateral hand to assess sympathetic tone. Nonparametric statistics were applied ($p < 0.05$). Following cup removal, both PPG-derived perfusion and CHb increased significantly at the stimulated site, confirming local vascular congestion. Continuous recordings revealed increased perfusion not only locally, but also in unstimulated regions of both upper limbs. These changes were accompanied by

reductions in EDA and pulse, suggesting systemic vasodilation and autonomic modulation.

Conclusions

These findings support the hypothesis that localized forearm cupping elicits widespread cardiovascular effects, potentially mediated by neurovascular reflexes. Further studies are warranted to elucidate the underlying mechanisms and clinical implications of this response.

A 08 | INFLAMMATORY CELLS AND MECHANISMS

A 08-01 Neutrophil extracellular trap formation in the placenta and peripheral blood in gestational diabetes mellitus

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Gestational diabetes mellitus (GDM) affects ~14% of pregnancies globally, with rates up to 25.8% in South Africa. Characterized by insulin resistance, hyperglycaemia, and chronic inflammation, GDM is associated with neutrophil extracellular trap (NET) formation. While NETs are implicated in various inflammatory conditions, their role in the placenta remains unclear.

Aim

This study investigated placental histology and NET presence in GDM and assessed NET formation by exposing isolated neutrophils from healthy, non-pregnant women to serum from healthy and GDM pregnancies.

Methods & Results

Placental samples were prepared for light- and confocal laser scanning microscopy (CLSM) using standard techniques. Sections were stained with H&E for histological evaluation and immunolabelled with antibodies against myeloperoxidase and citrullinated histone 3 to detect NETosis. Additionally, serum was used to assess NET induction in neutrophils isolated from a healthy non-pregnant donor via density gradient centrifugation. After serum exposure, NET formation was quantified and evaluated using the same immunostaining markers.

Histological assessment of placental tissue indicated increased syncytial knot formation and fibrin deposits associated with inflammation. NET formation was increased in the GDM placentas. Interestingly, the stimulation of healthy neutrophils with serum from women without GDM decreased NET formation, while serum from women with GDM increased NET formation.

Conclusions

The findings of this study suggest a role for circulating inflammatory mediators in driving placental dysfunction through NET formation, which supports the growing evidence of immune dysregulation in GDM. Targeting NET formation or its upstream activators may represent a novel therapeutic approach to mitigating complications in GDM.

A 08-02 Inflammatory Mediators in the Development and Progression of Peripheral Neuropathy in Male UCD-Type 2 Diabetic Rats

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Diabetic peripheral neuropathy (DPN) is a debilitating complication of type 2 diabetes mellitus (T2DM), often diagnosed only after irreparable nerve damage has occurred. Mechanical allodynia, where previously nonpainful stimuli become painful, precedes this damage. Inflammation is increasingly recognized as a key factor in DPN's development and progression. This study aimed to quantify inflammatory mediators during DPN's onset and progression. Hemoglobin A1c was assessed bimonthly in male University of California Davis (UCD)-T2DM rats (n=18) to detect diabetes onset (threshold ≥5.6%). Age-matched, healthy Sprague-Dawley rats (n=18) served as controls. Monthly measures determined mechanical allodynia (via

paw withdrawal threshold, PWT), insulin, and inflammatory mediators (via ELISA and Multiplex kits). Data were analyzed using unpaired student's t-test or two-way repeated measures ANOVA with Holm-Sidak post hoc analyses. Before diabetes onset, the pain threshold was similar between UCD-T2DM and control rats (T2DM: 1.56±0.23 vs. CTL: 1.59±0.22, p=0.564). And, UCD-T2DM had significantly higher levels of IL-6 (T2DM: 76±40 vs. CTL: 39±39, p=0.009) and IL-1β (T2DM: 51±45 vs. CTL: 12±23, p=0.002). Two months post disease onset, PWT significantly decreased in UCD-T2DM rats (PreD: 1.6±0.2 vs. 2mo: 1.4±0.2, p=0.013) and CRP significantly increased (PreD: 565±122 vs. 2mo: 729±185, p=0.027). However, in CTL rats, PWT was significantly increased (PreD: 1.6±0.2 vs. 2mo: 1.9±0.2, p<0.0001) and CRP remained unchanged (PreD: 483±125 vs. 2mo: 453±118, p=0.938). No significant differences were found in other inflammatory mediators with disease progression (p>0.05). Although pro-inflammatory cytokines did not appear to influence DPN development or progression, further research into the role of CRP is warranted.

A 08-03 The pro-inflammatory effects of PAH air pollutant phenanthrene on hepatic transcriptomics and cardiovascular dysfunction at the single cell level

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70% of worldwide air pollution deaths are attributed to cardiovascular (CV) disease. Phenanthrene, a polyaromatic hydrocarbon (PAH) component of air pollution, is the chief contributor to PAH-induced CV dysfunction in mammalian models. Phenanthrene exists in the gas and particle phase via adsorption to particulate matter. Translocation of particulate matter below 2.5 μm in diameter into the bloodstream enables phenanthrene to reach the liver as the main metabolising organ. The specific immune response to phenanthrene within the liver, and whether this may act systemically and contribute to CV dysfunction is not understood. Here, we use single nucleus RNA sequencing to identify changes in hepatic transcriptomes from mice chronically exposed to phenanthrene. Liver nuclei from mice exposed daily via intraperitoneal injection to high (30 μg/kg) or low (3 μg/kg) doses of phenanthrene for 6 weeks were sequenced (n = 3/4). In mice exposed to high dose phenanthrene, inflamed hepatocyte populations had significantly increased expression of pro-inflammatory-related genes like STAT3 (FC = 1.5, p-value < 0.01, Wilcoxon) compared to control. Pathway enrichment analysis revealed that hepatic immune cells were significantly enriched in antigen receptor-mediated pathways (GO:0050851, FC = 8.5, p-value < 0.01, Wilcoxon). ELISAs from grouped exposure showed a significant increase in IL-33 protein expression in liver lysate (FC = 1.6, p-value < 0.05, t-test) and plasma (FC = 1.8, p-value < 0.05, t-test) versus control. This liver-originating immune response may act systemically through plasma, contributing to phenanthrene-induced CV dysfunction which itself contributes to millions of premature deaths annually due to air pollution.

A 08-04 Human immunomodulatory endothelial cells contribute to T cell recruitment and activation through antigen presentation on MHC class II

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The author has objected to a publication of the abstract.

A 08-05 Dopamine D2 receptor on CD4+ T cells is protective against inflammatory responses and signs in a mouse model of rheumatoid arthritis

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The author has objected to a publication of the abstract.

A 08-06 Involvement of Pannexin-1/P2X7 Receptor in SARS-CoV-2 ORF3a-Induced Inflammatory Cell Death.

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Aim

Involvement of Pannexin-1/P2X7 Receptor in SARS-CoV-2 ORF3a-Induced Inflammatory Cell Death.

Methods & Results

SARS-CoV-2 protein ORF3a has been identified as a key player in the virus's pathogenesis, interacting with proteins involved in inflammation and cell death. In this study, we investigated the interaction between ORF3a and the Pannexin-1 (Panx1) ion channel and its downstream effects in HEK-293T and Neuro 2a (N2a) cell lines. When ORF3a was expressed in the HEK-293T cell line, it interacted with endogenous Panx1, as confirmed by co-immunoprecipitation experiments. Furthermore, ORF3a expression led to elevated levels of both Panx1 (t-test; mean ± SD, N=3; p= 0.026) and P2X7 receptor (P2X7R) (t-test; mean ± SD, N=3; p=0.0003). ORF3a-expressing cells showed increased permeability to YO-PRO dye (one way anova; mean ± SD, N=3; p=0.021) and significant ATP release (one way anova; mean ± SD, N=3; p=0.04) and both of which were reduced upon treatment with carbenoxolone (CBX), a Panx1 inhibitor, suggesting the involvement of Panx1 in this process (one way anova; mean ± SD, N=3; p=0.0672) and (one way anova; mean ± SD, N=3; p=0.002). Additionally, ORF3a expression disrupted cellular calcium homeostasis, leading to elevated basal cytosolic calcium levels (t-test; mean ± SD, N=3; p=0.012). Apoptotic pathway analysis revealed a higher Bax/Bcl-2 ratio (t-test; mean ± SD, N=3; p=0.002), increased levels of caspase-3 (t-test; mean ± SD, N=3; p=0.011). Dysregulated calcium homeostasis and apoptosis activation follow Panx1/P2X7R signaling activation events.

Conclusions

These findings suggest that Panx1/P2X7R may be a potential therapeutic target for treating SARS-CoV-2 infection.

A 08-07 Exploring the Role of Resistin in Inflammatory Pathways in Circadian Rhythm Disruption: A Translational Approach from Animal Models to Shift Workers

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Aim

The study aimed to exploring the role of resistin in inflammation in animal models obesity and human who work in shift work especially at the night shift

Methods & Results

The animal models was rat model obesity which is fed with high carbohydrate and fat. There are three groups consist of sixth rats each including morning, afternoon and control rats with low intensity aerobic exercise conducted for four weeks. In human shift work employess was comparative repeated cross-sectional design of female workers in hospitals who worked shifts and non-shifts. Data collection was carried out from June to December 2021 and obtained 272 female workers in the

city and district of Bandung. Based on the inclusion criteria, there were 64 shift workers and 54 non-shift workers. Characteristics assessed were not different based on age and others health parameters, PSQI score, DASS score, and nutritional status and intake.
In animal model obesity was found anova test there was significant differences of resistin gen expression on each group compared with control (p<0,01). In human shift work study found from the calculation of categorical regression analysis, Cyclic Guanosin monophosphate, ESR, and triglycerides affected endothelial microparticles represented by CD31+/CD62e+ with a path coefficient é = 0.82. GMFs, ESR, and triglycerides were shown to influence 67% of endothelium microparticles, based on the path coefficient of 0.82.
Conclusions
Resistin have ability as a pro-inflammatory factor, inhibiting endothelial nitric oxide synthase in endothelial cells and enhancing foam cell formation in macrophages via the cGMP pathway.

A 08-08 Gas6-AIM axis inhibits NLRP3 inflammasome activation and enhances autophagy and efferocytosis in acute lung inflammation in mice

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Aim
Growth arrest-specific 6 (Gas6) signaling is crucial for immune homeostasis and inflammation regulation. Gas6 enhances the production of apoptosis inhibitor of macrophages (AIM), an anti-inflammatory protein. This study investigates whether Gas6-induced AIM alleviates acute lung inflammation in mice by modulating inflammasome activation, autophagy, ROS generation, and efferocytosis.
Methods & Results
Methods: Acute lung inflammation was induced in wild-type (WT) and *AIM*$^{-/-}$ mice via intratracheal lipopolysaccharide (LPS) administration, and recombinant Gas6 (rGas6) was injected intraperitoneally to assess the Gas6-AIM axis in lung inflammation. Inflammatory responses were evaluated using ELISA, cell-sizing, and bicinchoninic acid assays. Lung pathology was analyzed with H&E staining. NLRP3 inflammasome activation and autophagy were assessed using western blot, qPCR, and immunofluorescence. ROS levels in alveolar macrophages were measured by fluorescence microscopy, and efferocytosis was evaluated in bronchoalveolar lavage (BAL) cells and macrophages co-cultured with apoptotic Jurkat cells.
Results: rGas6 administration reduced proinflammatory cytokines, neutrophil infiltration, and protein concentration in BAL fluid of WT mice but not in *AIM*$^{-/-}$ mice. It also decreased IL-1β, IL-18, caspase-1 activity in alveolar macrophages. Additionally, rGas6 enhanced autophagy and efferocytosis in alveolar macrophages while reducing ROS levels through AIM production. These protective effects were absent in *AIM*$^{-/-}$ mice.
Conclusions
Gas6-induced AIM protects against LPS-induced acute lung inflammation by inhibiting NLRP3 inflammasome activation, promoting autophagy and efferocytosis, and reducing oxidative stress. These findings highlight the Gas6–AIM axis as a potential therapeutic target for inflammatory lung diseases.

A 08-09 The endocannabinoid anandamide mediates anti-inflammatory effects through activation of NR4A nuclear receptors

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The author has objected to a publication of the abstract.

A 08-10 Exploring the Role of TGF-β and BMP9 in Modulating HIF-1α Expression in Human Leukocytes

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Members of the transforming growth factor beta (TGF-β) superfamily are regulatory cytokines. While the role of TGF-β in the immune response is well studied, increasing evidence suggests that other members, such as bone morphogenetic proteins (BMPs), also modulate immunity. However, their interaction with the transcription factor hypoxia inducible factor (HIF), which orchestrates cellular adaptation to low oxygen levels, remains largely unexplored. HIF-1α-deficient leukocytes are dysfunctional, and patients with mutations in TGF-β/BMP9 pathway genes, such as hereditary haemorrhagic telangiectasia (HHT), show impaired immune responses and reduced *HIF1A* gene and HIF-1α protein expression in leukocytes.

We isolated human peripheral blood mononuclear cells (PBMCs) from buffy coats and treated them with varying concentration of TGF-β or BMP9 under normoxic and hypoxic conditions. Our results show that both cytokines influence HIF expression in human leukocytes. Interestingly, TGF-β treatment upregulated HIF target genes without increasing HIF-1α protein levels, suggesting regulation via alternative mechanisms such as post-translational modification. In a T cell line, we confirmed that TGF-β treatment enhances phosphorylated HIF protein levels. Since PBMCs represent a heterogeneous cell population, with potentially divergent responses among subsets, we aim to investigate TGF-β effects in a monocytic cell line to gain a better understanding of cell-type-specific mechanisms.

Our findings reveal a previously underappreciated connection between TGF-β signalling and HIF regulation in human leukocytes, offering potential insights into immune dysfunction in HHT and broader implications for immune regulation under hypoxic conditions.

A 08-11 IL-21 regulates Cathelicidin expression during latent tuberculosis infection

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Aim

LTBI has been identified as the leading source of new TB disease and the leading obstacle for the end TB strategy. Tuberculosis control relies on the identification and preventive treatment of individuals who are latently infected with Mycobacterium tuberculosis. Cathelicidin is essential for innate and adaptive immunity against tuberculosis (TB) infection. These are upregulated during bacterial infection or as response to inflammatory cytokines. We aimed at compare the association between LL37 and cytokines among LTBI and healthy controls.

Methods & Results

[Stored samples of 41 LTBI individuals and 35 healthy controls from the Kampala TB cohort were retrieved. The enzyme linked immunosorbent assay was used to assess LL-37 levels. The cytokines were analysed using an 11-analyte Bio- Plex Pro™ human cytokine bead array. These consisted of interferon gamma (IFNγ),TNFα, IL-4, IL-5, IL-13, IL-10, IL-17a, IL-17f, IL-21, and IL-22. The Mann-Whitney U test was used to compare differences between the two groups. The Spearman correlation test was used to determine association. **Results:** LL-37 levels were significantly higher in LTBI (232.6 ng/mL) compared to healthy controls (162.0 ng/mL) p-value,0.0443. Additionally, IFN (Interferon-Gamma 1) levels were notably higher in the LTBI group (615.2) than in healthy controls (93.2, p = 0.0029). Among the cytokines analyzed, IL21 in LTBI showed a significant negative correlation with LL37 expression (Rho = -0.4972, P = 0.0083).

Conclusions

[Our study reveals a negative association between LL37 and IL 21 in LTBI suggesting immunological alterations that may inform development of a novel biomarker.

A 09 | HORMONES AND ENDOCRINE CELLS

A 09-01 Thyroid hormone induced up-regulation of Na+/K+-ATPase and thus of metabolism can be explained as a consequence of enhanced neuronal activity in postnatal rat brain

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The most prominent effect of thyroid hormone (T3) is its effect on oxygen consumption and thus metabolism. Since the activity of the Na^+/K^+ATPase contributes to a large extent to cellular energy consumption further studies showed that T3, indeed, upregulates the expression of several subunits of the Na^+/K^+-ATPase. This led to the concept, that thyroid hormone receptor occupancy regulates the expression of the sodium pump. On the other hand, it is known, that thyroid hormone increases the excitability of muscle cells (Brodie and Sampson, 1988) and neurons (Potthoff and Dietzel. 1997) via an upregulation of voltage-gated sodium currents. In neurons from postnatal rats, an increase in voltage-gated sodium currents is only observed in the presence of satellite cells and can be explained by a thyroid hormone induced release of protein factors, such as FGF-2 out of astrocytes (Niederkinkhaus et al., 2009; Igelhorst et al., 2015). In mice lacking a thyroid gland, voltage-gated sodium currents as well as the expression of Na^+/K^+-ATPases are severely reduced (Sundaram et al., 2022). Here we show with *western-blots* as well as *[^{3}H]-ouabain binding*, that the upregulation of the Na^+/K^+-ATPase by T3 or FGF-2 is reduced if currents through voltage-gated sodium channels are blocked by tetrodotoxin. This suggests that the primary effect of thyroid hormone is an upregulation of cellular excitability. The more well known effects of thyroid hormone on metabolism might thus at least partially result from an increased intracellular sodium load which in turn triggers an enhanced ATP consumption to restore equilibrium conditions.

A 09-02 Beta-type estradiol receptor modulates *in vivo* and *in vitro* responses to metabolic challenges

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Aim

Non-selective therapy improves metabolic dysfunction in postmenopausal but is less effective over time and unsuitable for hormone-dependent cancer genetic background. In contrast, estradiol receptor beta (ERβ) shows benefits in energy overload and antitumor effects. Our aim is to investigate the ERβ activation effect in hepatocytes, as an alternative to estradiol, in ovariectomized (OVX) rats and under energy overload conditions.

Methods & Results

In vivo, Wistar rats (90 days) underwent OVX surgery with isoflurane (2%) anesthesia and with Tramadol (20 mg/kg) post-surgery. After 15 days, they received DPN (1 mg/kg s.c.) for 15 more days. *In vitro*, AML12 cells were exposed to high glucose (20 mM) and palmitate (200 μM) for 24 h with or without DPN (0.1 or 1 nM). OVX DPN treated rats showed improved glucose levels and lipid profiles. Increased pancreatic islet circularity and reduction in retroperitoneal fat were also observed. While liver triglyceride accumulation remained unchanged, ketone body production was recovered. *In vitro*, treated hepatocytes in overload media showed metabolic flux improvement in epinephrine responses, and decreased ketone body production (compatible with *in vivo* data), suggesting direct effects on liver. Further analysis revealed that DPN reduces metabolic flux regulatory checkpoints, such as mitochondrial complex I, pyruvate dehydrogenase, electron transfer flavoprotein and carnitine palmitoyl transferase (western blot). Substrate oxidative flexibility decreased, indicating reduced substrate use by mitochondria.

Conclusions

These results suggest that ERβ can improve metabolic indicators *in vivo* and alter hepatocyte responses to physiological and pharmacological challenges, offering intriguing results as alternatives for non-selective estradiol therapy.

A 09-03 The thyroid hormones are necessary for the stability of circadian rhythms

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Thyroid hormones control energy balance in several ways. While they promote energy expenditure by increasing metabolic rate, its action is also necessary to stimulate energy intake. Thyroid hormones act centrally to maintain feeding behavior and body weight. In this work we analyzed the effect of thyroid hormone deficiency on the feeding pattern of rats. We induced hypothyroidism to a group of rats by administering a low methimazole dose (60 mg/kg) for 7 weeks and registered their feeding behavior. When kept under a conventional lighting schedule (light:dark 12:12 h; 150 lux), hypothyroid rats gradually reduced their food consumption and body weight gain but maintained feeding and activity rhythms similar to untreated controls. When put under constant dark, control rats maintained near-24 h rhythmicity (free-running), while hypothyroid rats became arrhythmic in both activity and feeding. The inter-daily stability and intra-daily variability analysis of the desynchronization patterns suggest that thyroid-hormone deficiency does not affect the clock mechanism within the circadian pacemakers, but instead it reduces the strength of their coupling. Our results suggest that thyroid activity is necessary for circadian robustness in the absence of light cues, so that thyroid hormones seem to intervene in energy homeostasis as both metabolic regulators and circadian entrainers.

A 09-04 Serum Phoenixin-14 Levels Correlates Negatively with Serum Follicle Stimulant Hormone in Obese Women

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Phoenixin-14, an anti-inflammatory hypothalamic peptide, regulates metabolism, food intake, reproduction, anxiety, acts via gonadotrophin releasing hormone receptors. Serum levels decrease in diabetes, in hypertension whereas increases in polycystic ovary syndrome irrespective of body mass index (BMI). We investigated impact of obesity on serum PNX-14 levels, glucose metabolism and gonadotrophic hormones.

Consented non-diabetic 53 obese females (OF, BMI 36,1 32,5-40) seen in obesity outpatients' clinic and 21 healthy females (HF, BMI 24,1 19,7-26,3) were enrolled to this case-control study. BMI and body fat content was measured. Hospital anxiety depression questionnaire was filled out (HAD). Follicle stimulant hormone (FSH), luteinising hormone (LH), oestrogen, PNX-14 were measured by electrochemiluminescence immunoassay (ECLIA) and ELISA respectively. Data was expressed as median (quartiles), compared with Mann Whitney U, and Kruskal Wallis test. Correlations were analysed by Spearman's test. $p<0.05$ was accepted. OFs were older as compared to HFs (40 34-47 vs 23 21-34, years $p<0.001$). PNX-14 levels were lower in OFs (524 417,4-641 vs 742, 5 523-1361, $p<0.005$). Overall (n=71) serum PNX-14 negatively correlated with age (r-0,3, $p<0.005$), with platelet count (r:-0.27, $p=0.033$), with FSH (r:-0.27, $p=0.047$) with BMI (r-0.23, $p=0.057$), with HAD-D (r:-0.2, $p=0.079$). There was no relationship between PNX and indices of

body fat content as was glucose, insulin, and insulin sensitivity. Menopause and phase of menstrual cycles did not alter serum PNX level. Negative correlation between serum PNX, one of the central regulators of fertilisation and FSH in obese females may possibly reflects disturbed hypothalamic function and deserves further elucidation.

A 09-06 Distribution and regulation of renin expression in adrenal glands of mice

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The systemic Renin-Angiotensin System (RAS) regulates blood pressure and electrolyte homeostasis. In addition to the renal juxtaglomerular cells as the main source for circulating renin, local RAS systems exist in various tissues, including adrenal glands. Main aim of our study is to clarify the (patho-)physiological role of adrenal renin. As a first step, we investigated the distribution and identity of adrenal renin-expressing cells using a combination of in situ hybridization, transgenic renin-reporter mice and immunofluorescence.

Renin-expressing cells are located in all three zones of the adrenal cortex, challenging the prevailing view that they are confined to the aldosterone-synthesizing zona glomerulosa. Marked expression in the zona fasciculata suggests involvement in glucocorticoid synthesis. A high-potassium diet, known to stimulate aldosterone synthesis, induced a redistribution of renin-positive cells toward the zona glomerulosa, indicating dynamic recruitment or phenotypic plasticity in response to physiological cues.

As expected, renin expressing cells in the zona fasciculata did not express CYP11B2 (aldosterone synthase), disproving the long-standing assumption that adrenal renin-expressing cells are identical to aldosterone-producing cells. However, renin in all adrenal zones co-localized with CYP11A1 (cytochrome P450 monooxygenase), which catalyzes the first step in steroid hormone synthesis. No overlap of renin expression with markers for immune cells, interstitial cells, or stem cells was observed.

Taken together, a subset of steroid-synthesizing adrenal cells can produce renin, which could be the basis for a local RAS. The functional role of this local RAS in aldosterone and glucocorticoid synthesis is investigated in ongoing *in vitro* and *in vivo* studies.

A 09-07 Calcium signals in the human zona glomerulosa

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The zona glomerulosa (ZG) of the adrenal gland releases the mineralocorticoid aldosterone through a calcium-dependent synthesis pathway rather than controlled vesicle fusion. In mouse models, stimulation with angiotensin II or potassium triggers electrical oscillations that activate voltage-gated calcium channels, producing characteristic intracellular calcium oscillations.

Primary aldosteronism, a disorder caused by uncontrolled aldosterone release, stems most often from gain-of-function mutations in ion channel genes, including in T- and L-type voltage-gated calcium channels. These mutations lead to increased calcium signaling, as demonstrated in murine models.

However, the composition of expressed ion channels and transporters differs between humans and mice. A notable example is *KCNJ5*, which is absent in murine ZG but is the most frequently mutated ion channel in human aldosterone-producing adenomas.

To understand these functional differences, we generated acute slice preparations from human adrenal gland samples from patients undergoing (partial) adrenalectomies to investigate calcium signals. Our findings show that human ZG cells exhibit intracellular calcium fluctuations in response to angiotensin II, but with frequency and patterns distinct from murine ZG cells. Preliminary experiments suggest that, unlike in mice, T-type voltage-gated calcium channels are not essential for maintaining calcium signaling in human ZG cells.

Our results demonstrate significant functional differences between human and murine ZG cells, highlighting the need for further investigation of human-specific calcium signaling mechanisms in both healthy and diseased adrenal glands.

A 09-08 Hormonal Influence on Adipose Health During Menopause: Insights from the HoCa Study Using Surgical Menopause and Adipose Organoids

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Aim

The Hormones and Cardiometabolic Health (HoCa) study investigates how ovarian hormones and their loss during menopause affect adipose tissue, aiming to combat postmenopausal metabolic disorders and obesity.

Methods & Results

The study involves 120 premenopausal women undergoing bilateral oophorectomy, resulting in sudden menopause and a significant drop in estradiol levels. Whole-body metabolism will be assessed using indirect calorimetry at rest and during exercise, systemic metabolism with NMR metabolite assessments, and circulating non-coding RNAs (ncRNA-seq). Adipose tissue samples from various depots will be used to develop adipose tissue organoids (adipoids). These adipoids will be exposed to pre- and postmenopausal sera or pure estradiol to study their responses through morphological changes, functional assays, and secretome analysis using multiplex-based assays, proteomics, and ncRNA-seq. Preliminary results from the Olink Target 96 Cardiovascular II assay showed that of the 98 Olink analytes, 21% were expressed in the adipoid secretome, with 6 showing differential expression after LPS exposure. For example, Interleukin-6 (LPS-treated: 5.5±1.7 vs. controls: 2.0±1.2, p=0.007) and Death Receptor 5 (LPS-treated: 1.2±0.4 vs. controls: 0.5±0.3, p=0.021) were upregulated, while Lipoprotein lipase (LPS-treated: 2.9±0.3 vs. controls: 2.7±0.3, p=0.371) did not respond to LPS treatment.

Conclusions

The HoCa study is ongoing, with preliminary results validating the Olink method for studying the adipose secretome. LPS treatment induced inflammatory and apoptotic responses. Future research will explore the effects of estrogen or its absence on different adipose tissue depots.

A 09-09 Unraveling the Role of LRRC8B in Pancreatic β-Cell Insulin Secretion and Calcium Dynamics

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Aim

Pancreatic β-cell dysfunction and impaired insulin secretion are key contributors to diabetes, with cytosolic calcium dynamics playing a crucial regulatory role. LRRC8B, a member of the leucine-rich repeat-containing 8 (LRRC8) family, has been implicated in ER Ca^{2+} leakage in HEK293 cells beyond its known function in volume-regulated anion channels (VRAC). However, its role in β-cell insulin secretion remains unclear. In this study, we investigated the impact of LRRC8B modulation on glucose-stimulated insulin secretion (GSIS) in INS-1 cells

Methods & Results

LRRC8B overexpression significantly increased fold change in insulin secretion at 30 minutes following 20 mM glucose stimulation (control: 15.58 ± 3.4 ng insulin/mg protein; LRRC8B overexpressing cells 34.99 ± 16.2 ng insulin/mg protein; n = 3; p = 0.03; student's t test). Similar trends in insulin increase were observed at 5 and 60 minutes in LRRC8B overexpressing cells. Conversely, LRRC8B knockdown enhanced basal insulin secretion but reduced secretion at 5 minutes of glucose stimulation. However, insulin secretion increased at 30 and 60 minutes, mirroring

overexpression effects. Additionally, Fura-2 AM-based cytosolic Ca^{2+} imaging revealed higher cytosolic calcium levels and enhanced calcium oscillation amplitude in LRRC8B-overexpressing INS-1 cells (Fura 340/380: 0.3 AU, n = 120) compared to controls (Fura 340/380: 0.11 AU, n = 120). This suggests that LRRC8B influences calcium signaling, which may regulate insulin release.

Conclusions

These findings highlight the novel role of LRRC8B in β-cell function, suggesting its mechanistic role and potential therapeutic implications for diabetes mellitus.

A 09-10 Spatially Organized Heterogeneity Shapes the Modular Architecture and Functionality of Beta Cell Networks

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Aim

Multicellular structures interpret external stimuli through the collective activity of their constituent cell populations. This is also true for the islets of Langerhans, where networks of beta cells coordinate their responses to changes in extracellular nutrient concentrations to regulate insulin secretion. However, due to their inherent functional heterogeneity, the spatiotemporal activity patterns within these networks are complex and incompletely understood.

Methods & Results

Functional beta cell networks were reconstructed based on multicellular calcium dynamics recorded by confocal microscopy in mouse (8-12 weeks old NRMI) tissue slices and isolated human donor islets. Our analyses show that beta cells with similar signaling traits tend to cluster spatially, and activation during glucose stimulation (10 mM) progresses through smaller groups. The networks are highly modular, with community structure shaped by cell positions and closely aligned with cellular activity patterns and with simultaneously activated cell groups in response to elevated glucose. These patterns suggest shared organizational principles in mouse and human islets, highlighting a broadly conserved organizational framework. Specific subpopulations, including hub, wave-initiator, and first-responder cells, are spread across communities, with their distribution driven by spatial factors in mouse islets and activity of regions in human islets.

Conclusions

Our findings not only enhance our understanding of beta cell networks but also provide a valuable framework for future studies aimed at unraveling the complexities of collective beta cell behavior and function in both health and disease, with potential implications for the design and composition of stem cell-derived islets.

A 09-11 Temporal Persistency in Pancreatic Beta Cell Networks: Do Specialized Subpopulations Retain Their Roles?

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Aim

Healthy pancreatic islets contain hundreds of β cells that act in synchrony to generate pulsatile insulin secretion, thereby ensuring metabolic homeostasis. Their collective behavior is shaped by the interplay of three specialized subpopulations: (i) first-responder cells, which initiate the first-phase response to glucose stimulation; (ii) wave-initiator cells, which trigger intercellular Ca^{2+} waves; and (iii) hub cells, which facilitate intercellular communication in the multicellular network. Yet, important questions remain regarding the stability and interplay of these subpopulations

Methods & Results

In this study, we investigated the temporal stability of these subpopulations using

functional multicellular Ca^{2+} imaging in acute mouse (NMRI, 8-25 weeks old) pancreatic tissue slices, combined with methods from complex network theory. We employed a protocol consisting of three consecutive 20-minute stimulations with 10mM glucose, interspersed with recovery intervals of 20 and 180 minutes in substimulatory 6mM glucose. Our findings suggest that, over this time frame, the roles of the three subpopulations are largely maintained across successive stimulations, albeit with some exceptions. However, there were important distinctions in their temporal dynamics. Hub cells consistently retained their network-central positions both during and between stimulations. In contrast, the identities of first-responder and wave-initiator cells showed more variability, with roles occasionally shifting between cells and regions within and across stimulations.

Conclusions

These findings suggest that the role of hub cells is more strongly determined by stable cell-autonomous parameters, whereas the emergence of wave-initiators and first-responders is more flexible, shaped by non-cell-autonomous parameters, like spatial location and the dynamic socio-cellular context.

A 09-12 Effects of Arginine Vasopressin on Pancreatic α and β Cells: Glucose-Dependent Modulation and Receptor-Specific Responses

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Problem statement: The role of arginine vasopressin (AVP) in the regulation of pancreatic α and β cell function has been controversial. We investigated the effect of AVP and its designed receptor agonists and antagonists on α and β cells.

Methods: Acute pancreatic tissue slices from C57BL/6J mice were imaged with confocal microscopy. Slices were stimulated with 8mM glucose with AVP or AVP receptor agonist/antagonist and cAMP modulating agents, cytosolic Ca^{2+} events were recorded and analyzed as described before[1].

Results: AVP exerts glucose-dependent effects on both cell types. At low glucose concentrations AVP selectively activated α cells, without significantly affecting β cells. At stimulatory glucose concentrations AVP enhanced β and α cell activity, leading to increased intracellular Ca^{2+} activity. Epinephrine-dependent depletion of cAMP levels in β cells suppressed the AVP effects. AVP displayed a bell-shaped concentration dependence, with lower concentrations stimulating and higher concentrations diminishing responses, consistent with IP_3 receptor activation and inactivation properties. Furthermore, our results indicate that AVP acts primarily through V_{1b} receptors.

Conclusion: The effects of AVP are contingent on the intracellular cAMP environment enabling it to significantly modulate the response of α and β cells to other stimuli. These findings provide new insights into the glucose-dependent modulation of pancreatic hormones by AVP, highlighting its potential role in metabolic regulation.[1]Postic S, Sarikas S, Pfabe J, Pohorec V, Krizancic Bombek L, Sluga N, et al. High-resolution analysis of the cytosolic Ca(2+) events in beta cell collectives in situ. Am J Physiol Endocrinol Metab. 2023;324(1):E42-E55.

A 10 | SMOOTH MUSCLE AND SKELETAL MUSCLE AGING

A 10-01 Basal opening of K_V7 and BK_{Ca} channels provide a hyperpolarizing brake to activation of L-type Ca^{2+} channels in male mouse urethral smooth muscle

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Aim

L-type Ca^{2+} channels (LTCC) contribute to excitation-coupling in urethral smooth

muscle cells (USMC) in numerous species. In contrast, LTCC do not contribute to tone, or responses to adrenergic agonists or nerve stimulation in mice, despite expression of the channels. Reasons for non-contribution of LTCC to murine USMC contractility remain unclear. We hypothesized Kv7 or BK_{Ca} channels, when opened, might reduce USMC excitability, inhibiting LTCC.

Methods & Results

In isometric tension experiments, when urethral rings were pre-contracted by phenylephrine (PE, 1 μM, α1-adrenoreceptor agonist), or contracted by electrical field stimulation (EFS), neither XE991 (10 μM, Kv7 inhibitor) nor iberiotoxin (300nM, BK_{Ca} inhibitor) had any effects. However, a combination of iberiotoxin and XE991 induced phasic contractions superimposed on PE responses and enhanced EFS-evoked contractions, which were all reversed by nifedipine (LTCC inhibitor). During in situ Ca^{2+} imaging of USMC, combination of XE991 and iberiotoxin converted asynchronous, localized intracellular Ca^{2+} signals to coordinated, propagating intercellular waves, which were abolished by nifedipine. Ca^{2+} influx via Orai channels is critical for USMC Ca^{2+} signalling, and pre-contracted urethral rings were relaxed by GSK-7975A or Synta 66 (Orai inhibitors). Under these conditions, nifedipine-sensitive phasic activity was evoked by XE991, while iberiotoxin addition had no effect.

Conclusions

Above findings suggest that Ca^{2+} influx via Orai was linked to BK_{Ca} activation. Our data suggest that LTCC do not contribute to mouse USMC contractions under normal conditions due to basal activation of Kv7 and BK_{Ca} channels whose latter activation is linked to Ca^{2+} influx via Orai channels.

A 10-02 Prostaglandin E_2 inhibits agonist-induced contractions of mouse urethral smooth muscle

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Aim

Prostaglandins (PG) contribute to inflammation during urinary tract infection, and high PG levels correlate with malfunctions in urethral function, possibly leading to stress urinary incontinence of women or urethral obstruction in men. While drug therapies targeting PG might provide avenues to treat urinary tract disorders, the mechanism of how PG control urinary smooth muscle is largely unknown.

Methods & Results

We used isometric tension recordings of mouse urethral smooth muscle (USM) rings, to investigate mechanisms of PG effects on urethra. Mouse USM rings were mounted to force transducers in organ baths containing oxygenated Krebs solution @ 37°C. Inhibiting basal PG production with the cyclooxygenase inhibitor indomethacin (10 μM) led to 200.1% enhancement in area under the curve (AUC) of male USM contractions evoked by the adrenergic α1 agonist phenylephrine (PE, 1 μM, n=12). Conversely, PE-evoked contractions of male USM were inhibited by exogenous PGE_2 (3 μM, n=7). Male USM contractions evoked by electrical field stimulation exhibited a small, but statistically significant increase in amplitude in response to indomethacin (5, 10, 20Hz, n=21). In female USM pre-contracted with arginine vasopressin (AVP, 10 nM), indomethacin increased contractions by 181% (n=8; 10 μM). In female USM pre-incubated with indomethacin and subsequently contracted with AVP, addition of PGE_2 (100 pM to 300nM) led to dose-dependent inhibition of contractions, which was largely reversed by an antagonist of EP_2 receptors (PF-04418948, 1 μM) but not EP_4 receptors (ONO-AE3-208, 1 μM).

Conclusions

These data suggest PGE_2 inhibits agonist evoked contractions of USM via EP_2 receptor mediated pathways.

A 10-03 Exploring the function of Orai channels in regulating cholinergic contractions of detrusor

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Activation of muscarinic receptors (MRs) by Acetylcholine (ACh) on the detrusor smooth muscle cells (DSMCs) – cholinergic pathway – is the main pathway responsible for detrusor contraction during micturition. Murine detrusor cholinergic contractions are dependent on extracellular Ca^{2+} influx (~70%) via Transient receptor potential channels (TRPCs) and L-type channels and SR Ca^{2+} release (~30%) via Inositol trisphosphate receptors (IP_3Rs). SR refilling is achieved through store-operated Ca^{2+} entry (SOCE) via the activation of SR sensory protein STIM and opening of plasmalemmal Orai channels. However, little is known about the role of SOCE and Orai channels in mediating cholinergic responses.

Transcriptional studies on murine detrusor RNA and immunocytochemistry on DSMCs indicated the presence of all Orai isoforms at RNA and protein level. Cholinergic responses were induced using the MR agonist, Carbachol (CCh). CCh-induced contractions were reduced in the presence of Orai inhibitor, GSK7975A, by 40% or the L-type channel blocker, Nifedipine, by 60%. Orai blockers GSK7975A and BTP2 reduced Cholinergic-isolated Electrical Field Stimulation (EFS)-induced contractions in a frequency-dependent, with effects observable at 16Hz and 32Hz (15.07% and 14.14% decrease respectively) in GSK7975A or 8Hz, 16hz and 32Hz (12.28%, 21.3% and 30.26% decrease respectively) in BTP2. In Fluro4-AM loaded DSMCs, preincubation of cells with GSK7976A or BTP2 reduced CCh responses (81.35% and 81.24% respectively). Preincubation with Nifedipine or 2APB decreased CCh responses (34.1% and 42.73% respectively). The Nifedipine- or 2APB- resistant responses were abolished by BTP2. Our data indicates that Orai channels play a significant part in cholinergic-mediated responses of detrusor smooth muscle.

A 10-04 MicroAge II: Mitochondria as Key Regulators of Muscle Mass in Microgravity and during Ageing

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The MicroAge II project is the second phase of the University of Liverpool's space-based muscle physiology research, in collaboration with the UK Space Agency and Kayser Space Ltd. Building upon findings from MicroAge I, which demonstrated significant microgravity-induced muscle atrophy, with alterations in mitochondrial function and cellular signalling pathways. The primary objective of MicroAge II is to elucidate the mechanisms by which microgravity affects mitochondrial dynamics, bioenergetics, and disruption of mitochondrial-cytoskeletal linkages, while assessing the efficacy of mechanical loading as a countermeasure to muscle deterioration.

Engineered human skeletal muscle constructs are cultured in bioreactors and will be subjected to controlled mechanical tension and stimulation onboard the International Space Station (ISS). These bioreactors are equipped with advanced microfluidic systems to deliver media changes throughout the experiment and fixative solutions enabling biological testing upon their return to Earth. Studies to date have established optimum culture conditions for the 3D constructs to maintain viability and contractility during unpowered upload to the ISS (up to 7 days) and established the levels of passive stretch that maintain muscle construct function for the duration of the mission.

Post-flight, these constructs will undergo comprehensive analyses, including transcriptomic, proteomic, mitochondrial bioenergetic, and electron microscopy studies, to characterize molecular pathways linked to mitochondrial dysfunction and cytoskeletal changes in microgravity-induced muscle loss.

MicroAge II aims to provide a thorough understanding of muscle deterioration mechanisms in space, potentially informing the development of targeted interventions for sarcopenia, including mitochondria-targeted therapeutics and

biomechanical rehabilitation strategies, benefiting both astronauts and aging populations on Earth.

A 10-05 MicroAge Mission: Effects of Microgravity and HSP10 Overexpression on the Proteome of Human Tissue-Engineered Muscle Constructs.

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Aim

Demographic shifts are increasing the number of older adults with poor health. Age-related muscle loss, along with poor exercise adaptation, impairs muscle mass, function, and quality of life. Astronauts in microgravity (µg) face similar challenges, experiencing rapid muscle loss and reduced exercise responses. Heat Shock Proteins (HSPs), crucial molecular chaperones, play a key role in muscle adaptation. Overexpression of the mitochondrial chaperone HSP10 in mice has been associated with a reduction in age-related muscle loss and cross-sectional area by limiting the accumulation of oxidatively damaged proteins. As part of a UK Space Agency mission to the ISS, we conducted *in vitro* studies using tissue-engineered human muscle to explore the effects of µg on the proteome and test whether HSP10 overexpression can mitigate microgravity-induced changes.

Methods & Results

Muscle constructs were fabricated using immortalised human myoblasts (AB1167) encapsulated in fibrin hydrogels and integrated into bioreactors developed in collaboration with Kayser Space Ltd. After returning from the ISS, we performed a ground-matched study, followed by LC-MS analysis. Proteomic comparison of AB1167 constructs exposed to microgravity showed 445 differentially expressed proteins (216 upregulated and 229 downregulated). In AB1167-HSP10 overexpressing constructs, only 338 proteins were differentially expressed, indicating a reduced response to microgravity.

Conclusions

Pathway analysis of 284 proteins changed in microgravity, but not when HSP10 was overexpressed, revealed enrichment of the unfolded protein response, glycosphingolipid and fatty acid catabolism and nitric oxide regulation, suggesting HSP10 overexpression protects the cells by mitigating the stress response and altering metabolic pathways in microgravity.

A 10-06 The use of ^{23}Na-MRI and ^{1}H-MRI to quantify damage in the gastrocnemius muscle of young male volunteers

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Aim

Robust markers of muscle damage following high-load eccentric exercise remain elusive, and those available provide limited insight into the aetiology and time-course of deficits in muscle function. This study investigated the use of ^{23}Na-MRI and ^{1}H-MRI to quantify local muscle damage following eccentric exercise.

Methods

Five physically inactive males (age: 26±5y; body mass index: 23±2kg/m^2) completed 5×50 dominant leg unilateral eccentric calf raises (1s concentric, 3s eccentric phase; each set separated by 2min rest), whilst wearing a weighted vest (30% body mass). Resting gastrocnemius muscle ^{23}Na-MRI scans, ^{1}H-MRI DIXON structural images, and transverse (T_2) mapping were performed in both legs before and 24, 48, and 96h after eccentric exercise. Additionally, plasma creatine kinase (CK) concentration, perceived muscle soreness, ankle range of motion and plantar flexion isometric strength were assessed 72h and 7 days after eccentric exercise.

Results

A main effect of leg (P=0.039) was observed for sodium, but neither time nor time*group interaction was significant (P>0.05). Sodium concentration peaked 96h post eccentric exercise in the exercised leg (25.5±9.7mmol/L). Compared to the contralateral leg, isometric strength in the exercised leg was less between 24-72h (P<0.05), whilst muscle soreness was greater from 24-96h (P<0.01). Delta changes revealed associations between muscle sodium concentration and ^{1}H-MRI T_2 (r=0.97, P<0.001)and plasma CK concentration (r=0.83, P<0.001).

Conclusions

Based on ^{23}Na-MRI and ^{1}H-MRI measurements, these data suggest that high-load eccentric exercise increases local muscle cell permeability, which may be linked to changes in plasma CK concentration and deficits in muscle function following eccentric muscle damage.

A 10-07 Lipofuscin as a marker for skeletal muscle biological aging

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Aim

Aging reduces skeletal muscle mass and function, but different people age at different rates. Lipofuscin is an intracellular accumulation of lipid-containing residues of lysosomal digestion. It is formed under conditions of impaired autophagy or proteasomal function. Lipofuscin accumulates progressively in neurons and cardiomyocytes with chronological age, but whether this also happens in skeletal muscle is unclear. The aim is to investigate the accumulation of lipofuscin in skeletal muscle as a marker of biological age in health, myalgic encephalomyelitis/chronic fatigue syndrome (ME/CFS) and post-COVID patients.

Methods & Results

In this study, we studied the accumulation of lipofuscin in the vastus lateralis muscle in relation to the biological age of 16 healthy participants and compared this with patients with chronic diseases, associated with oxidative stress, including post-COVID (n=8), and ME/CFS (n=4). Immunohistochemistry and electron microscopy were used to assess lipofuscin accumulation in skeletal muscle.

Lipofuscin accumulation in skeletal muscle of healthy controls is increased with chronological age (P=0.002, R^2=0.50, age range: 18-63years). Lipofuscin accumulation was higher in patients with post-COVID (1.66±0.68, P<0.001) and ME/CFS (3.94±0.48, P<0.001) compared to healthy controls (0.56±0.57). The age-dependent relation was absent in patients with post-COVID (n=8, P=0.33) and ME/CFS (n=4, P=0.28).

ConclusionsThese results suggest that lipofuscin accumulates in skeletal muscle with increasing age, and its autofluorescence complicates immunohistology experiments. Skeletal muscle of post-COVID and ME/CFS had more lipofuscin accumulation. These results suggest lipofuscin-linked accelerated aging of skeletal muscle in post-COVID and ME/CFS and shows the potential of lipofuscin as a physiological biomarker of muscle aging.

A 10-08 Acute caloric restriction in aged mice influences diaphragm sarcopenia and the circadian clock transcriptome

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Background: Age-related declines in skeletal muscle mass and strength, termed sarcopenia, affect both limb and respiratory muscles. Diaphragm weakness is associated with worse symptoms and pulmonary complications in older people. Whereas extended periods of caloric restriction (CR; 25-40%) improve limb

sarcopenia in aged rodents, the effects of CR on diaphragm sarcopenia remain unclear.

Objective: To characterise the effects of an acute and modest bout of CR on diaphragm structure, function, and transcriptome in aged mice.

Methods: Young (9 months old; n=9) and old (23 months old; n=9) male C57BL/6J mice were subjected to four weeks of modest CR (10-25% of ad-libitum levels) and compared to age-matched controls (chow ad-libitum fed; n=7 young and old). Diaphragm bundles underwent global transcriptome profiling (RNAseq), immunohistochemistry, or *in vitro* functional analysis (directly stimulated across force-frequencies).

Results: Ageing was associated with a 13% decrease in total and type IIa myofibre cross sectional area (P<0.05), but this was attenuated by CR (P<0.05). Compared to aged-controls, contractile function (specific twitch force) was increased by 24% in aged mice following CR (P<0.05). Following CR in aged mice, RNAseq revealed enriched core circadian clock genes CRY1/2, PER2/3 and CLOCK (p.adj<0.05) compared to aged controls.

Conclusion: An acute and modest bout of CR in aged mice attenuated diaphragm atrophy and increased contractile function, which was associated with increased expression of circadian clock genes. Overall, these data suggest that modest, short-term CR may have therapeutic potential for treating respiratory muscle sarcopenia in ageing.

A 10-09 Potential of Edible Bird Nest Extract as Antioxidant and Anti-Sarcopenia : An In Vitro Analysis

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Aim

[This study aims to explore the antioxidant and anti-sarcopenic effects of Edible Bird Nest (EBN) extract via in vitro analyses.]

Methods & Results

[EBN was prepared using ethanol to maintain the bioactive compounds. The antioxidant capacity was measured using DPPH, ABTS, and FRAP assays. To evaluate its anti-sarcopenia effects, C2C12 myotubes were stimulated with dexamethasone (DEX) to mimic muscle atrophy, and then EBN was applied. MTT assay was performed to determine cell viability, and western blot analysis was conducted to examine the levels of sarcopenia-associated proteins (Myostatin, TGF-β, and NF-κB) to assess the effect on muscle regeneration. EBN showed very high antioxidant activity with the IC50 values of 56.90 µg/mL (FRAP), 48.55 µg/mL (DPPH), and 59.58 µg/mL (ABTS), which are almost equal to that of the standard antioxidant, Trolox. When testing the anti-sarcopenia effects in the in vitro study, the treatment of EBN was found to enhance the viability of C2C12 cells to 101.30% and 105.45% with 50 mg/mL and 100 mg/mL, respectively, as opposed to 86.75% in DEX treated cells. In addition, EBN suppressed the Myostatin, TGF-β and NF-κB protein levels, which indicates its efficiency in preventing muscle atrophy and inflammation]

Conclusions

[The EBN extract possesses excellent antioxidant activity and protects the muscle from atrophy. Therefore, it is suggested that EBN could be used as a functional food for the management of oxidative stress and age-related muscle weakness and atrophy. However, it is essential to conduct further in vivo and clinical trials to substantiate these findings]

A 10-10 Like physical activity, phlorotannin-enriched extracts protect against oxidative stress.

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Physical activity (PA) is a major determinant of good human health. In addition to its role in maintaining physical fitness, physical activity is known to prevent the development and recurrence of chronic diseases (cardiovascular disease, type II diabetes, osteoporosis,...). Although the beneficial effects of PA are recognised, a sedentary lifestyle is an increasingly widespread phenomenon which has a number of significant deleterious effects on muscular health and mitochondrial function. These changes may trigger various pathologies in particular metabolic diseases such as insulin resistance, and contribute to chronic low-grade inflammation. Particularly, a sedentary lifestyle disrupts mitochondrial biogenesis and dynamics, leading to a reduction in aerobic capacity and an increase of oxidative stress. In this context, phlorotannins, polyphenols produced by brown macroalgae, have attracted interest for their antioxidant and anti-inflammatory properties. In this study, we explore the use of phlorotannin-rich algal extracts as an alternative to regular PA to induce similar beneficial effects on skeletal muscle and mitochondrial function. To this end, extractions of three brown seaweeds living on rockyshores in Brittany were carried out and the effect of phlorotannin-enriched extracts was studied *in vitro* on myoblasts (L6 cell-lines). A decrease in intracellular radical production and an increase in cell viability were observed in a model of oxidative stress induced by hydrogen peroxide (H_2O_2) after a 24-hour preventive treatment with these extracts. These findings highlight the protective effects of these extracts against oxidative stress, supporting their potential role in preserving skeletal muscle health.

A 10-11 β-Hydroxy-β-Methylbutyrate supplementation prevents capillary regression and fiber-type shift in rat soleus muscle during hindlimb unloading

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Aim

Prolonged inactivity reduces skeletal muscle capillarization via angio-adaptive imbalance and fiber-type shift. β-Hydroxy β-methylbutyrate (HMB) mitigates disuse atrophy by improving protein metabolism and fiber-type modulation. However, its effects on capillary regression and fiber-type shifts remain unclear. This study aimed to investigate whether HMB supplementation prevents unloading-induced capillary regression and fiber-type shift in rat soleus muscle.

Methods & Results

Twenty female Wistar rats were randomly divided into 4 groups: control (CON), HMB supplementation (HMB), 2-week hindlimb unloading (HU), and 2-week hindlimb unloading with HMB supplementation (HU+HMB) groups (n=5/group).

After anesthesia with 4% isoflurane, soleus muscles were excised. Histological analyses were performed to assess the capillary-to-fiber (C/F) ratio and the proportion of slow-twitch fibers. Protein expression levels of Akt, FoxO1, VEGF, TSP-1 and eNOS were evaluated via Western blotting. Each blot was repeated three times per protein. Statistical comparisons were conducted using one-way ANOVA followed by Tukey's post hoc test ($P<0.05$). Compared to the HU group (C/F ratio: 1.60 ± 0.02; slow-twitch fibers: 74.9 ± 2.9 %), HU+HMB group exhibited a significantly higher C/F ratio (1.85 ± 0.01) and preservation of slow-twitch fibers (81.9 ± 1.8 %), accompanied by increased phosphorylation of Akt and FoxO1. HMB also increased the VEGF/TSP-1 ratio and prevented unloading-induced reductions in eNOS expression.

Conclusions

These findings suggest that HMB supplementation preserves skeletal muscle microvascular integrity and fiber-type profile during unloading, potentially through

Akt–FoxO1-mediated modulation of angio-adaptive signaling. HMB may serve as a promising nutritional strategy to mitigate microvascular dysfunction and muscle quality decline during disuse.

A 11 | RENAL TRANSPORT PROCESSES

A 11-01 Kidney sodium handling and blood pressure control orchestrated by renal macrophages in salt sensitive hypertension

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Aim

A third of people are salt-sensitive, with exaggerated blood pressure response to high salt intake. Salt-sensitivity increases cardiovascular risk, but underlying causes are unresolved. Immune cells, like monocytes and macrophages, are important regulators of both salt homeostasis and blood pressure physiology. Here, we examined the impact of macrophage depletion on salt-sensitive hypertension in the mouse.

Methods & Results

Male FVB-CD11b-DTR transgenic mice, expressing human diphtheria toxin receptor on CD11b$^+$ myeloid cells, were fed a high salt diet (3% Na) for 4 weeks. During the final week we gave diphtheria toxin, which significantly reduced the macrophage count in renal cortex and induced complete macrophage depletion in the renal medulla. Radiotelemetry showed that high salt diet significantly increased both systolic and diastolic BP over baseline (0.3% Na). Following macrophage depletion, blood pressure increased by a further ~15mmHg, showing salt-sensitivity. In other mice, we found macrophage depletion reduced urinary Na^+ excretion. At the molecular level, mRNA encoding subunits of the epithelial sodium channel (ENaC) was elevated in kidney cortex after macrophage depletion. We also found that the natriuretic response to the *ENaC* blocker benzamil was reduced by high salt intake, showing downregulation of ENaC: in macrophage-depleted mice, benzamil was natriuretic, suggesting functional ENaC. Aldosterone was downregulated by high salt intake in all groups.

Conclusions

Macrophage depletion in kidney activates ENaC to reduce salt excretion. This is independent of aldosterone and contributes to the development of salt-sensitive hypertension.

A 11-02 Link between G-protein-coupled receptor kinase 6 and AQP2 trafficking

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The author has objected to a publication of the abstract.

A 11-03 Aquaporin-2 Trafficking Dynamics: Investigating Protein Kinase A Localization upon cAMP Elevation

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The kidney rapidly adjusts urine concentration in the collecting ducts through arginine vasopressin (AVP)-regulated trafficking of aquaporin-2 (AQP2) between intracellular vesicles and the plasma membrane. Dysregulated AQP2 shuttling contributes to water balance disorders, leading to impaired urine concentration or water retention. Understanding the molecular mechanisms of AQP2 vesicle shuttling is crucial for developing targeted treatments.

This study investigates the relationship between AQP2 and protein kinase A (PKA) in response to changes in cAMP levels. AVP-mediated cAMP elevation activates PKA, leading to AQP2 serine 256 phosphorylation, which promotes membrane insertion, however, the exact mechanism is unclear.

We hypothesized that PKA must co-localize with AQP2 endosomes/vesicles for phosphorylation. To test this, MDCK cells expressing AQP2 were stimulated with forskolin to elevate cAMP, fixed at different timepoints, and analyzed using 4.5x

Expansion Microscopy (ExM) for improved resolution Colocalization analysis revealed partial overlap between AQP2 and PKA in intracellular compartments at baseline but showed minimal colocalization following 30 minutes of cAMP elevation. ExM revealed some colocalization of AQP2 and PKA on endosomes in control and following 2-4 minutes of cAMP elevation. After 2-4 minutes of cAMP elevation, large AQP2-containing endosomes diminished, and by 10 minutes, only small vesicles remained, showing only little or no colocalization with PKA. After 30 minutes, AQP2 predominantly localized at the plasma membrane, while PKA remained dispersed below the membrane.
In summary, some colocalization of AQP2 and PKA was observed before cAMP elevation but was not maintained on vesicles following stimulation, suggesting that potential PKA and AQP2 co-localization is transient.

A 11-04 Regulation of the Na-K-2Cl cotransporter NKCC2 by ubiquitylation in a novel MDCKI cell line

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NKCC2 is localized in cells of the renal thick ascending limb, facilitating ~25% of filtered NaCl reabsorption, and is essential for water, salt, and ion homeostasis. NKCC2 is ubiquitylated on at least 15 lysine residues, with the E3 protein ligase Nedd4-2 proposed to play a major role in the ubiquitylation process. Studies examining the role of NKCC2 ubiquitylation, have been limited by challenges in expressing NKCC2 in mammalian cell lines. Therefore, the aims of this study were to: 1) generate a polarized mammalian epithelial cell line with stable full-length NKCC2 expression, 2) detail the role of Nedd4-2 in NKCC2 ubiquitylation, and 3) determine the functional impact of site-specific ubiquitylation of NKCC2. A polarized mammalian cell line was generated by stably transfecting human FLAG-NKCC2 into tetracycline-inducible FRT-MDCKI (Madin-Darby Canine Kidney type I) cells. Site-directed mutagenesis introduced ubiquitylation site mutations in NKCC2, and CRISPR/Cas9 was applied to generate Nedd4-2 knock-out cells. Western blotting following surface biotinylation assessed total and membrane protein abundances. Immunoprecipitation was performed using FLAG-M2 affinity beads, and NKCC2 activity was measured via a FluxOR Potassium Ion Channel Assay. The MDCKI cell line demonstrated stable, inducible expression of full-length human NKCC2. Nedd4-2 co-immunoprecipitated with NKCC2. Knockout of Nedd4-2 increased total, but not membrane, NKCC2 abundance (n=12). Mutation of K871, increased NKCC2 membrane abundance, enhanced NKCC2 uptake activity, and reduced NKCC2 internalization rates (n=9-12).
The MDCKI-NKCC2 cell line is an excellent model for studying NKCC2. Nedd4-2 directly alters NKCC2 degradation, and ubiquitylation at K871 plays an important role in NKCC2 regulation.

A 11-05 The molecular mechanism of augmented renal HCO_3^- excretion during respiratory alkalosis

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Background: During respiratory alkalosis, the kidneys compensate for this acid-base imbalance by increasing urinary HCO_3^- excretion as seen at high altitudes. However, the underlying molecular mechanism remains unknown. Interestingly, augmentation of renal HCO_3^- excretion during metabolic alkalosis was recently shown to depend on pendrin and CFTR in the collecting duct (CD) β-intercalated cells (β-IC). Furthermore, secretin was shown to activate β-ICs via the secretin receptor. We hypothesized that β-IC pendrin and CFTR are necessary for renal compensation of respiratory alkalosis, and that secretin plays a role in mediating the response.
Methods: Under anaesthesia (ketamine-xylazine, i.p., 66.7 µg and 6.67 µg/g/h, respectively), bladder catheterization experiments were conducted in mechanically ventilated pendrin wild-type (WT) and knockout (KO) mice to assess the renal response to hyperventilation-induced respiratory alkalosis. Arterial blood gas analyses were performed to confirm respiratory alkalosis. Additionally, pendrin WT and KO mice were subjected to hypoxia using intermittently-closed-flow respirometry.

Results: A sharp increase in urine pH occurred shortly (i.e., 10-15 minutes) after initiation of hyperventilation in pendrin WT mice. This effect was completely absent in pendrin KO mice. Arterial blood gas analyses confirmed respiratory alkalosis during hyperventilation in both genotypes. Furthermore, pendrin KO mice displayed a reduced hyperventilatory response and larger body temperature decreases during hypoxia in intermittently-closed-flow respirometry.
Conclusions: Our results imply that lack of pendrin impairs the ability to increase urinary HCO_3^- excretion during hyperventilation-induced respiratory alkalosis and that β-IC pendrin function is crucial to renal compensation of respiratory alkalosis. Finally, they suggest that the renal response initiates immediately.

A 11-06 Aquaporin-2 Transport Vesicles: Characterization of Spatial Organization in Cells and Tissue Using Expansion Microscopy

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Aquaporin water channels (AQPs) play a critical role in water homeostasis by facilitating water diffusion across cellular membranes following an osmotic gradient. AQP2 is localized in subapical vesicles and the plasma membrane of renal collecting duct principal cells. Arginine Vasopressin (AVP) stimulates AQP2 accumulation in the apical plasma membrane, increasing water permeability and urine concentration. Dysregulation of AQP2 trafficking is linked to disorders such as chronic kidney disease, nephrogenic diabetes insipidus and congestive heart failure. A major challenge in studying AQP2 vesicles is their small size ~40 nm. We therefore optimized expansion microscopy to resolve single vesicles in 3D in both cells and tissue.
AQP2 in kidney collecting ducts are localized in the cytoplasm within small transport vesicles and large endosomes and also in the basolateral plasma membrane. In saline treated control rats, the transport vesicles were more concentrated in the apical membrane and cytoplasm of the cells. Large endosomes were observed in the perinuclear regions. AQP2 phosphorylated on Serine 256 (p256AQP2) was observed in transport vesicles and the apical membrane but not in endosomes and the basolateral membrane. Upon dDAVP treatment AQP2 was no longer observed in large endosomes. The number of transport vesicles decreased and AQP2 in the apical membrane increased. p256AQP2 was exclusively observed in the apical plasma membrane in the tissue.
Future work will incorporate advanced AI-based image analysis to characterize the AQP2 vesicle population and interacting partners, with the aim of uncovering new regulatory patterns and vesicle behavior.

A 11-07 Effects of Parathyroid Hormone and Vitamin-Induced Hypercalcemia on Renal Calcium Handling and Metabolic Changes

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Central to calcium homeostasis is the calcium-sensing receptor (CASR), predominantly located in the parathyroid glands and kidneys. In the parathyroid glands, CASR regulates parathyroid hormone (PTH) release. Increased PTH activates vitamin D, both raising blood calcium levels. This study investigates the impact of hypercalcemia induced by the PTH fragment teriparatide and the vitamin D analog (DHT) on kidney calcium transport.
PTH and DHT were administrated for 3 days to C57BL/6 mice. Electrolyte excretion was determined. 1.5 % Isoflurane inhalation was used asanesthesia. Protein- and gene expression levels were determined by qPCR and immunohistochemistry. Plasma calcium levels were significantly elevated after PTH and DHT administration in comparison to controls (control 1.3 ± 0.01 mM, DHT 2.1 ± 0.07 mM, PTH 1.9 ± 0.05 mM). Urinary calcium excretion was only significantly elevated in DHT-treated mice. DHT-treated mice had a markedly higher expression of claudin-14 than PTH-treated mice. Notably, large calcium deposits were observed exclusively in PTH-treated mice in segments outside the collecting system. PTH-treatment caused the development of metabolic acidosis and increased urine ammonium and phosphate excretion. However, urine pH remained unchanged. We observed no visual change in protein expression of the H^+-ATPase, the anion exchanger AE1, or Pendrin. No change in gene or protein expression of NHE3 was detected between groups. These results highlight that while PTH and DHT similarly elevate plasma calcium,

their effects on renal calcium handling and associated metabolic changes differ significantly. Further studies will be conducted to explore the mechanisms underlying these pathways.

A 11-08 Spatial and Temporal Expression of the Calcium-Sensing Receptor in Renal and Extrarenal Tissues

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The Calcium-Sensing Receptor (CASR) regulates calcium balance by controlling parathyroid hormone release and renal divalent cation transport. While CASR is abundant in the thick ascending limb (TAL) of the kidney, its precise distribution across nephron segments and other tissues is unclear, partly due to inconsistent antibody staining. In addition, the developmental expression profile of the CASR is still not fully defined.

To map CASR localization by immunohistochemistry, we used transgenic mice expressing EGFP driven by the *Casr* promoter, allowing detection of both EGFP and endogenous CASR. We also employed Casrtm1b(KOMP)Mbp mice carrying a LacZ cassette in exon 3 of *Casr* to determine beta-galactosidase activity. CASR antibody specificity was validated using various *Casr* knockout mice. Animals were euthanized under 1.5% Isoflurane inhalation.

EGFP fluorescence was strongest in the TAL, particularly in the cortex and outer stripe of the outer medulla, with reduced signal in the inner stripe. CASR immunostaining mirrored this pattern, co-localizing with NKCC2-positive TAL cells, and showing weaker expression in NCC-positive distal tubules and some Aquaporin-2-labeled collecting ducts. Notably, no EGFP or CASR signal was detected in the proximal tubules or inner medulla. Outside the kidney, EGFP was present in pancreatic islets, parts of the gastrointestinal tract, and bone of adult animals, while E16.5 embryos showed strong expression within the primordial cartilage and olfactory epithelium. In kidneys of Casrtm1b beta-galactosidase activity was visible only in the TAL.

Our findings delineate spatial localization of the CASR to understand its important roles in renal and extrarenal tissues.

A 11-09 Potassium depletion deactivates β-IC-mediated HCO_3^- excretion

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Aim

The combination of hypokalemia and metabolic alkalosis is a commonly encountered phenotype in different clinical settings. Whilst it is well-understood that potassium depletion enhances renal acid excretion, it is yet to be elucidated how K^+ depletion impairs the kidneys' large capacity to excrete excess base that contributes to the maintenance of metabolic alkalosis. Recent studies have clarified the pivotal role of proper β-IC function in the excretion of excess HCO_3^- and we here hypothesize that in states of K^+deprivation, β-IC-mediated HCO_3^- excretion might be impaired and thus contribute to the phenotype of hypokalemic metabolic alkalosis.

Methods & Results

All experiments were performed in C57/Bl6J mice. To assess β-IC function, net base excretion was quantified after an acute oral HCO_3^- challenge in animals exposed to different K^+ dietary regimens. In further experiments mice on a control diet were pretreated with eplerenone. Subsequently, the acute oral HCO_3^- challenge was given to investigate a possible aldosterone-dependent effect on the ability to excrete the base into the urine.

We find that 1) Net base excretion following an acute HCO_3^- load was markedly impaired after 3 days of K^+ deprivation and it was increased in states of excess K^+ supplementation, 2) Eplerenone pretreatment reduced net base excretion following an acute HCO_3^- load.

Conclusions

1) Net base excretion following an acute HCO_3^- load was markedly impaired after 3 days of K^+ deprivation and it was increased in states of excess K^+ supplementation,

2) Eplerenone pretreatment reduced net base excretion following an acute HCO_3^- load.

A 11-10 Systemic and renal responses to acetazolaminde do not require functional NHE3 in the kidney

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Aim

NHE3 has been implicated for maintaining acid-base balance by mediating Na^+ reabsorption and H^+ secretion in the proximal tubule, the latter is required to facilitate HCO_3^- reabsorption. HCO_3^- reabsorption requires the enzymatic activities of luminal carbonic anhydrase (CA) IV and intracellular CA II. We hypothesized that NHE3 is required to mediate the effects of CA inhibitors.

Methods & Results

Control (Con, n=8-10) and tubule-specific NHE3 knockout mice ($NHE3^{KS-KO}$, n=8-10) were randomized to application of vehicle (Veh, 0.85% saline, 0.2% of bw i.v.), acetazolamide (ACZ, cell-permeable CA inhibitor; 50 mg/kg i.v.) or C18 (cell-impermeable CA inhibitor; 5 mg/kg i.v.) and blood gas analysis (1 hour) and metabolic cage (3 hour) experiments were performed. Veh treatment did not significantly alter blood pH, HCO_3^- or Cl^- levels in either genotype. ACZ significanlty decreased blood pH (Con: 7.39±0.01 vs. 7.26±0.01, P<0.05; $NHE3^{KS-KO}$: 7.35±0.01 vs. 7.24±0.01, P<0.05) and blood HCO_3^- (Con: 23.2±0.5 vs. 19.2±0.5 mmol/L, P<0.05; $NHE3^{KS-KO}$: 22.7±0.3 vs. 19.1±0.5 mmol/L, P<0.05) in both genotypes without significant differences between Con and $NHE3^{KS-KO}$ mice. Plasma Cl^- levels similarily increased in Con (114±0.3 vs. 116±0.4 mmol/L, P<0.05) and $NHE3^{KS-KO}$ (114±0.3 vs. 116±0.4 mmol/L, P<0.05) mice after ACZ treatment. Neither C18 nor Veh treatment altered blood pH, HCO_3^- or Cl^- levels in either genotype. ACZ treatment made urinary pH significantly more alkaline in Con (6.9±0.2 vs. 8.5±0.1, P<0.05) and $NHE3^{KS-KO}$ (7.5±0.2 vs. 8.4±0.1, P<0.05) mice.

Conclusions

Luminal blockade of CA IV does not affect blood acid-base parameters and the effects of ACZ on acid-base status do not require functional NHE3.

A 11-11 Sex differences in the natriuretic response to an acute increase in plasma potassium in Sprague Dawley rats

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Aim

Salt intake is typically high and causes poor health outcomes for many. Higher potassium intake can negate the hypertensive effect of salt-rich diets and higher urinary potassium excretion associates with lower blood pressure and reduced cardiovascular risk. Why dietary potassium has beneficial effects is not fully defined but may be due to enhanced sodium excretion and improved sodium balance. Ingesting potassium salts promotes natriuresis and, at a molecular level, dephosphorylates and deactivates the thiazide-sensitive NaCl co-transporter, NCC. This study aimed to measure the effect of an acute rise in plasma potassium on urine sodium excretion and the response to thiazide diuretic in Sprague Dawley rats.

Methods & Results

Anaesthetised rats (Inactin, 120mg/kg IP) were randomised to an intravenous infusion of 140mmol/l KCl or NaCl. KCl infusion increased plasma potassium to ~6mmol/l, significantly higher than the ~4mmol/l in the NaCl-infused control group. Renal plasma flow and glomerular filtration rate were not different between groups. Sodium excretion was higher in KCl-infused male rats than controls, associated with a lower abundance of T53-phosphorylated NCC measured by western blot in kidney cortex at the end of the experiment. In female rats, KCl infusion also reduced T53-phosphorylated NCC but did not promote natriuresis. In other male rats, KCl infusion significantly diminished the acute natriuretic effect of the NCC inhibitor bendroflumethiazide; this was not seen in female rats.

Conclusions

This study suggests that plasma potassium differentially influences renal epithelial sodium transport in male and female rats.

A 11-12 Characterising novel transport mechanisms for corticosteroid hormones in the murine collecting duct

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Background: Within the renal collecting duct (CD), Na^+ reabsorption and K^+ secretion are stimulated by aldosterone (ALDO) in response to hyperkalaemia, hyponatremia, or hypovolemia. Although the lipophilic nature of ALDO enables passive diffusion across target cell membranes, corticosteroids may also undergo active transport in tissues such as brain and adipose. Preliminary data suggest similar mechanisms may transport ALDO across CD cells.

Aim: To evaluate the ALDO distribution dynamics across CD cells- comparing apical (AP) vs basolateral (BL) application; distribution over 48h; and in the absence of Na^+/K^+-ATPase activity.

Methods: $mCCD_{cl1}$ were cultured on filters (9-10d) to model polarised epithelia[MM1] . [ALDO] was measured across three compartments: AP, BL and cell lysate (CL). Equivalent short-circuit current (I_{eq}) was quantified by epithelial volt-ohm-meter. [ALDO] was determined *via* targeted LC-MS/MS.

Results: ALDO distribution following BL application (300nM, 3h) was unequal: AP 14.1±2.0%, BL 85.3±1.8%, CL 0.6±0.7%, whilst AP application reversed this (AP 91.4±1.5%, BL 8.3±1.4%, CL 0.3±0.3%), consistent with previous findings using 3nM ALDO or 150nM CORT (n=6). I_{eq} stimulation was similar to BL treatment.

BL application (300nM, 3–48h) demonstrated unequal distribution; AP increased and BL decreased (3–12h), then plateaued (AP: 30.4±3.4%, BL: 69.2±4.9%, CL: 0.5±0.2%; n=6). I_{eq} was stimulated above control values at all timepoints (3–48h).

Ouabain pre-treatment (2mM, 30min) near-abolished I_{eq}; subsequent ALDO (300nM, 3h) did not alter I_{eq} nor [ALDO] distribution: AP: 9.3±2.8%, BL: 90.3±2.7% and CL: 0.4±0.3% (n=6).

Conclusions: These data suggest cellular bioavailability of ALDO may be maintained by active transport, independently of mechanisms in the CD.

A 11-13 Empagliflozin-induced microalbuminuria, aminoaciduria and myo-inositol loss: Evidence for a general reduction of proximal tubule reabsorption under SGLT2 blockade?

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SGLT2 inhibitor empagliflozin (EMPA) has substantial beneficial effects on renal function in patients with diabetic and non-diabetic chronic kidney disease (CKD). However, the cellular consequences of SGLT2 inhibition on proximal renal tubules are not yet well understood. In this study, we investigated the acute and long-term effect of EMPA on proximal tubule reabsorption processes in healthy C57BL/6J mice (WT) and LLCPK1 cells, a proximal tubule cell line. Despite the overwhelming effects of EMPA on lowering existing albuminuria in patients with CKD, EMPA (30 mg/kg/d) significantly increased the urinary albumin-creatinine-ratio in healthy WT animals both during acute and long-term treatment, while the glomerular filtration rate was not affected by EMPA. In LLCPK1 cells, grown on filter membranes, EMPA (1µM) inhibited the uptake of fluorescence-labeled albumin by 25% pointing towards an albumin reabsorption defect upon SGLT2 inhibition, possibly by interference with the megalin/cubilin endocytosis pathway and/or the Na^+-H^+-exchanger NHE3. In addition to albumin loss, EMPA caused a mild aminoaciduria in WT mice. Most pronounced was the loss of neutral amino acids leucine, valine and isoleucine. An unbiased urine metabolome analysis further revealed increased levels of myo-inositol in the urine of EMPA treated WT animals. Notably, myo-inositol transporters are strongly expressed in renal proximal tubules and LLCPK1 cells. EMPA-treated LLCPK1 cells showed reduced expression of myo-inositol transporter SMIT2 and accumulation of myo-inositol in the supernatant. The observed tubular effects may also be involved in renal protection by EMPA, particularly the protection of intact proximal tubules from reabsorption overload thereby preventing long-term tubular damage.

A 12 | KIDNEY DISEASE

A 12-01 DNAJB4 Activates NETosis and Exacerbates Inflammation in Chronic Kidney Disease

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Aim:

Dnajb4, a member of the DNAJ/heat shock protein (HSP) 40 family, serves as the co-chaperone of HSP70 and regulates protein homeostasis and cellular functions. However, the molecular mechanism underlying the biological significance of dnajb4 on the development of the process of chronic kidney disease (CKD) remains elusive.

Methods & Results:

The Gene Expression Omnibus (GEO) datasets showed that mRNA expression of the dnajb4 gene was upregulated in CKD patients. In this study, C57BL/6 wild type (WT) mice and dnajb4 knockout (dnajb4$^{-/-}$) mice were fed with the 0.2% adenine diet for 4 weeks and followed by the regular diet for 3 weeks. Western blots analysis revealed that protein expression of dnajb4 was increased in the kidneys of WT mice fed with an adenine diet. Moreover, genetic deletion of dnajb4 retarded CKD progression in mice, as evidenced by the decreases in plasma levels of creatinine and blood urea nitrogen, increased endothelial cell integrity and autophagy, and reduced oxidative stress, consequently ameliorated inflammation and fibrosis in the kidneys of CKD mice. The results of LC-MS/MS and canonical analysis showed that genetic deletion of dnajb4 deregulated the formation of neutrophil extracellular trap (NETosis) in the CKD kidneys. *In vitro* studies showed that genetic knockdown of dnajb4 using small interfering RNA in neutrophils abrogated indoxyl sulfate-induced NETosis.

Conclusions:

Our findings suggest that dnajb4 upregulates oxidative stress and promotes NETosis and leukocyte infiltration, ultimately exacerbating inflammation and accelerating CKD progression.

A 12-02 P2Y6-signaling in renal fibrosis

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Aim

Extracellular nucleotides function as paracrine or autocrine signals in normal physiology and act as damage-associated molecular patterns in disease. While P2Y receptors are widely expressed in the nephron and vasculature, their function in renal interstitial cells remains unclear. In this study, we aimed to investigate the importance of purinergic signaling in renal interstitial fibroblasts and renal fibrosis.

Methods & Results

We performed RNA *in situ* hybridizations of several key players of the purinergic signaling pathway, including several $G_{q/11}$ protein-coupled P2Y receptors and the ectonucleotidases *Entpd1* (Cd39) and *Nt5e* (Cd73) in mouse kidney slices. We observed co-expression of *P2ry1* ($P2Y_1$), *P2ry6* ($P2Y_6$) and both ectonucleotidases with the interstitial cell marker *Pdgfr*. Interestingly, mRNA expression of *P2ry6* and *Entpd1* was increased in fibrotic mouse kidneys, indicating a role of purinergic signaling in fibrosis.

To functionally analyze the importance of P2Y receptors in interstitial fibroblasts, we performed Ca^{2+} measurements on isolated renal fibroblasts using the ratiometric Ca^{2+} indicator Fura2. Superfusion of the cells with the P2Y agonists ATP, ADP, UTP or UDP resulted in transiently elevated Ca^{2+} signals indicative of functionally active P2Y receptor signaling.

Conclusions

Renal interstitial cells express components of the purinergic signaling pathway such as $P2Y_6$, Cd39 and Cd73 in interstitial fibroblasts. Since some of these components are upregulated in renal fibrosis, we would like to study the importance of purinergic signaling for the progression of fibrosis and their therapeutic potential in future studies.

A 12-03 Toxicological Mechanisms of Uranium-Induced Damage in HK-2 Cells: A Proteomics and Metabolomics study

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Aim

Uranium is widely distributed in nature and can causing environmental pollutions. studies showed that soluble uranium compounds have selective toxicity to the kidneys, yet the specific toxicological mechanisms are not fully understood. This research aims to study the specific toxicological mechanisms of Uranium to HK-2 cells.

Methods & Results

CCK8, flow cytometry, DAPI staining to demonstrate that the primary mode of cell death in uranium exposure is apoptosis. The Comet assay、KEGG (Kyoto Encyclopedia of Genes and Genomes) pathway enrichment diagram and the proteomic interaction network analysis are all associated with DNA damage. The abnormal expression in ABC transporters, ABCB7 protein and the mitochondrial related proteins illustrate that uranium exposure can cause mitochondrial dysfunction in HK-2 cells. The COG(Cluster of Orthologous Groups of proteins) chart and the PPI (Protein-protein interaction) network diagram shows that uranium exposure can cause abnormal protein synthesis. The GO (Gene Ontology) functional annotation indicate that uranium can trigger apoptosis in cells through extrinsic signaling pathways. The down-regulation of UBL5 protein suggests that uranium can induce apoptosis in cells through endoplasmic reticulum stress.

Conclusions

This research shows that uranium exposure mainly induces apoptosis in HK-2 cells through intrinsic pathways by damaging cellular DNA and mitochondria as well as interfering with cellular protein synthesis. In addition, roles for endoplasmic reticulum stress-induced apoptosis and extrinsic apoptosis also play certain roles in this process.

A 12-04 The potential role of secretin and its receptor in kidney disease progression

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Aim

Secretin, a hormone released in the postprandial phase, has recently been demonstrated to modulate renal glomerular filtration. Congruently, loss of the receptor leads to hyperfiltration, a pathophysiological condition that entails irreversible kidney damage.

This study aims to investigate whether secretin and its receptor play a role in the development of renal disease.

Methods & Results

The project is centered around the secretin receptor (SCTR) KO mouse model. The mice will be examined during aging and after subjection to a hypertensive and a western diet challenge.

A broad range of methods will be utilized, including transcutaneous GFR measurements, blood pressure measurements, echocardiography, urine and blood analysis, western blotting and tissue staining for renal damage patterns. 2% isoflurane is used for anesthesia.

Under baseline conditions, KOs and WTs had comparable blood pressure and cardiac function at 1 year of age. Despite this, KOs presented with marked proteinuria compared to controls.

KO mice had elevated GFR when young but showed a pronounced age-dependent GFR decline compared to WTs. In kidney tissue from 1-year-old KO and WT mice, KOs presented with elevated mRNA levels of several fibrosis markers, indicative of ongoing renal fibrosis and damage.

Conclusions

Based on these first results, SCTR KO mice exhibit a phenotype indicative of progressive renal disease. The phenotype is present during normal aging in both functional and ex vivo measurements.

The results will deepen the understanding of secretin's potential involvement in renal disease development and provide information concerning the SCTR as a potential therapeutic target.

A 12-05 The pathogenic human variant G165A of claudin-10 compromises tubular function and tight junction assembly

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Claudin-10 mutations have been linked to HELIX syndrome (hypohidrosis (H), electrolyte (E) imbalance, hypolacrimia (L), ichthyosis (I) and xerostomia (X)). A new pathogenic variant of claudin-10 (G165A) was described in two unrelated patients characterized by salt-losing tubulopathy with polyuria, hypermagnesemia, hypocalciuria, hypokalemia and hypochloremic alkalosis. In a mouse model, we investigated the effect of this mutation on claudin-10 localization and its functional electrophysiological consequence along the proximal tubule (PT) and thick ascending limb (TAL), where claudin-10 is expressed. Freshly isolated single murine PT and TAL segments of C57BL/6-Claudin10-Gly165Ala knock-in mice were investigated by fluorescence microscopy and electrophysiological measurements of transcellular (transepithelial voltage and resistance) and paracellular (diffusion potential) properties. Claudin-10-G165A mice presented hypocalciuria and hypermagnesemia and, unlike the patient, the mice showed nephrocalcinosis. In PT, similar to the knock-out situation (1), anion selectivity was lost along the entire proximal tubular axis and the paracellular pathway showed a strong preference for Ca^{2+}, Mg^{2+} and Na^{+}, indicating the incorporation of more claudin-2 into the tight junction (TJ). In the TAL, the increase in transepithelial voltage and resistance was less pronounced and more variable in comparison to the KO situation (2), but TAL showed loss of paracellular Na^{+} selectivity. In both segments tubules from heterozygous mice showed an intermediate state. Immunofluorescence revealed that G165A mutation disturbs the correct TJ strand assembly in both PT and TAL. The mouse and patient's phenotype might be explained by the lower ability of the mutant G165A protein to form functional TJ strands in the native tubule.

A 12-06 Proteasome Dysfunction and Immunoproteasome Induction in Diabetic Nephropathy: Therapeutic Implications beyond SGLT2 Inhibition

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The author has objected to a publication of the abstract.

A 12-07 Preexisting cancer is associated with a higher incidence of acute kidney injury and mortality in the critically ill which is not mediated by neutrophil extracellular traps (NETs)

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Introduction

Sepsis is a life-threatening syndrome with multiple organ failure due to an infection. Neutrophil activation and subsequent NETs formation have been suggested to play a role in the organ failure seen in sepsis. NETs have also been associated with morbidity and mortality in cancer. We hypothesized that critically ill patients with cancer have a higher rate of AKI and mortality than those with no cancer, which are associated with higher levels of NETs.

Methods & Results

Positive covid-19 patients admitted to the ICU at Uppsala University Hospital were assessed for the primary outcomes: AKI, in-hospital mortality, and 30-day mortality.

Secondary outcomes were plasma levels of NETs by the biomarkers: neutrophil elastase - NE, cell free DNA - cfDNA, growth arrest specific protein 6 - GAS6, histone 3 - H3. Chi-Square, multiple logistic regression, and t-test were used for statistical analysis.

N=372 patients were included in the cohort, n=35 (9%) with cancer and n=337 (91%) without. Incidence of AKI was higher in the cancer group relative to the non-cancer group (63% vs 48%, p<0.0001). Mortality was twice as high in the critically ill patients with preexisting cancer than non-cancer patients. 29% vs 14% died before ICU discharge (p=0.013), and 37% vs 16% died within 30 days. (p<0.001). Plasma levels of NETs did not differ between the cancer and non-cancer group.

Conclusions

Sepsis-patients with preexisting cancer have higher rates of AKI and twice as higher mortality rate than sepsis patients without cancer. This is however not mediated by higher levels of NETs.

A 12-08 Autoantibody-triggered vesicle formation in podocytes as a novel pathomechanism in membranous nephropathy

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Aim

In membranous nephropathy (MN), circulating autoantibodies target podocyte foot process antigens, such as THSD7A and PLA_2R1, leading to immune-complex deposition, podocyte injury, and progressive kidney dysfunction. The mechanisms behind podocyte responses to this autoimmune attack remain unclear. This study investigated whether podocytes form and release extracellular vesicles (EVs) into the urine in response to autoantibody binding and explores their potential as non-invasive biomarkers in MN patients.

Methods

Urinary EV formation and characteristics were investigated by microscopical, ultrastructural and biochemical approaches. Proteomic and flow cytometry analyses provided insights into molecular EV composition. Protein accumulation and EV release were assessed in $THSD7A^+$-MN mice and in diagnostic and follow-up $THSD7A^+$-MN and PLA_2R1^+-MN patient material.

Results

Binding of autoantibodies to podocyte foot process antigens induced formation of EVs and their release into the urine through plasma membrane budding. These EVs, termed autoimmunoglobulin-triggered (AIT)-EVs, carry immune complexes, stress markers, and podocyte-specific proteins, including MN antigens. AIT-EVs differed in their protein composition from EVs of patients with other nephrotic diseases. Importantly in patients, immunoblot demonstrated autoantibody binding to AIT-EVs even if the autoantibodies were no longer detectable in plasma or kidney tissue. This indicates the potential of AIT-EVs for monitoring disease activity in MN patients.

Conclusions

This study describes a novel pathobiological mechanism in MN, in which podocytes release AIT-EVs to clear immune complexes and maintain their function. By shedding AIT-EVs into urine, they offer a valuable diagnostic tool for assessing disease progression and monitoring in MN patients.

A 12-09 Polyamine supplementation partially restores kidney function in adenine-induced nephropathy

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Kidney injury is characterized by a sudden or sustained decline in excretory kidney function. Rather than representing a single disease entity, the etiology and pathophysiology of acute kidney injury and chronic kidney disease are highly heterogeneous, encompassing conditions such as hypoxia, inflammation, and metabolic disturbances. Our previous findings indicate that the kidney responds to various forms of injury with a downregulation of polyamine synthesis and activation of the polyamine degradation pathway. The polyamines putrescine, spermidine, and spermine are key regulators of injury and repair processes, including cell proliferation, protein synthesis, and autophagy. Polyamine metabolism, including their synthesis, interconversion, and breakdown, is tightly regulated by enzymatic mechanisms. Notably, serum putrescine levels are markedly elevated in kidney injury.

Here, we hypothesized that impaired polyamine homeostasis could be therapeutically targeted by exogenous polyamine supplementation. Nephropathy was induced in male C57BL/6J mice through a 14-day adenine-enriched diet (0.2% w/w), followed by supplementation with either 30 mM spermidine or 30 mM spermine in the drinking water. Mice receiving no supplementation served as controls. Kidney function, as assessed by serum cystatin C and creatinine, was restored to normal levels in animals receiving either spermidine or spermine. Blood urea nitrogen levels were normalized by spermidine, but not by spermine, supplementation. Moreover, serum putrescine levels were reduced to baseline levels with both treatments. However, urinary albumin levels remained elevated and were not normalized by polyamine supplementation.

These findings suggest that spermidine and spermine supplementation can partially restore kidney function and polyamine homeostasis in a mouse model of nephropathy.

A 12-10 Role of the cGAS-STING pathway in the progression of diabetic kidney disease

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Aim

Diabetic kidney disease (DKD) is the leading cause of chronic renal pathology. We have demonstrated previously that Type 2 Diabetic Nephropathy (T2DN) rats develop renal and physiological abnormalities similar to clinical observations in humans with DKD, indicating these rats are an excellent model for studying the progression of renal injury in DKD. The Aim of this study was to provide some mechanistic insights into the progression of DKD.

Methods & Results

We utilized young (12-week-old) and aged (>48 weeks old) type 2 diabetic nephropathy (T2DN) rats. To delineate transcriptional changes, RNA-Seq analysis was performed in the kidney cortex of T2DN rats of both sexes at a younger and older age. Western blotting, immunohistochemistry, and flow cytometry analyses were further used to identify specific pathways contributing to progression of DKD in T2DN rats. We have revealed that the cyclic GMP-AMP synthase (cGAS) / Stimulator of interferon genes (STING) signaling pathway is upregulated in T2DN rats and in type 2 diabetic human kidneys. The expression of key proteins in the cGAS-STING pathway was significantly different between male and female T2DN rats and following the progression of DKD. Proinflammatory genes were also upregulated in male T2DN rats compared to female rats of the same age, and their levels were further elevated in aged rats. The transcriptional analysis revealed a number of critical molecules, including genes in the cGAS-STING pathway.

Conclusions Our study provides critical insights into the progression of DKD and identifies the cGAS-STING pathway as an essential contributor to disease development.

A 12-11 Pathophysiology of the kidney in a mouse model lacking both isoforms of claudin-10

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Aim: Claudin-10 (*Cldn10*) has significance in nephrology due to its function in chloride and sodium transport within different nephron segments. While previous studies investigated the individual Claudin-10 isoforms (Claudin-10a and Claudin-10b), a knowledge gap is the effect of the absence of both isoforms. Biallelic mutations in *CLDN10* cause HELIX syndrome in humans, while monoallelic mutations are associated with an increased risk of chronic kidney disease. Since patient mutations typically affect Claudin-10b or both isoforms, we investigated the renal phenotype by comparing mice deficient in both isoforms (*Pax8-Cre cKO*) or only in Claudin-10b (*Ksp-Cre cKO*).

Methods & Results: We employed Alizarin Red staining to visualize nephrocalcinosis and RNAscope analysis of mouse kidney sections to identify regional expression of damage markers. Both cKO models were viable and showed nephrocalcinosis (3 replicates); *Ksp-Cre cKO* displayed a "two-line" calcification pattern, while *Pax8-Cre cKO* presented a unique "one-line" pattern, whereas no calcification was observed in heterozygous mice. *Cldn10* expression (replicates =2) was lost in all nephron segments (*Pax8-Cre cKO*) or only in the distal nephron (*Ksp-Cre cKO*). In contrast, *Cldn2* and *Cldn12* expression remained unchanged. Among injury associated markers, *Gpnmb* was significantly upregulated around areas of calcification in *Pax8-Cre cKO* kidneys, while *Aoc1* and *Lcn2* also showed increased expression in the medulla.

Conclusions: These results contribute to understanding distinct nephrocalcinosis patterns in Claudin-10 deficient mouse models and identify damage markers. Although heterozygous mice did not show early calcifications, it may be necessary to look at aged animals to detect an increased risk for kidney injury.

A 12-12 podocytotic evs influence tubular metabolism and redox state

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Aim

Extracellular vesicles (EVs) are nanoparticles that play an important role in cell-cell communication by carrying RNA, DNA and proteins through extracellular fluids. Under pathological conditions, adjustment of the quantity of released EVs and their cargo was reported. The proximal tubular epithelial cells are strictly aerobic, but during kidney disease they start to perform glycolysis. Whether podocytotic EVs may influence this metabolic switch and thus the progression of kidney disease is yet unknown.

Methods

We collected the EVs from conditionally immortalized podocytes, in which we mimicked different glomerular diseases by applying different stressors: cold stress (6h/4°C/SCS) followed by warm reperfusion (24h/37°C/Rep), inflammation (48h/LPS), nephrotic syndrome (48h/PAN) and high glucose (48h/300mg/dL Glu). EVs were harvested by dual centrifugation (900xG, 3000xG) and size exclusion chromatography. EVs were characterized using Amnis flow cytometry and Cryo transmission electron microcopy. Starved HK2 cells were treated with the obtained EVs for 16h. Changes in the metabolism and redox status was analyzed using resazurin reduction assay, seahorse metabolic flux analysis, fluorescence lifetime imaging, qPCR and Western Blot.

Results

The EVs surface marker analysis revealed a clustering of the EVs in the different groups. Glucose-treated EVs correlated with LPS, indicating some kind of inflammatory response in these groups. Surprisingly, HK2 cells treated with EVs from healthy control podocytes showed a significantly lower capability to reduce resazurin after 16 hours of incubation.

Outlook

13C-isotopic labelling of proteogenic lysine will also be used to investigate which proteins enter the HK2 cells that can facilitate these metabolic changes.

A 12-13 Heterogeneity in lysosomal dynamics and metabolic functions along the kidney proximal tubule

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Aim

The kidney proximal tubule transports and processes metabolites to conserve valuable nutrients and maintain body homeostasis. Lysosomes play a key role in intracellular metabolite digestion and lysosomal damage is increasingly recognized as an important cause of kidney diseases. Elucidating the dynamic behavior of renal lysosomes in the proximal tubule is thus integral to deciphering the metabolic consequences of lysosomal dysfunction.

Methods & Results

Here, using targeted sensors, live cell and intravital imaging, we have tracked the behavior of acidified lysosomes along the mouse proximal tubule and uncovered the existence of two distinct populations. In the early part, cathepsin-rich lysosomes frequently fuse and divide with apical endosomes to receive and degrade filtered plasma proteins, respectively. Conversely, in the later region, lipase-containing lysosomes traverse across cells to mobilize and degrade mitochondria-associated lipid droplets and facilitate extrusion of their contents into the tubular lumen. Acutely de-acidifying lysosomes dramatically alters their dynamics, causing major changes in tubular protein and lipid processing.

Conclusions

Thus, proximal tubules exhibit striking axial heterogeneity in lysosomal distribution, characteristics and organellar interactions, which may be important for understanding the origins of metabolic phenotypes frequently arising in disease states.

A 13 | UTERUS AND PLACENTA PHYSIOLOGY

A 13-01 Distinguishing fetal from maternal macrophages to understand their role in human placental vascular development

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Aim

The placenta supports fetal development in pregnancy, where its branched villous structure containing fetal blood vessels provides critical exchange of nutrients and oxygen between the maternal and fetal circulations. Impaired placental vascular development is linked to fetal growth restriction (FGR). Hofbauer cells (fetal-derived macrophages) contribute to this vascular development, but their study has been limited by contamination from maternal macrophages in placental isolates. This study aimed to determine whether incorporating MHC class I markers into high-dimensional flow cytometry could distinguish maternal and fetal macrophage populations for accurate phenotyping.

Methods & Results

Placentae from normal pregnancies (n=10) were processed by a) washing maternal blood from tissue and b) enzymatic digestion of villous cores. A 20-colour flow cytometry panel was developed, including HLA-A2, HLA-A3, and HLA-B7 serotypes alongside immune, endothelial and trophoblast markers. Serotype markers allowed identification of HLA mismatches (instances where maternal and fetal cells have different allele expressions of these markers) between maternal and fetal cells. HLA mismatches were detected in 80% (8/10) of samples, validating the use of this technique and enabling exclusion of maternal macrophages from Hofbauer cell analysis. Varying degrees of contamination were evident, with 0.33-12.67% of myeloid cells in villous digests of maternal origin (n=4).

Conclusions

Incorporating MHC class I markers into our flow cytometry panel allowed reliable discrimination of maternal and fetal macrophages in placental samples. This enables more accurate phenotyping of Hofbauer cells, paving the way for improved

understanding of their role in vascular development in both healthy and FGR-affected placentae.

A 13-02 Effects of Rotenone Exposure on Placental Development and Related Protein Expression in Rats During Pregnancy

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Aim

To investigate the effects of rotenone (ROT) exposure during pregnancy on placental development and cell cycle-related protein expression levels in rats.

Methods & Results

SD rats were cage with male: female = 1:3 ratio. Pregnant rats were randomly divided into control group (Con), vegetable Oil group (Oil) and rotenone group (oil +2mg/kg ROT). At 20 days of gestation, the drug was given by gavage, and the placental blood flow was detected by Doppler color ultrasound. The placental permeability was detected by intravenous injection of Evans blue tail. Tissue and serum estradiol (E3) were detected by ELISA. The expression levels of C-myc, Cyclin D2 and other proteins were detected by Western blotting. The results showed that the weight of pregnant rats in ROT group increased slowly, the weight of placenta decreased and the weight of fetus decreased. Doppler color ultrasonography showed that peak systolic blood flow velocity, end-diastolic blood flow velocity (S/D), resistance index (RI) and fluctuation index (PI) of umbilical artery spectrum increased in Rot group ($P<0.01$). The content of Evans blue in Rot group was increased ($P<0.01$). The serum and tissue E3 levels were decreased ($P<0.01$) and INF-γ levels were increased ($P<0.01$) in Rot group. WB showed that the expressions of C-myc, Cyclin D2 and MMP9 in ROT group were all decreased ($P<0.01$).

Conclusions

Rotenone can induce inflammatory injury of rat placental tissue through oxidative stress, and lead to down-regulation of cyclin expression.

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A 13-03 Endocrine-disrupting compounds and their impact on human placental function: Evidence from placenta organ-on-chip studies

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Introduction: The placenta serves a critical function in xenobiotic protection, nutrient transport, and immunoregulation. The adverse effects of endocrine-disrupting compounds (EDCs) in pregnancy have been well documented, especially those targeting the placenta leading to adverse pregnancy outcomes. However, reliable models to evaluate physiologically disrupting mechanisms by EDCs are limited.

Methods: In this study, an advanced human placenta organ-on-chip (2TPLA-OOC) modeling the second trimester was created by photo and soft-lithography, which subsequently utilized gestational cells to mimic the placenta *in utero*. Cellular markers, cytotoxicity, and cellularity, and endocrine factors were measured. Four classical EDCs (i.e., bisphenol A [BPA], bisphenol S [BPS], and polybrominated

diphenyl ethers [PBDE]-47 and -99) were added to the placenta vessel chamber, and their effects at a concentration of 150 ng/mL were evaluated via measurements of antioxidant capacity, cell signaling activation, cytokine and hormone production, immune cell movement, apoptosis/necrosis, and glucose transport. **Results:** Gestational cells (decidua, placental vessel, cytotrophoblast, syncytiotrophoblast, placental stroma, umbilical vein endothelium, outlet) on chip maintained their morphology, cell-specific markers, production of endocrine factors, and viability for up to 72 hours. The EDCs induced differential, cell specific responses; overall glucose transport however was not perturbed post-exposure to these compounds.

Conclusions: Overall, concentrations of EDCs in maternal biological compartments associated with adverse pregnancy outcomes are unlikely to disrupt placental function. Different placental cells exhibit unique pathophysiologies; but compensatory mechanisms may exist due to intercellular interactions leading to overall refractoriness to exposures. Future studies may utilize this device for other potential toxicological studies.

A 13-04 Establishment and comparison of human-term placenta-derived trophoblast cells

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INTRODUCTION: Choriocarcinoma-derived cell lines like BeWo are widely used in placental research, but their physiological relevance is limited. This study aimed to generate, immortalize, and characterize primary placental trophoblast cells (PTCs) to provide a more accurate model for placental function.

METHODS: Term placental cells were isolated via enzymatic digestion, Percoll gradient separation, and immunomagnetic purification, yielding HLA-ABC(–) PTCs. These were immortalized using SV40T transfection to form cytotrophoblasts ($hPTC^{CTB}$) and differentiated into syncytiotrophoblasts ($hPTC^{STB}$) using forskolin. Transcriptomic differences were assessed via RNA sequencing and principal component analysis (PCA), comparing hPTCCTB with primary PTCs (pPTC), BeWo, and commercial PTCs. Marker expression was evaluated by immunocytochemistry, and functional responses to LPS and cigarette smoke extract were analyzed through NF-κB and p38MAPK activation.

RESULTS: PCA showed that $hPTC^{CTB}$ clustered with pPTC and commercial PTCs, distinctly separated from BeWo. Both $hPTC^{CTB}$ and pPTC displayed similar epithelial morphology and marker expression (cytokeratin-7, E-cadherin, GATA3). Upon differentiation, $hPTC^{STB}$ expressed syncytial markers GCM1 and CGB. Functional assays showed comparable p38-MAPK and p-NFκB activation. Genes related to adhesion, basement membrane, MAPK, and TLR signaling were upregulated in $hPTC^{CTB}$ versus BeWo.

CONCLUSION: The term placenta is a viable source of functional trophoblasts. Immortalized $hPTC^{CTB}$ provide a reliable and physiologically relevant model for placental studies, offering significant advantages over BeWo cells in mimicking true placental biology.

A 13-05 TGF-β1 disrupts primary ciliary prostaglandin E2-mediated uterine receptivity

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Aim

Infertility affects one in six individuals globally, with endometriosis contributing to 25–50% of female infertility. However, the underlying mechanism of endometriosis-associated infertility remains to be investigated.

Methods & Results

By performing immunofluorescence staining of endometria, we found that women who failed to conceive after in vitro fertilization-embryo transfer (IVF-ET) exhibited significantly fewer and shorter primary cilia in the endometrium compared to those who achieved pregnancy, suggesting a link between ciliogenesis and uterine receptivity. Decidualization of endometrial stromal cells (ESCs) is crucial for implantation, and we identified primary cilia as essential regulators of this process via prostaglandin E2 (PGE_2) signaling. The E-type prostanoid receptor 4 (EP4) localizes to primary cilia, where it activates the cAMP/PKA/CREB pathway and upregulates the decidualization marker IGFBP1 in response to PGE_2. We further demonstrate that TGF-β1, elevated in women with endometriosis, suppresses ciliogenesis by downregulating COUP-TFII and KIF3B, impairing PGE_2-mediated decidualization. Knockdown of COUP-TFII or KIF3B disrupts primary cilia formation and reduces decidualization markers. In vivo, intrauterine administration of TGF-β1 in mice diminishes ciliogenesis, impairs decidualization, and lowers pregnancy rates, whereas blocking TGF-β1 signaling restores these processes.

Conclusions

These findings highlight the critical role of primary cilia in uterine receptivity and reveal that TGF-β1-mediated ciliogenesis defects contribute to endometriosis-associated infertility. Targeting TGF-β1 signaling may be a promising therapeutic strategy to improve implantation success in women with endometriosis.

A 13-06 Gestational exposure to *Citrus limon* juice and its bioactive components reduce placental lipid metabolism and efficiency in Wistar rats

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Aim

Lipids are essential in pregnancy homeostasis. *Citrus limon* juice (CLJ) and its bioactive components; oleic (OA), palmitic (PA), and stearic acids (SA) are documented hypolipidemic agents. The relationship between these hypolipidemic agents on placental and foetal outcomes was investigated

Methods & Results

Twenty-five pregnant female Wistar rats (100-120g) assigned in to five groups (n=5) were used in the study: Group 1 (control) received distilled water (1.00 mL/kg), Group 2,3,4,and 5 received CLJ (1.00 mL/kg), OA (0.31 mL/kg), PA (0.26 mL/kg) and SA (0.19 mL/kg) respectively orally on gestation days 1-20. Foetal crown-rump length, abdominal circumference, placental; thickness, lipid profile, Small Neutral Amino Acids (SNAAT-1), Glucose Transporter (GLUT-1), Fatty Acid Transport Protein (FATP-1), Peroxisome Proliferator-Activated Receptor Gamma (PPAR-γ), Leptin Receptor (LEPR) and Insulin Receptor Substrate-1 (IRS-1) were assessed. Data were analysed using ANOVA at $p<0.05$. Crown-rump length was reduced in CLJ, OA and SA groups. Abdominal circumference was reduced in all groups. Placental thickness increased in the CLJ group. Lipid profile was reduced in all groups compared with control. SNAAT-1, FATP-1 and GLUT-1 were reduced in CLJ, OA and SA groups. Expression of PPAR-γ and LEPR reduced in CLJ, OA and SA groups while IRS-1 was increased in CLJ and SA when compared to the control rats.

Conclusions

It was deduced from the study that maternal *Citrus limon* juice administration reduced placental nutrient transporters and efficiency, thereby reducing foetal morphometric indices. This effect is linked to the oleic and stearic acids constituents of the juice.

A 13-07 One Health Perspectives on Calcium Carbide-Ripened Banana Consumption during Pregnancy: Potential Risks for Infertility in Female Offspring of Rats

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Aim

[The use of commercial-grade calcium carbide (CaC_2) for artificial fruit ripening poses a public health riskdue to its impurities, including arsine, which has been linked to low birth weight and fetal loss. With the growing reliance on artificial ripening methods due to changing agricultural patterns, this study examines the potential effects of maternal consumption of CaC_2-ripened banana pulp on offspring development through a One Health approach, integrating human, animal, and environmental health perspectives.]

Methods & Results

[Sixteen pregnant rats were divided into two test groups and two control groups. Test groups received pelletized feed mixed with CaC_2-ripened banana pulp (50g/5kg and 100g/5kg), while controls received either naturally ripened banana pulp or pelletized feed alone. Feeding continued ad libitum throughout pregnancy. At birth, male offspring were separated, and each dam nursed eight female pups. After weaning, reproductive development was assessed using vaginal opening day (VOD), hormonal assays, and fertility tests at sixth week.Trace amounts of arsenic (0.35 ppb) were detected in the 100g/5kg CaC_2 group, correlating with delayed puberty, decreased serum follicle-stimulating hormone (FSH), and reduced fertility rates ($p<0.05$)]

Conclusions

[These findings suggest that exposure to CaC_2 contaminants through food may disrupt reproductive health, with potential transgenerational effects. From a One Health and climate perspective, the increasing use of artificial ripening agents raises concerns about food safety, environmental contamination, and human health, emphasizing the need for stricter regulations and sustainable agricultural practices.]

A 13-08 Oral Supplementation of Gum Arabic Ameliorates Obesity and Ovarian Oxidative-Nitrosative Stress in Female Rats with Cafeteria Diet-Induced Obesity

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Aim

This study aimed to explore the effects of Gum Arabic on reproductive outcomes in female rats with obesity induced by a western diet.

Methods & Results

Female Westar rats, weighing 120-130 grams, are housed in standard cages and treated in compliance with the guidelines for the care and use of animals. This study was approved by the IRB (Sudan). The Control group (n=10) was fed standard rodent chow diet. The Obese group (n=20) was fed a cafeteria diet for 8 weeks. Body weight was recorded weekly. By the end of the 8th week, 10 obese rats were randomly selected (a 20% increase in initial weight was used as the obesity threshold). These rats were administered 10% Gum Arabic dissolved in tap water (100 g/l). Food intake, weight gain, (GTT), and (ITT) results were recorded every 2 weeks, and serum lipid profile analysis was performed. The rats were then placed in mating cages . The mating, fertility, and fecundity indices were calculated. The number of live pups and their body weights were recorded. In the first estrous phase, the rats were anesthetized and dissected. Ovaries were removed for antioxidant and nitric oxide measurements.The Gum Arabic group demonstrated a statistically significant enhancement in ovarian oxidative capacity and reproductive outcomes.

Conclusion Oral administration of Gum Arabic improved reproductive outcomes disrupted by obesity induced by a Western diet, primarily through its antioxidant effects on ovarian tissues, which were linked to weight reduction and improvements in glucose intolerance, insulin resistance, and lipid profile.

A 13-09 Climacteric symptoms during mid reproductive age-group among women in Lagos, Nigeria: a focus on perimenopause

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Aim

Study assessed prevalence and determinants of climacteric symptoms in women aged 35 – 60 years residing in Lagos, Nigeria. Study also investigated interplay between the levels of reproductive and stress hormones with established perimenopausal symptoms in these women.

Methods & Results

Study questionnaires were distributed to women aged 35 – 60 years attending primary healthcare centres in Lagos metropolis to document perimenopausal symptoms using the menopausal rating scale (MRS). Women were categorized as premenopausal, perimenopausal or postmenopausal using the Stages of Reproductive Aging Workshop (STRAW) criteria. Blood sample to assay follicle stimulating hormone (FSH), luteinizing hormone (LH), progesterone, estrogen was collected from the participants. Severity of perimenopausal symptoms across the 3 groups was related using Kruskal–Wallis test and multiple linear regression analysis was used to compare significant variables obtained from bivariate analyses. A total of 304 women were recruited for the study, 193 (63.48%) were premenopausal, 63 (20.72%) perimenopausal, while 48 (15.79%) were postmenopausal. Menopausal Rating Scale (MRS) score ranged from mild to severe in 60.3% of premenopausal, 85.7% of perimenopausal and 91.6% of postmenopausal women. MRS score was significantly higher ($p<0.001$) in perimenopausal and postmenopausal women compared to premenopausal women. Hypertension, tribe, and memory problems were all significantly associated ($p<0.05$) with higher MRS score.

Conclusions

Climacteric symptoms were highly prevalent in perimenopausal women residing in Lagos with higher comorbid disorders like hypertension and memory problems increasing the risk. Further studies are needed to determine the mechanisms involved and identify other determinants of these symptoms.

A 13-10 The PEPP Study: Pregnancy's Effect on Physical fitness and Pain

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Background: During pregnancy, the body undergoes significant changes in circulation and hormone levels. Alterations in these factors can impact a woman's physical performance, but research on the effects of pregnancy on skeletal muscle and pain threshold is lacking. A deeper understanding of the changes in the female body before and during pregnancy can optimize health recommendations. **Aim:** To investigate pregnancy's effect on physical fitness in terms of muscle size, strength, and VO2 max, as well as physical activity levels and pain perception before anpregnancy.

Methods: This is an observational study: 20 women are examined before, during and after pregnancy to investigate the changes that pregnancy induces in a woman's body. Assessments include questionnaires regarding physical activity levels (IPAQ) and quality of life (SF-36), pain threshold and tolerance testing, and physical fitness tests: measurements of muscle size in the hand, finger grip, handgrip, and elbow flexor strength, estimations of VO2max, and blood samples, measuring sex hormones, lipid profile and more. Additionally, physical activity levels are measured over a week, using an accelerometer. The pregnant woman is compared with herself before pregnancy and a control group (20 women).

Results: The study is ongoing (recruitment began December 2024). We expect to present results comparing prepregnancy and 1st trimester at IUPS 2025.

Perspectives: This study aims to contribute to the foundation for recommendations on physical activity during pregnancy and to generate more knowledge in this field to promote well-being, welfare, and health for pregnant women.

A 13-11 Linking Serum Ferritin and Hepcidin to Glucose Dysregulation in Pregnancy

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The author has objected to a publication of the abstract.

A 14 | VASCULAR ENDOTHELIUM

A 14-01 Microparticles and cGMP as Biomarkers of Endothelial Dysfunction and Cardiovascular Risk in Shift Work Employees: From Circadian Disruption to Inflammation

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Aim

This study aims to determine of microparticle endothel and cGMP as a sign of endothelial dysfunction in shift workers.

Methods & Results

This study used a comparative repeated cross-sectional design of female workers in hospitals who worked shifts and non-shifts. Data collection was carried out from June to December 2021 and obtained 272 female workers in the city and district of Bandung. Based on the inclusion criteria, there were 64 shift workers and 54 non-shift workers. Characteristics asseessed were not different based on age, nutritional status, blood pressure, physical activity, history of smoking, family planning history, cholesterol, blood sugar, triglycerides, erythrocyte sedimentation rate, PSQI score, DASS score, and nutritional intake. Statistical analysis to distinguish the two groups shift and non-shift was using the Mann Whitney test.

The results showed that there was a significant difference between shift workers and non-shift workers in resistin levels p = 0.004 (1.78 ng/mL vs. 3.33 ng/mL), leukocyte count (p = 0.005; 7182 µL vs. 5900 µL), monocyte count (p = 0.016; 475.45 µL vs. 371.98 µL), the number of CD31+ (p < 0.001; 588.68 u/L vs. 286.44 u/L), the number of CD31+CD62e+ (p = 0.031; 236.16 u/L vs. 185.58 u/L), and levels of cGMP (p <0.01; 8.97 pmol/mL vs. 23.92 pmol/mL).

Conclusions

It was concluded there were the occurrence of endothelial dysfunction which is characterized by a decrease in GMFs and an increase in the number of CD31+ in shift workers.

A 14-02 Protective Effects of Physical Activity on Endothelial Function in Healthy Adults: Associations with Endothelial Progenitor Cells, Endothelial Microparticles, and Flow-Mediated Dilation

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The author has objected to a publication of the abstract.

A 14-03 The inflammation-regulated microprotein miP-PSTPIP2 modulates endothelial cell proliferation and endocytosis

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Non-canonical microproteins (miPs), encoded by small open reading frames (smORFs) and consisting of 100 or fewer amino acids, are expressed in human endothelial cells. However, their function remains largely unexplored. This study characterized the function of miP-PSTPIP2, a novel 46-amino-acid endothelial cell miP encoded by a smORF within the coding sequence of the proline-serine-threonine phosphatase interacting protein 2 (PSTPIP2) transcript, albeit in a different reading frame. A custom antibody enabled localization of miP-PSTPIP2 to actin-rich membrane domains, cytoskeleton, cytosolic vesicles, and the nucleus. Expression of miP-PSTPIP2 was increased in interleukin (IL)-1β-treated endothelial cells and inflammation-activated murine carotid arteries (i.e., ligated carotid arteries from atherosclerosis-prone apolipoprotein E-deficient mice). Additionally, miP-PSTPIP2 was detected in the endothelium of carotid arteries from patients with atherosclerosis. Immunoprecipitation combined with mass spectrometry identified the interaction between miP-PSTPIP2 and caveolar proteins, proteins involved in cytoskeletal regulation, intracellular transport, clathrin adaptor activity, as well as nuclear proteins involved in DNA repair and core components of paraspeckles. Additionally, miP-PSTPIP2 co-localized with clathrin heavy chain and AP2 adaptor complex. Adenovirus-mediated overexpression of miP-PSTPIP2 resulted in a significant reduction in the expression of genes associated with cell cycle progression and decreased endothelial cell proliferation. Endocytosis and uptake of transferrin and low-density lipoprotein were increased in miP-PSTPIP2 overexpressing cells. Furthermore, miP-PSTPIP2 overexpression impaired endothelial cell activation, as evidenced by the downregulation of IL-1β expression and reduced monocyte adhesion. These findings indicate that miP-PSTPIP2 is an IL-1β-regulated miP that modulates endothelial cell function through its involvement in intracellular transport and transcriptional regulation.

A 14-04 Identification of long non-coding RNAs that control endothelial regeneration

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The author has objected to a publication of the abstract.

A 14-05 Uncovering the role of two nuclear endothelial cell microproteins encoded by "non-coding" RNAs

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Aim:

Microproteins (miPs) encoded by long non-coding RNAs (lncRNAs) are an emerging class of molecules with potential roles in vascular biology. However, their function remains largely unknown. This study focuses on the characterization of two human endothelial cell miPs: a 71 amino acid miP encoded by the lncRNA NEAT1, i.e miP-NEAT1, and a 29 amino acid miP encoded by the uncharacterized lncRNA

SERTAD4-AS1, i.e miP-SERTAD4-AS1, whose expression was downregulated in inflammatory conditions.

Methodes & Results:

To elucidate their function in human endothelial cells, gain-of-function (via adenovirus-mediated overexpression of FLAG-tagged miP) as well as loss-of-function approaches (using CRISPR/Cas9-mediated knockout) were combined with transcriptomic, proteomic, and functional assays. Both miPs localized predominantly to the endothelial cell nucleus (FLAG immunofluorescence). FLAG-miP immunoprecipitates from nuclear extracts of human endothelial cells were analysed by mass spectrometry to assess the interactome of each miP. These analyses identified interactions with proteins involved in numerous nuclear processes, including transcriptional regulation. MiP-NEAT1 partially co-localized with the promyelocytic leukaemia protein and its overexpression led to the disruption of promyelocytic leukaemia nuclear bodies. miP-SERTAD4-AS1 co-localized with the key paraspeckle-associated protein Non-POU domain-containing octamer-binding protein. Transcriptome analysis of miP-SERTAD4-AS1 overexpressing cells revealed marked alterations in genes associated with inflammation and cell cycle regulation. Functionally, cell proliferation and migration were decreased in miP-SERTAD4-AS1 overexpressing endothelial cells, and increased following the lncRNA SERTAD4-AS1 knockout.

Conclusion:

These results suggest that miP-NEAT1 and miP-SERTAD4-AS1 play distinct roles in the endothelial cell nucleus and regulate nuclear body organization, transcriptional activity, and endothelial function.

A 14-06 Novel GPCR in the lung endothelium regulates VEGF-mediated angiogenic processes.

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Introduction

Respiratory diseases account for ~10% of European hospital admissions. Angiogenesis is implicated in various lung pathologies, notably pulmonary hypertension, and cancer, but also conditions including COPD and pulmonary fibrosis. The bitter taste receptor T2R14 is expressed in the lung microvascular endothelium, and its agonists disrupt endothelial barrier function, however, there are no studies which investigate the impact of T2R14 on angiogenic processes.

Aim

To investigate the role of the novel T2R14 antagonist, LW129, on angiogenic processes within primary human pulmonary microvascular endothelial cells (HPMECs).

Methods & Results

Primary HPMECs were treated with varying concentrations of LW129. Cell viability was assessed using MTT and proliferation assays. Angiogenic processes were evaluated via tube formation assays (Matrigel™) and cell migration assays. The impact of LW129, in the presence and absence of VEGF, was examined. MTT assays revealed LW129 toxicity at concentrations above 10 μM, likely due to off-target effects. At concentrations from 1 nM -1 μM, LW129 increased HPMEC viability which was largely mirrored by proliferation assay findings. Both tube formation and cell migration assays showed no significant impact of LW129 alone, however, the antagonist significantly reduced VEGF-induced tube formation and migration.

Conclusions

While LW129 increases HPMEC proliferation at low concentrations, it does not significantly promote angiogenesis. Furthermore, it appears to attenuate VEGF-mediated angiogenic responses. These findings suggest a role for T2R14 in regulating angiogenesis in the lung microvasculature and indicate a potential role for antagonists for pro-angiogenesis pathologies.

A 14-07 SECS, drugs and Rac1&Rho: regulation of EnNaC in vascular endothelial cells

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The endothelial ENaC (EnNaC) plays a pivotal role in regulating the biomechanical properties of the endothelial cell surface, including its responsiveness to hemodynamic shear stress, thereby contributing to vascular homeostasis. A strong correlation exists between EnNaC surface expression, the mechanical behavior of the cortical actin cytoskeleton, and nitric oxide bioavailability, suggesting an intrinsic structure-function coupling. Mechanical flexibility of the endothelial surface has been associated with proper vascular function while chronic stiffening leads to endothelial dysfunction and the so-called 'Stiff Endothelial Cell Syndrome' (SECS).

We investigated the underlying cellular mechanisms and signaling pathways of EnNaC-dependent endothelial behavior by using atomic force microscopy (AFM)-based nanoindentation and immunofluorescence staining *in vitro* and *ex vivo*.

We were able to show that the interplay between EnNaC and the cortical cytoskeleton is mediated by the small GTPases RhoA and Rac1. Inhibition of Rac1 (NSC23766) or RhoA (CT04) led to a significant reduction of cortical stiffness by -14.5 ± 0.7% and by -13.0 ± 0.6%, respectively and directly impacted on EnNaC membrane abundance (RhoA-inhibition: -35.6±0.2%, Rac1-inhibtion: 23.9±0.2%). The functional inhibition of EnNaC by benzamil led to membrane removal of the channel by clathrin-mediated endocytosis within minutes (-8.2±0.4%). Furthermore, we could show the involvement of SGK1 and Nedd4-2 in the regulation of EnNaC membrane insertion/retrieval and endothelial nanomechanics.

Our study provides additional insights into the intricate regulation of EnNaC expression and activity and elucidates its interplay with the actin cytoskeleton - highlighting this interface as a critical modulator of vascular homeostasis under both physiological and pathophysiological conditions.

A 14-08 Endothelial cytochrome P450 reductase-derived cholesterol limits angiogenesis

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The cytochrome-P450 reductase (POR)/ CYP51 monoxygenase is important for sterol synthesis. Cholesterol is an key membrane constituent and is involved in cell signaling. Cellular cholesterol is determined by uptake and de novo synthesis. High-circulating cholesterol is linked to cardiovascular diseases but the role of endogenous cholesterol synthesis for endothelial function is unknown and was studied here. To inhibit cholesterol synthesis in endothelial cells, POR and CYP51 CRISPR-knockout was performed in human aortic endothelial cells (HAEC) and in human umbilical vein endothelial cells (HUVEC). Furthermore, an endothelial-specific tamoxifen-inducible POR knockout mouse (ecPOR$^{-/-}$) was generated. Knockout of POR and CYP51 in HAEC led to an accumulation of the CYP51 substrate lanosterol, whereas its product, desmosterol was reduced. Loss of endogenous cholesterol synthesis was linked to an increased basal and VEGF-stimulated angiogenic sprouting in HUVEC. Similarly, endothelial-sprouting from aortic segments was increased in ecPOR$^{-/-}$ mice as compared to control mice. Importantly, increased angiogenesis was also observed *in vivo* in retina of ecPOR$^{-/-}$ mice. Cholesterol levels are sensed by the SREBP2 (sterol regulatory element-binding proteins) system, and indeed, SREBP2 activation was increased after deletion of POR in cells and *in vivo* (*en face* of aorta). Overexpression of the active SREBP2 in cells increased angiogenesis akin to the knockout of POR. RNAseq of POR$^{-/-}$ HAEC showed significant upregulation of cholesterol

related genes LRP1, VLDLR and ABCG1, as well as pro-angiogenic genes such as VEGFA and ADM2. Altogether, inhibition of endothelial POR/CYP51 impairs endogenous cholesterol production which correlates with the transcription of genes that promote angiogenesis.

A 14-09 NFAT5-controlled heat shock protein expression limits senescence in hypoxia-exposed lung endothelial cells

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Chronic hypoxia causes detrimental structural alterations in the lung, which are partially evoked by stress responses of the endothelium. In this context, *in vitro* analyses revealed that hypoxia-exposed murine lung endothelial cells (MLEC) activate nuclear factor of activated T-cells 5 (NFAT5/TonEB) - a transcription factor that adjusts the cellular transcriptome to cope with multiple environmental stressors. However, *Nfat5*-deficient MLEC reinforced energy- and protein-metabolism-associated gene expression under normobaric hypoxia (10% O_2) for seven days as evidenced by microarray- and scRNA-seq-based analyses. We identified *Hspa1a* and *Hspa1b* as transcriptional targets of NFAT5 in hypoxic MLEC encoding subunits of heat shock protein 70 (HSP70). Considering the important role of HSP70 protein, we assumed that shortage of HSP70 as observed in *Nfat5*-deficient MLEC impairs their capability to adequately cope with hypoxia. In fact, hypoxia-exposed *Nfat5*-deficient (vs. control) MLEC failed to increase HSP70 levels. Subsequent experiments exposing *Hspa1a*-deficient MLEC to hypoxia showed increased expression of cellular senescence markers including *Cdkn2a*, *Tert1*, and *H2afx* (vs. control). We also observed a decline in *p53* expression and of its downstream target *Mdm2*, suggesting a dysregulated stress response pathway under these conditions. Moreover, besides senescence-associated morphological features, β-galactosidase activity was significantly elevated in *Hspa1a*-deficient MLEC. Collectively, we identified *Nfat5*-regulated HSP70 and specifically *Hspa1a* as a protective mechanism that supports adequate cellular adaptation to hypoxia and prevents premature senescence. It thus may serve as a crucial determinant for preserving the function of the lung endothelium in a hypoxic environment as caused by ventilation disorders or other chronic obstructive pulmonary diseases.

A 14-10 Bradykinin-mediated actin cytoskeleton rearrangement drives endothelial barrier disruption

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Aim

The actin cytoskeleton is crucial for maintaining endothelial barrier function, remaining cortically oriented under normal conditions. During barrier disruption, increased calcium levels and modulation of small Rho GTPases trigger radial reorientation, leading to stress fiber formation and increased tension on cell-cell junctions. During angioedema, the release of vasoactive substances is enhanced. In hereditary angioedema, a genetic defect in the SERPING1 gene reduces C1 esterase inhibitor function, resulting in increased bradykinin release. However, the effect of bradykinin on the actin cytoskeleton is still largely unclear.

Methods

Human umbilical vein endothelial cells were cultured on semipermeable filters, and bradykinin was added to assess its effect on barrier function. Impact was measured by transendothelial electrical resistance and permeability, with actin cytoskeleton orientation analyzed by phalloidin staining. The role of calcium signaling and small Rho GTPases was explored through pharmacological modulation.

Results

Bradykinin significantly increased endothelial permeability, accompanied by actin

cytoskeleton reorganization and stress fiber formation. Pharmacological modulation of calcium and small Rho GTPases inhibited these effects, reducing permeability.

Conclusions

Bradykinin induces increased stress fiber formation in the actin cytoskeleton through activation of the calcium signaling pathway and disruption of small Rho GTPase homeostasis. This represents a key aspect of bradykinin-mediated endothelial barrier dysfunction. In the future, this could play a crucial role in the development of new therapeutics.

Disclosures

This study was supported by the Basic Clinician Scientist Program of the Medical Faculty (University of Ulm) (CASCADE 1.0).

A 14-11 SHP-2 inactivation disrupts endothelial barrier integrity under inflammatory conditions via downregulation of tight junction protein expression

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Aim

In a previous study, we demonstrated that the tyrosine phosphatase SHP-2 negatively regulates inflammatory endothelial cell (EC) activation via interaction with the adaptor molecule MyD88 at Y257, thereby preventing downstream signalling. Moreover, inflammation inactivated SHP-2 resulting in increased vascular permeability *in vivo*. Here, we investigated the underlying mechanisms *in vitro*.

Methods & Results

Wild type (WT), dominant negative (CS), constitutively active SHP-2 (E76A), MyD88 WT or MyD88 with a mutation of Y257 (Y257F) were over-expressed in human umbilical vein endothelial cells (HUVEC). Endothelial permeability *in vitro* was measured by electric cell-substrate impedance sensing and immunofluorescent staining of VE-Cadherin. Expression of tight junctional (TJ) proteins were assessed by western blot and qRT-PCR.

SHP-2 CS overexpression for 48h resulted in enhanced basal and IL1β mediated EC permeability compared to SHP-2 WT (all $p<0.05$, n=5), whereas constitutively active SHP-2 EA maintained the barrier integrity (n=5). This was confirmed by VE-Cadherin staining (n=5). Moreover, SHP-2 CS cells showed reduced expression of TJ proteins (claudin-5, occluding, ZO-1) compared to SHP-2 WT (WB: all $p<0.05$, n=8-10; qRT-PCR: all $p<0.05$, n=4-8). Regarding short-term effects, pharmacological SHP-2 inhibition (SHP099) enhanced IL-1β mediated EC permeability within a few hours ($p<0.05$, n=3). Interestingly, mutation of the SHP-2 binding site (Y257) on MyD88 increased IL-1β mediated EC permeability ($p<0.05$, n=6).

Conclusions

SHP-2 activity maintains EC barrier integrity via interaction with the SH2-binding site on MyD88 and promotes expression of tight junctional proteins. SHP-2 inactivation by inflammatory conditions may thus contribute to the progression of diseases involving vascular leakage.

A 14-12 Gap junctional interaction of endothelial progenitor cells with endothelial cells induces angiogenic network formation *in vitro*

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Aim

Endothelial progenitor cells (EPC) support neovascularization and endothelial repair by incorporation into newly formed or injured vessels and by secreting proangiogenic factors. By forming gap junctions (GJ), connexins (Cx) allow the direct exchange of ions and small molecules between adjacent cells. Here, we studied the interaction of EPC with endothelial cells (EC) and the role of GJ in the formation of capillary-like structures *in vitro.*

Methods & Results

EPC and EC (HUVEC, PAEC, HMEC) were co-cultured and the angiogenic network formation was studied in long-term co-cultures or on Geltrex. The gap junctional

coupling (GJC) was investigated by dye-injection studies and FACS analysis. GJC was inhibited by heptanol combined with meclofenamic acid (GJB) or carbenoxolone (CBX). Cx43 localisation was assessed by immunofluorescence stainings (IF). Angiogenic networks were formed in monocultures of EC and in EC/EPC co-cultures on Geltrex, but remarkably also in co-cultures after 3-6 days on uncoated culture dishes (n=4-7). IF stainings demonstrated a Cx43 localisation in the membrane at contact sites between EC and EPC. FACS analysis and dye-injection studies confirmed a time dependent GJC of EC with EPC (n=4, $p<0.01$). The angiogenic network formation was significantly reduced by inhibition of GJ in EC/EPC co-cultures (n=4-7, $p<0.05$ vs. control).

Conclusions

Our results suggest that the GJC involving Cx43 in co-cultures is necessary for the spontaneous formation of angiogenic networks. Therefore, Cx43 is a potential target for regulating angiogenesis and exploring new cell-based treatment approaches in clinical therapy.

A 15 | ENDOTHELIUM AND SMOOTH MUSCLE

A 15-01 Long non-coding RNA-mediated control of the endothelial proliferation–quiescence switch

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Healthy endothelial cells line the vascular wall and remain in a metabolically active but quiescent (non-proliferative) state to maintain vascular homeostasis. Disruption of quiescence can lead to uncontrolled proliferation and vascular disease. Although quiescence is tightly regulated through distinct transcriptional and epigenetic programs, the molecular mechanisms controlling the endothelial proliferation-quiescence switch remain poorly defined. Recent evidence, including our own, highlights the involvement of long non-coding RNAs (lncRNAs) in governing endothelial state. We hypothesise that endothelial-specific lncRNAs are involved in driving cells into and out of quiescence.

RNA-sequencing of human umbilical vein endothelial cells (HUVECs) identified lncRNAs differentially expressed in quiescence versus proliferation. One of the top quiescence-enriched lncRNAs (60-fold induced) was subsequently found to attenuate angiogenic sprouting. Analysis of publically available single-cell RNA-seq datasets demonstrated a differential expression of quiescence-enriched lncRNAs between vascular disease states. Next steps will include an analysis of chromatin accessibility and transcription factor binding at candidate lncRNA gene loci. RNA, DNA and protein interaction studies using Red-C (RNA ends on DNA capture), CUT&RUN, and pulldown-mass spectrometry experiments will be employed to investigate how lncRNAs influence chromatin state and epigenetic profiles to maintain endothelial quiescence.

We will investigate whether lncRNAs dynamically control the endothelial proliferation-quiescence switch through their maintenance of chromatin accessibility and epigenetic state. This will offer insight into the precise molecular mechanisms of a fundamental cellular process that remains relatively uncharacterised. Targeting lncRNAs in such a process may provide novel therapeutic opportunities in vascular diseases characterised by dysregulated endothelial proliferation.

A 15-02 Exercise alters circadian clock in skeletal muscle and heart endothelial cells

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Aim

Function of all tissues is dependent on appropriate vascularization. Metabolic diseases are associated with capillary rarefaction and endothelial cell (EC) dysfunction, but exercise promotes angiogenesis and metabolic health. However, the role of ECs in exercise benefits is still largely unexplored. Our aim was to study

the effects of exercise on skeletal muscle and heart EC transcriptome and how this relates to the health benefits of exercise.

Methods & Results

We conducted exercise intervention with young male (8-9 weeks, n=4) and aged female (82-104 weeks, n=10) wild-type C57BL/6J mice. Exercise (EXE) groups were trained on a treadmill, two weeks in young and four weeks in aged mice, while sedentary (SED) mice stayed in their cages. Gastrocnemius muscles and hearts were collected for further analyses and ECs and fibroblasts were isolated. ECs from young were used for single-cell RNA sequencing (scRNASeq).

The scRNASeq data revealed that circadian clock genes, especially *Nr1d1* and *DBP*,were upregulated both in heart and skeletal muscle ECs following exercise, and circadian rhythm was one of the enriched pathways. Expression of *DBP* increased in aged muscle ECs and fibroblasts *P*=0.0044 and *P*=0.0210 (t-test), respectively, in the EXE-group. Exercise increased *Nr1d1* in muscle ECs *P*=0.0405, while there was no significant change in fibroblasts. Immunofluorescent staining showed Nr1d1 colocalization with ECs in skeletal muscle, while DBP was associated with oxidative muscle fibers.

Conclusions

Exercise alters circadian clocks in ECs. Nr1d1 seems to be enriched in vasculature and could have a potential role in exercise-induced health benefits in the vasculature.

A 15-03 Establishing a perfusion system for endothelial cells under physiological flow

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Artificial blood in the form of lecithin-modified nanoscale oxygen carriers (LENOX)[1] offers potential as an alternative to erythrocyte concentrates in terms of oxygen transport capacity. This study aimed to investigate the influence of LENOX on endothelial cells under physiological flow as intended use implies intravenous administration.

To evaluate the effect of shear stress and LENOX under flow we set up a perfusion system using an ibidi µ-Slide Luer channel, a fluid reservoir and a peristaltic pump. Murine endothelial cells (C166) were cultured and exposed to shear stress of 2 increasing to 10 dyn/cm^2 over 1 h at 37 °C in an incubator for 2 or 24 h, using either DMEM or 4 % LENOX in DMEM. ATP levels were measured using the Cell Titer Glo assay, with static cultures as control.

ATP measurements showed no difference at baseline before perfusion. After 2 hours, perfused cells (with or without LENOX) showed a slight decrease in RLU compared to static controls. After 24 h of treatment, luminescence increased in all groups compared to the 2 hour values, with static samples showing a 1.8 fold change and perfused cells showing a 1.7 fold increase.

We established a perfusion setup for characterizing endothelial cells under flow. Initial toxicity tests showed no effect of LENOX on cell viability. Slight ATP-decreases under perfusion could be attributed to adaptation to flow-induced shear stress, as metabolism recovered after 24 hours. Future tasks include the investigation of immune markers and endothelial-specific parameters such as VCAM-1.

[1] LENOX (2022) EP-22193221.3

A 15-04 *In vivo* chemogenetic generation of hydrogen peroxide by endothelial cells induces cardiac remodelling and vascular dysfunction

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Aim

Increased level of reactive oxygen species (ROS) results in oxidative stress, a hallmark for cardiovascular diseases. ROS are important signalling molecules,

which impact on the function of enzymes through thiol modification, referred to as redox-signalling. Redox-signalling is central to homeostasis but also to the damage response in the cardiovascular system. However, the dynamics of redox-signalling and its functional contribution has been difficult to study.

Methods & Results

To understand how redox-signalling contributes to cardiovascular homeostasis and damage response, we generated an inducible, endothelial cell-specific knock in mouse expressing a chemogenetic probe based on the yeast D-amino acid oxidase enzyme (ecDAO mouse). DAO generates H_2O_2 as a by-product in the conversion of D-amino acids into their imino acids allowing for manipulation of H_2O_2 production. Mice received D-Alanine (D-Ala, 0.5 M) through drinking water. In a damage model using minimally invasive myocardial infarction (MI), there was no difference between control and ecDAO mice in the outcome of MI. Next, we evaluated the acute and chronic cardiovascular effects of endothelial-derived H_2O_2. Three days of D-Ala had no effect on cardiac function but slightly improved vascular function as demonstrated by an increase in the diameter of the carotid artery *in vivo* and a decreased vessel constriction to phenylephrine. Chronic endothelial generation of H_2O_2 induced cardiac remodelling, associated with an increase in peripheral resistance and overoxidation of peroxiredoxins.

Conclusions

In conclusion, we have generated a functional and inducible, endothelial cell-specific chemogenetic mouse model to manipulate H_2O_2 production. Chronic intracellular production of H_2O_2 by endothelial cells results in cardiac remodelling and vascular dysfunction.

A 15-05 Kynurenine aminotransferases salvage methionine via glutamine transamination in endothelial cells

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Kynurenine aminotransferases (KYAT) are enzymes well known for the transamination of kynurenine to kynurenic acid in tryptophan metabolism. Interestingly, we found that a single nucleotide variant (SNV) in the KYAT3 gene that decreases its expression in human arteries is significantly associated with an increase in plasma levels of imidazole lactate and 2-hydroxy-4(methylthio)butanoic acid (KMBA), the α-keto acids of histidine and methionine respectively. The isoenzymes KYAT1 and KYAT3 transaminate glutamine using α-keto acids as co-substrates to generate α-ketoglutaramate and replenish amino acids. Given that endothelial cells use glutamine at high rates we investigated here the importance of KYAT1/3 for the endothelial metabolome and normal endothelial cell function.

We generated KYAT1/3 double knockouts in primary endothelial cells by CRISPR/cas9 and performed untargeted metabolomics. In fact, methionine was the sixth most downregulated metabolite in KYAT1/3$^{-/-}$ as compared to non-targeted control cells (NTC). Targeted LC-MS/MS confirmed that methionine was indeed decreased whereas KMBA was increased, suggesting that endothelial KYAT1/3 are important for methionine salvage using glutamine. Functionally, deletion of KYAT1/3 decreased protein translation (SUnSET assay) and endothelial sprouting, altered genes related to cell cycle progression and induced endothelial senescence. Moreover, methionine deprivation decreased proliferation in both NTC and KYAT1/3$^{-/-}$ cells but upon supplementation of KMBA (100 µM) proliferation was rescued exclusively in NTC cells. Altogether, we present KYAT1/3 as key enzymes for the methionine salvage pathway that was, so far, not characterized in mammalian cells and is important to maintain normal endothelial cell function.

A 15-06 Single Nucleotide Polymorphisms and their role in (Epi)genetic Regulation of Human *NOS3* Gene Expression

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Nitric oxide (NO), a key vasodilator and anti-inflammatory agent, is synthesized by endothelial nitric oxide synthase (NOS3), whose expression is maintained by unidirectional shear stress (USS). A single nucleotide polymorphism (SNP T-786C) within the promoter of human *NOS3* gene limits its responsiveness to USS and is a strong independent risk factor for coronary heart disease and related inflammatory disorders. Two additional SNPs (A-922G, T-1468A) are in strong linkage disequilibrium with the T-786C SNP. Bioinformatic analysis suggests a co-binding of CCCTC-binding factor (CTCF) and transcription factor Yin Yang 1 (YY1) to the $^{-922}$GG allele, potentially affecting chromatin remodeling and *NOS3* expression.

To identify the functional consequences of these SNPs in USS-dependent *NOS3* expression, immortalized human umbilical vein endothelial cells (HUVEC) were used for reporter gene analyzes. The knockdown experiments were performed on primary HUVECs.

Mutation of the CTCF binding site significantly disinhibited USS-dependent reporter gene expression in cells transfected with the $^{-922}$GG/$^{-786}$CC construct. Point mutation of a signal transducer and activator of transcription binding motif at position -854 to -835 abrogated USS-mediated reporter gene expression. Chromatin accessibility analysis indicated position -786 and -922 as a hotspot for the histone mark H3K27ac. The histone deacetylase inhibitor trichostatin A disinhibited USS-dependent *NOS3* expression in cells transfected with the $^{-922}$GG/$^{-786}$CC in a time-dependent manner.

Temporal differences in chromatin remodeling in the promoter region between position -922 and -786 may be linked to differential accessibility of the *NOS3* promoter. CTCF may act as an inhibitory regulator, potentially influencing chromatin remodeling thereby modulating *NOS3* transcription.

A 15-07 Lipid droplet formation is associated with augmented store-operated Ca^{2+} entry and impaired barrier function in senescent vascular endothelial cells

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Aim

Endothelial cells play a crucial role in vascular health. Endothelial senescence contributes to pathogenesis of age-associated diseases. It was reported that high-fat dieting caused lipid droplet formation and endothelial dysfunction. However, the role of lipid droplet formation in the senescent endothelial cells remains unknown. The present study utilized replicative senescent porcine aortic endothelial cells (PAEC) as a model of senescent endothelial cells and investigated the formation of lipid droplet and endothelial function.

Methods & Results

PAEC at passages 11-19 and 23-32 were used as younger and senescent cells, respectively. Senescent cells exhibited greater activity of senescence-associated β galactosidase activity than that seen in younger cells. Staining with Bodipy498/503 revealed a significant lipid droplet formation in senescent cells compared to younger cells. The lipid droplet deposition was significantly reduced by treatment with 10 μM forskolin for 48 h. The store-operated Ca^{2+} entry (SOCE) was evaluated using thapsigargin, an inhibitor of sarcoplasmic/endoplasmic reticulum Ca^{2+} ATPase. In the absence of extracellular Ca^{2+}, thaprigargin induced a transient increase in cytosolic Ca^{2+} concentrations ($[Ca^{2+}]i$), followed by a sustained increase in $[Ca^{2+}]i$ after repletion of extracellular Ca^{2+}. SOCE was significantly increased in senescent cells. Thrombin induced actin filament formation at cell periphery as an early event in young cells at confluence with mature cell-cell junction, while it induced stress fiber formation in those with lose cell-cell junction. Senescent cells exhibited instantaneous actin stress fiber formation even at confluence, thus suggesting impaired barrier function.

Conclusions

Lipid droplet was associated with augmented SOCE and impaired barrier function.

A 15-08 Bradykinin increases endothelial permeability by restructuring tight and adherens junctions

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Aim

Endothelial barrier function is crucial for intravascular fluid homeostasis and is maintained primarily by tight and adherens junctions. In angioedema, this barrier is disrupted by vasoactive substances, such as bradykinin. In hereditary angioedema, a mutation in the SERPING1 gene reduces C1 esterase inhibitor formation or activity, which leads to increased bradykinin concentrations. The exact mechanisms by which bradykinin affects cell-cell contacts remain largely unclear.

Methods

Bradykinin was added to Human Umbilical Vein Endothelial cell culture, a common endothelial cell model. Its impact on the barrier structures was investigated by immunohistochemistry in fixated and live cells, alterations in gene expression and post-translational modifications were detected by RT-PCR and Western Blot. On a biophysical level, trans-endothelial electrical resistance and permeability for water and macromolecules were measured.

Results

Raised bradykinin levels increased endothelial permeability significantly. This was accompanied by changes in expression and post-translational modifications of cell-cell contacts, especially claudin 5 in tight junctions and VE-cadherin in adherens junctions. Inhibition of VE-cadherin phosphorylation antagonized the effects of bradykinin entirely.

Conclusions

Bradykinin impairs endothelial barrier function by reduction of claudin 5 expression and phosphorylation of VE-cadherin. This increases vascular permeability and promotes edema. This mechanism may be central to angioedema pathophysiology, diagnostics, and treatment.

Disclosures

This study was supported by the Basic Clinician Scientist Program of the Medical Faculty (University of Ulm) (CASCADE 1.0).

A 15-09 Free fatty acid receptor 4 activation reduces pulmonary arterial tone and smooth muscle cell growth

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Human data suggest that high dietary uptake of the omega-3 polyunsaturated fatty acid docosahexaenoic acid (DHA) reduces systemic blood pressure and cardiovascular events. While the exact mechanism is still unknown, binding of DHA and other long-chain fatty acids (LCFAs) to the G-protein-coupled free fatty acid receptor 1 (FFAR1) and FFAR4 has been demonstrated. Therefore, we wondered if LCFAs can modulate pulmonary vascular tone and cell growth and thus be of therapeutic relevance in pulmonary hypertension (PH), and which signaling pathway might be involved.

Several ex vivo methods were used to determine the effect of DHA or synthetic FFAR4 agonists on murine pulmonary vascular tone. Murine pulmonary artery smooth muscle cells (mPASMC) were used to analyze FFAR4 effects on PDGF-stimulated cell growth. In all animal experiments isolated tissue of female and male C57BL/6 mice were used.

Isometric force measurements of mouse pulmonary arteries (PAs) revealed a right shift of serotonin (5-HT) dose response curves after DHA pre-incubation. Interestingly, the synthetic FFAR4 agonists compound A and TUG-891 induced similar effects, which were prevented by the FFAR4 antagonist AH7614, but not the FFAR1 antagonist GW1100. Furthermore, FFAR4 activation could relax large but also small mouse PAs after 5-HT pre-constriction. DHA and the FFAR4 agonists reduced mPASMC cell growth in a scratch assay after PDGF stimulation.

Our data reveal a dual effect of FFAR4 activation, induction of pulmonary vasorelaxation and reduction of cell growth and could therefore be of therapeutic relevance for PH treatment.

A 15-10 Inactivation of G_q, but not G_{11} protein, in smooth muscle cells prevents hypoxia-induced pulmonary hypertension

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Aim

Pulmonary hypertension (PH) is a progressive disease characterized by chronic pulmonary vasoconstriction and vascular remodeling leading to right heart dysfunction and hypertrophy. Proteins of the Gq family are involved in both, the vascular and cardiac (patho)physiology of PH. To identify the responsible Gq family member and its specific localization, we compared the effect of pharmacological pan-Gq inhibition using FR900359 (FR) with genetic deletion of G_q or G_{11} in smooth muscle cells (SMMH-CRE ERT2; Gna11-/-, Gnaqfl/fl) on pulmonary vascular and right heart function and remodeling in mouse PH.

Methods & Results

For PH induction the standard Sugen 5416/hypoxia (SuHx) mouse model was used. During disease development, mice received intraperitoneal applications of solvent or FR. Transgenic mice received tamoxifen prior to SuHx. Cardiac function was analyzed by pressure-volume catheter measurements, while vascular remodeling and right heart hypertrophy were examined by histology.

FR abolished increases in right ventricular systolic pressure and the elevation of the slopes of the end-systolic- and end-diastolic-pressure-volume relations in the SuHx model. Furthermore, pulmonary vascular wall thickening and right heart hypertrophy were prevented. Interestingly, these effects could all be attributed to G_q inactivation in smooth muscle cells, whereas G_{11} deletion had no effect on PH development.

Conclusions

These data suggest that inactivation of G_q, but not G_{11}, in smooth muscle cells prevents the development of SuHx PH. The role of G_q and G_{11} in cardiomyocytes in PH will be determined in future studies.

A 15-11 Hypoxia-Inducible Factor 2α Promotes Hypertension and Vascular Remodeling via Smooth Muscle Cell Phenotypic Switching

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Background: Vascular smooth muscle cell (VSMC) phenotypic switching is a hallmark of hypertensive vascular remodeling. However, the molecular mechanisms driving this process remain poorly defined.

Methods: Single-cell RNA sequencing data (GSE149777) from normotensive (Wistar Kyoto) and spontaneously hypertensive rats were reanalyzed to identify hypoxia-inducible factor 2α (HIF2α) as a key regulator of remodeling-associated SMC subtypes. Cardiovascular phenotype was assessed in untreated and angiotensin II (Ang II)-treated wild-type and vascular smooth muscle cell-specific HIF2α-deficient or overexpressing male mice.

Results: HIF2α emerged as a hub gene in remodeling-associated SMC subtype (identified as proliferating SMC after injury, PAI_SMC), with elevated expression in internal mammary artery from hypertensive patients and aortic tissue from hypertensive mice. VSMC-specific *Hif2α* deletion attenuated Ang II-induced hypertension, vascular remodeling and mitochondrial oxidative stress, while *Hif2α* overexpression exacerbated these effects. Mechanistically, chromatin immunoprecipitation and dual luciferase assays demonstrated HIF2α directly bound

the *NOX4* promoter, driving its transcription and mitochondrial reactive oxygen species production. This pathway promoted VSMC dedifferentiation, as evidenced by reduced contractile markers (SM22α, calponin) in HIF2α-overexpressing SMCs. Pharmacological HIF2α inhibition with PT2385 blocked Ang II-induced hypertension and vascular remodeling.

Conclusion: Our findings establish the HIF2α-NOX4 axis as a key driver of hypertensive vascular remodeling through VSMC phenotypic switching, highlighting its therapeutic potential for hypertension management.

A 15-12 Impact of inositol hexakisphosphate kinases knockdown on vascular smooth muscle cell calcification

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The author has objected to a publication of the abstract.

A 16 | NEURAL PLASTICITY AND PHYSIOLOGY OF NEURAL CIRCUITS

A 16-01 THE POTENTIAL EFFECT OF CYANOCOBALAMIN SUPPLEMENTATION ON BRAIN NEUROPLASTICITY IN AN ANIMAL MODEL

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Aim

[This research aims to examine the differences in Nerve Growth Factor (NGF) levels in the hippocampus of rats treated with cyanocobalamin (VB12) supplementation via oral and intraperitoneal routes compared to the control group. We sought to determine whether VB12, known for its crucial role in maintaining neuroplasticity, could effectively prevent age-related cognitive decline. The hippocampus, a brain region critical for learning and memory formation, is particularly vulnerable to the effects of aging. NGF levels are often associated with cognitive impairment.]

Methods & Results

[This study was conducted with 39 male Wistar rats, which were divided into 3 groups: control group (C), treatment group with VB12 per-oral 10µg/day (PO), and treatment group with VB12 intraperitoneal 10µg three times a week (IP). After 6 weeks, the animals were anesthetized using 125 mg/kg ketamine + 10 mg/kg xylazine and then sacrificed. Brain hippocampal lobe tissue samples were taken, and then NGF levels were quantified. One-way ANOVA was used to analyze the data.

There was a significant difference in NGF level (p=000) from all three groups.]

Conclusions

[The conclusions showed a significant difference between the control group and both the VB12 intraperitoneal group and the VB12 per-oral group. These findings indicate that the route of cyanocobalamin administration plays an important role in the observed effects, with both intraperitoneal and oral administration methods demonstrating significant efficacy compared to controls.]

A 16-02 From experience to inhibition: Impact of environmental enrichment onto hippocampal synaptic inhibition

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Environmental enrichment (EE) enhances synaptic plasticity and improves hippocampus-dependent learning and memory. Soma-targeting parvalbumin (PV)- and dendrite-targeting somatostatin (SOM)-expressing interneurons are crucial for regulating excitability and plasticity of CA1 pyramidal neurons. While the effects of EE on hippocampal excitatory circuits are well-established, its influence on inhibitory circuit dynamics remains unclear.

In this study, we investigated how EE shapes inhibitory synaptic transmission in the hippocampal CA1 region. Using whole-cell patch-clamp recordings in acute

hippocampal slices from C57BL/6 mice (7-11 weeks old) we assessed evoked and miniature synaptic activity in animals housed under standard conditions (CON) or EE for 2–3 weeks. All experiments were approved by the Basel Cantonal Veterinary Office.

EE was found to lead to a proportional increase in excitatory and inhibitory synaptic inputs onto CA1 pyramidal neurons. Electrical stimulation of dendrite- and soma-targeting interneurons suggested that EE selectively enhanced dendritic inhibition onto CA1 pyramidal neurons, while perisomatic inhibition remained unchanged. Optogenetic stimulation in SOM-ChR2 and PV-ChR2 mice showed that EE selectively increased SOM-mediated inhibition of CA1 pyramidal neurons. Lastly, EE was found to enhance the synaptic recruitment of SOM-interneurons, evidenced by a significant increase in excitatory inputs and decrease in inhibitory inputs onto these cells.

Our findings suggest that EE shifts hippocampal CA1 circuit dynamics toward increased dendritic inhibition of pyramidal neurons, primarily via SOM-interneurons. This activity-dependent modulation of circuit dynamics likely serves as a compensatory mechanism that helps to stabilize network dynamics and memory encoding, even during periods of enhanced brain activity associated with EE.

A 16-03 Evidence for ultrafast synaptic engrams in parallel fiber-Purkinje cell synapses in mouse cerebellum

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Short-term plasticity is a key feature of synapses in the brain that contributes to information processing and storage. During repeated firing, synapses can either facilitate or depress, depending on their initial release probability. This may be determined by structural parameters such as the docking and priming state of synaptic vesicles (SVs) at the active zone (AZ) membrane, as well as the extent of the electron-dense material (EDM) underneath, containing the fusion machinery. To fully understand the mechanisms of short-term facilitation and depression, it is therefore essential to consider activity-dependent ultrastructural dynamics of SVs and their associated EDM at high spatial and temporal resolution. To this end, we performed electrical stimulation of parallel fiber–Purkinje cell (PF-PC) synapses in acute cerebellar slices, originating from mice anaesthetized with isoflurane using the bell jar method, followed by high-pressure freezing at different timepoints after stimulation, automated freeze substitution, and electron tomography. We found profound alterations in the SV pools, especially in those closest to the AZ membrane and their EDM 5 and 10 ms after stimulation. These changes not only reflect topographic information about the fusion and recycling of release-ready neurotransmitter-filled SVs, but presumably characterize the facilitated state of the PF-PC synapse.

By investigating characteristic activity-dependent changes to synaptic ultrastructure and in particular vesicle dynamics, we aim to arrive at morphological determinants of short-term plasticity that may serve as ultrafast synaptic engrams, physically encoding information on a millisecond timescale.

A 16-04 Impact of astrocyte-neuron lactate shuttle on LTP

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Aim: Neuronal activity is closely linked to metabolic processes, requiring precise regulation of local energy supply. A pivotal mechanism in this context is the astrocyte-neuron lactate shuttle, through which astrocytes supply lactate as an energy substrate during neuronal activity. Lactate release is mediated by adenosine activating A2B-receptors on astrocytes. Blockade of these receptors results in reduced lactate release, cognitive deficits in mice and impairment of synaptic long-term potentiation (LTP), a critical process in memory formation. However, the mechanisms linking lactate release and synaptic plasticity remain unclear. This

project aims to investigate the function of lactate during the induction and expression of LTP in the hippocampus of mice.
Methods & Results: Animals were handled in accordance with European and national guidelines. Coronal brain slices were prepared to record field potentials in the CA1 region of the hippocampus in an interface chamber. Our initial results indicate that LTP is impaired upon application of the A2B receptor blocker (PSB-603, 10 μM). Furthermore, A2B-receptor-blockade seemed to enhance short-term depression during the high-frequency synaptic transmission, which was used to induce LTP.
Conclusion: Our findings suggest that lactate supports high-frequency transmission and enables induction of LTP. Together with additional experiments, we aim to contribute to a better understanding of how metabolic support from astrocytes influences synaptic plasticity.

A 16-05 Developmental Profile of Neurturin in Rat Brain During The Postnatal Period

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Aim Neurturin is a neurotrophic factor belonging to the 4-member family of glial-derived growth factors. This study aimed to investigate the changes in the protein levels of Neurturin in different brain regions at various stages of the postnatal period.
Methods & Results Forty male Wistar Albino rats aged 1,3,6, and 12 weeks were used (10 animals per group). The subjects were sacrificed under 50 mg/kg ketamine + 10 mg/kg xylazine anesthesia. The prefrontal cortex, striatum, hypothalamus, thalamus, cerebellum, and hippocampus were removed. Neurturin levels were analyzed by ELISA method. One-way ANOVA and Tukey tests were applied statistically. All experimental procedures were performed with the approval of the Balıkesir University Animal Experiments Local Ethics Committee (Approval Reference No.: 2024/5-7).Neurturin levels of the cerebellum and hypothalamus were significantly higher at postnatal week 6. Neurturin levels were lower at week 3 than weeks 1 and 12 in the thalamus. Hippocampal levels were higher at week 12 than weeks 3 and 6. The highest neuturin levels were measured in the cerebellum at week 1, hypothalamus at week 3, cerebellum at week 6 and hippocampus at week 12.
Conclusions There are significant changes in neurturin levels in the cerebellum, hypothalamus, thalamus and hippocampus at different neurodevelopmental times. These differences in the levels of Neurturin, which is known to play a role in the development and differentiation of neurons, have the characteristics of guiding different studies in terms of reversal of neuronal damage and formation of new neurons that will occur in postnatal, adolescent and adulthood periods.

A 16-06 Transcriptomic Signatures of Age-Associated Endothelial Dysfunction in the Brain

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Aging is a major risk factor for neurodegenerative diseases and is associated with chronic, low-grade neuroinflammation. This condition is characterized by increased infiltration of peripheral immune cells and elevated levels of proinflammatory cytokines and chemokines within the brain microenvironment, primarily mediated by NF-κB signaling. Our previous studies demonstrated that caveolin-2 (Cav-2) expression is upregulated in brain endothelial cells of aged mice, facilitating leukocyte adhesion and transendothelial migration. To further investigate the functional role of Cav-2, we utilized a stable endothelial cell line overexpressing Cav-2 and assessed transcriptomic alterations and signaling pathway dynamics in response to inflammatory stimuli. Furthermore, we evaluated the therapeutic potential of Exo-srIκB, an engineered exosome-based NF-κB inhibitor previously shown to attenuate age-associated neuroinflammation, by analyzing its effects on endothelial cell phenotypes under inflammatory conditions. Single-cell transcriptomic profiling of brain endothelial cells from aged mice identified an expanded endothelial subpopulation, characterized by upregulated expression of

genes involved in vascular permeability and immune cell recruitment, alongside downregulation of key transporters essential for maintaining blood-brain barrier integrity. Notably, Exo-srIκB treatment reversed these transcriptional alterations, indicating restoration of endothelial homeostasis through targeted NF-κB inhibition in the aged brain. Collectively, these findings implicate the Cav-2–Notch1–NF-κB signaling pathway as a critical mediator of age-related endothelial dysfunction and highlight Exo-srIκB as a potential therapeutic strategy to preserve vascular integrity and attenuate neurodegenerative progression.

A 16-07 Investigation of Functional Integration of Cajal-Retzius Neurons into Maturing Cortical Networks

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Cajal-Retzius neurons (CRn) are a transient, early-born neuronal population that contributes to the proper development of the cortex. The mechanisms regulating their density and cell death are still not fully understood despite it being known that they are partially activity-dependent. At very early stages, CRn represent a major target of GABAergic synapses in the marginal zone. Therefore, we explored the functional integration of CRn into the immature cortical network through immunohistochemistry, electrophysiology, and calcium-imaging. Previous patch-clamp studies indicated that GABAergic inputs depolarize CRn. Therefore, we investigated the expression levels of chloride transporters NKCC1 and KCC2 in genetically labeled CRn ($\Delta Np73^{Cre/+}$ and $BaxCKO\Delta Np73^{Cre/+}$) at different ages using multiplexed FISH. We showed that CRn, unlike most neurons, fail to upregulate KCC2 expression during maturation, thereby retaining immature-like properties. This peculiar chloride homeostasis combined with the excitatory GABAergic effect on CRn could impact CRn death. Indeed, we showed that chronic bumetanide treatment of organotypic cultures significantly improved the survival of $\Delta Np73^{+}$ CRn. We then investigated the functional effect of early vs late activation of CRn. We found that late chronic optogenetic high-frequency stimulation of CRn significantly increased their death rate. Conversely, repeated acute optogenetic stimulation of CRn in early life increased network excitability. In conclusion, while late activation of CRn has a detrimental effect, the early activation of CRn influences cortical activity, highlighting a novel functional role during development. This study could thereby provide hints towards the consequences of persistent CRn under pathological conditions.

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A 16-08 Characterization of *in vitro* gamma oscillations in human neocortex

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Gamma-band oscillations (30-100 Hz, γ-Osc.) are local rhythmic network activity that emerge from fast inhibition by parvalbumin interneurons (PV INs). While being a cellular correlate for cognitive domains, aberrations in γ-Osc. and PV INs are strongly linked to various neuropsychiatric diseases of all ages. Despite electro-/magnetoencephalographic findings that connected γ-Osc. to certain brain functions, the underlying neurocellular mechanisms of γ-Osc. in the human brain remain elusive.

By utilizing acute neocortical slices of human brain resections from neurosurgical interventions we could establish a method to pharmacologically induce stable rhythmic network activity in the low-γ range (25-40 Hz) in *in vitro* field potential recordings. The reliability of this method enabled us to record up to 24 slices parallelly, allowing us to establish an initial dataset of over 650 slices from more than 50 patients.

Our data shows that neocortical γ-Osc. are subjected to developmental trajectories, as seen in age-dependent rise in power. They are highly relying on GABA-A-receptor-mediated inhibition. Blocking calcium-permeable AMPA receptors, specifically expressed in PV INs, markedly reduced the frequency of oscillations (from 25.47 ± 5.22 Hz to 11.65 ± 5.78 Hz; n = 43, mean ± SD), emphasizing the importance of net-excitatory drive of PV INs.

Concluding, we present a high-throughput method to induce stable and pharmacologically well-defined gamma oscillations. Our explorations on the

indispensable role of PV IN-dependent inhibition in γ-Osc. and its age-dependent development give hints to potential vulnerable loci in pathogenesis and set up for future investigations and possibly therapeutic research.

A 16-09 Structural and molecular reorganization of engram cells after spatial encoding

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Neuronal encoding and learning rely on activity-dependent changes in synaptic strength driven by the synchronous activation of excitatory inputs. In the dentate gyrus, granule cell (GC) activity is sparse, a feature essential for efficient contextual pattern separation. This low network activity is mainly due to the intrinsic properties of GCs and strong GABAergic modulation. How these properties contribute to the integration and stability of engram cells remains unclear. We hypothesize that during spatial encoding, GC dendrites undergo activity- and input-dependent changes in structure and physiology, influencing synaptic integration and contributing to the formation and stabilization of dentate gyrus (DG) engram cells. To test this, we investigate the molecular and physiological mechanisms by which dendritic excitatory and inhibitory inputs regulate DG engram emergence and maintenance, using quantitative high-resolution immunoelectron microscopy (iEM), in vitro electrophysiology, and computational modeling. Using cFOS-dependent labeling of engram cells after contextual fear conditioning, we observed notable changes in the synaptic and molecular architecture of engram cells. Seven days after learning, dendrites of cFOS-YFP$^+$ engram cells exhibited increased synapse size and decreased surface expression of NMDA receptors and Kv4.2 channels compared to non-engram GCs. In contrast, no changes in metabotropic $GABA_B$ receptor expression were observed. In vitro electrophysiological measurements of intrinsic excitability and NMDA/AMPA receptor ratios will be performed to support these results, while computational models will help explain how these changes might influence engram stability. Our current findings indicate that engram cells undergo morphological and physiological modifications during spatial encoding, potentially critical for memory formation and learning.

A 16-10 In vivo two-photon calcium imaging of the cortical response to sensory stimuli during a hibernation-like state in mice

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Some mammals enter hibernation to survive extreme environmental conditions by lowering their metabolic rate and body temperature. Despite this profound physiological suppression, hibernating animals can still be aroused by external stimuli, suggesting that certain sensory pathways remain functional. However, the mechanisms underlying sensory processing during hibernation remain unclear due to the difficulty of studying brain activity in natural hibernators. A breakthrough study by Takahashi et al. (Nature, 2020) demonstrated that activation of Qrfp-expressing neurons in the preoptic area (POA) of the hypothalamus induces a hibernation-like state—termed Q-neuron-induced hypothermia and hypometabolism (QIH)—in non-hibernators such as mice, offering a valuable model for studying sensory processing in a hypometabolic state. In this study, I aim to elucidate how thow animals under QIH respond to tactile stimulation.Using in vivo calcium imaging, I assessed the somatosensory cortex activity under awake, anesthetized, and QIH conditions. Sensory responses will be evoked by electrical stimulation applied to the hindpaw. Interestingly, QIH mice remained responsive to stimulation, indicating that sensory pathways remain active despite suppressed metabolism and lowered body temperature. Furthermore, cell-type-specific calcium imaging revealed that astrocytes in the somatosensory cortex responded more strongly to stimulation during QIH, whereas neurons were more activated during non-QIH state.These findings suggest that astrocytes may play a key role in maintaining sensory responsiveness during metabolic suppression. Overall, this work provides new insights into the functional adaptability of the sensory cortex during hypometabolism

and highlights astrocytic involvement in sensory processing under low-energy conditions.

A 16-11 Differential involvement of hippocampal pyramidal cell subtypes in spatial learning tasks

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Hippocampal pyramidal cells are involved in spatial coding and memory formation which requires the reliable integration of multiple synaptic inputs within single neurons. According to current understanding, this takes place at the soma which is directly connected with the axon, where supra-threshold inputs trigger action potentials. However, in about 50% of hippocampal CA1 pyramidal neurons the axon emerges from a basal dendrite (AcD, 'axon-carrying dendrite'). This particular dendrite is largely independent from somatic signal integration and can efficiently convert excitatory inputs into action potentials (APs). We therefore hypothesize that AcD cells are more active during the formation and consolidation of memories.

To test this hypothesis, we trained mice on a spatial memory task. Active neurons are expected to express immediate early genes (e.g. cFos), and can be identified by ex vivo staining. cFos expression of cells were analyzed at different time points of the training process and cell were classified into AcD and canonical cells. The number of cells expressing cFos was higher in dorsal compared to medio-ventral portions of the hippocampus. AcD cells and canonical neurons showed different learning-related time courses of cFos. While the number of cFos expressing cells in the dorsal hippocampus decreased within canonical cells during the learning process, it increased in AcD cells. Interestingly, the proportion of AcD cells in medio-ventral CA1 decreased during the learning protocol, indicating structural plasticity of axon initial segment location. Our findings indicate distinct roles of AcD and nonAcD cells during formation and consolidation of spatial memories in the hippocampus.

A 16-12 Employing human iPSC-derived sympathetic neurons to investigate regulatory mechanisms of sympathetic neurons

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Background and Aims

Increased activity of sympathetic neurons has been linked to various cardiovascular disease (e.g. hypertension, heart failure)[1]. Native sympathetic neurons express alpha-adrenergic[2] (αARs) and muscarinic receptors[3] but their mechanisms have not been studied in detail. We employ human iPSC-derived sympathetic neurons (hiPSC-SNs) as a human/scalable *in vitro* tool to dissect pathways and potential therapeutic targets in sympathetic neurons.

Methods

hiPSC-SNs were generated following a published protocol[4]. Their lineage was assessed by immunostaining and Ca^{2+} imaging (nicotine application). We employed whole-cell patch clamp recordings to study the effects of: norepinephrine(100μM), the α2ARs agonist UK14,304(10μM) and the muscarinic agonist carbachol (2μM) in absence/presence of the GIRK blocker Tertiapin-Q(100nM).

Results

Our data confirmed key characteristics of hiPSC-SNs: they expressed Phox2b, tyrosine hydroxylase and, responded to nicotine with Ca^{2+}-influx. Norepinephrine hyperpolarised the neurons (RMP: -67.48±2.14mV vs: -69.42±2.01mV, p=0.0014;n=10; Paired-Student's t-test), likely via α2ARs since application of UK14,304 resulted in similar changes (RMP:-62.71±1.64mV, vs -65.97±1.60mV, p=0.0052; rheobase: 72.31±17.03pA vs UK 100±17.54pA vs washout 90.91±17.96; p=0.01 n=13 Repeated-Measures one-way ANOVA). Similar to the adrenergic

agonists, carbachol reduced neuronal excitability (RMP:-63.85±2.25mV vs -66.63±1.82mV *p*=0.047; *Wilcoxon matched-pair;* rheobase: 40±11.34pA vs CCH 55±12.39pA vs washout 42.86±8.08, *p*=0.01; n=8 Repeated-Measures one-way ANOVA). Application of Tertiapin-Q reduced the carbachol-evoked effect: hyperpolarization and increased rheobase.

Conclusions

Our data show that hiPSC-SNs could be a powerful tool to dissect the regulatory mechanisms of sympathetic neurons. We show that they express functional α2ARs and muscarinic receptors and identified a possible contribution of GIRK channels downstream of muscarinic receptors.

[1]PMID38778747
[2]PMID29686017
[3]PMID10066893
[4]PMID32822546

A 16-14 Differential K_V4 channel functions among projection-defined dopamine neurons of the ventral tegmental area

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K_V4 channels are well established regulators of pacemaking frequency in dopamine (DA) midbrain neurons. Although previous studies have characterized K_V4 channel properties in substantia nigra (SN) and ventral tegmental area (VTA) DA neurons, the functional contribution of K_V4 in axonal projection-defined VTA DA subpopulations is still unknown.

To investigate K_V4s biophysical properties in projection-defined VTA DA subpopulations, we combined axonal retrograde tracing with nucleated outside-out patch-clamp recordings. In DA neurons projecting to the medial shell of nucleus accumbens (mNAc) the K_V4 steady-state activation curves were shifted to significantly more depolarized potentials compared to those in lateral shell (lNAc)-projecting DA neurons ($V_{50\text{-}A}$:mNAc:-1.4±6.0mV; lNAc:-11.7±3.5mV). Additionally, the K_V4 steady-state inactivation curves were more negative ($V_{50\text{-}I}$: mNAc:-88.6±2.4mV; lNAc: -72.2±2.3mV). These distinct K_V4 gating properties suggested functional differences regarding K_V4-mediated pacemaker control. To test this, we recorded the spontaneous activity of retrogradely traced VTA DA neurons before and after wash-in of the K_V4-specific inhibitor AmmTx3 (1µM). Consistent with the biophysical differences, K_V4 inhibition increased the mean firing frequency in lNAc DA neurons by ~60%, while it did not affect discharge rates in mNAc DA neurons.

These results demonstrate differential K_V4 channel functions for projection-defined VTA DA subpopulations. Like for SN DA neurons, K_V4 controls pacemaker frequency in lNAc DA cells. In contrast, in mNAc DA neurons K_V4 appears to operate only in the subthreshold voltage range.

We are currently undertaking single-unit recordings of chemogenetically-targeted VTA DA subpopulations using DREADDs in freely moving mice, to study the functional *in vivo* implications of K_V4-diversity across different DA projections.

A 17 | SYNAPTIC PHYSIOLOGY

A 17-01 Age-Related Changes in Calcium Signaling and Glucose Metabolism in Neurons and Glia of the *Drosophila* Brain

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Aim

During ageing, brain glucose utilisation declines, contributing to cognitive deficits. This may result from dysfunction of the noradrenergic system, which regulates brain metabolism and cognitive processes via Ca^{2+} and cAMP signalling. In astrocytes, noradrenergic signalling promotes glucose uptake, glycogen breakdown, and aerobic glycolysis, producing lactate that fuels neurons during heightened activity. Whether this regulation is impaired in the aged brain remains unclear. We

investigated age-related changes in noradrenergic regulation of neuronal and glial metabolism in *Drosophila* brain.

Methods & Results

We expressed fluorescent sensors for Ca^{2+}, cAMP, glucose, and lactate in neurons or glia of young and aged *Drosophila* brains and stimulated them with octopamine, an invertebrate analogue of noradrenaline. In young brains, octopamine triggered robust Ca^{2+} signalling in both neurons and glia; this response was markedly reduced in aged brains, indicating age-related impairment. Neuron-specific increases in cAMP and lactate were observed in both age groups, suggesting aerobic glycolysis occurs primarily in neurons and is maintained with age. Octopamine-induced glucose increases in glial cytosol were detected only in young brains, likely reflecting enhanced uptake. While both neurons and glia could take up extracellular glucose and lactate, glucose uptake was diminished in aged neurons. These metabolic impairments in aged brains were accompanied by neurodegenerative lesions.

Conclusions

Neurons are the main site of regulated aerobic glycolysis in the *Drosophila* brain. Age-related impairments in Ca^{2+} signalling and glucose handling in neurons and glia may disrupt brain energy metabolism and contribute to cognitive decline during ageing.

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A 17-02 *DMWD* induces social deficits via medial prefrontal cortex dysfunction in mice

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Aim

Sociability among animals can regulate their essential behaviors, which is also the criterion for assessing the core symptoms of many neuropsychiatric disorders, including autism spectrum disorder (ASD). The medial prefrontal cortex (mPFC) is believed to be involved in mediating social behavior, but the region that regulates this complex behavior and its underlying neural basis are diverse and unclear. Here, we demonstrate how Dystrophia myotonica, WD repeat containing (DMWD), a rarely studied gene, regulates the neural activity of the mouse mPFC to mediate typical social behavior.

Methods & Results

DMWD-deficient mice exhibited impaired social interaction, a reduction in the number of excitatory neurons in the medial prefrontal cortex (mPFC) via single-cell sequencing, loss of dendritic spines, and decreased neuronal activity. Selective activation of mPFC excitatory neurons through optogenetic and chemical genetic methods or the restoration of *DMWD* expression can rescue social deficits in mice. Via mass spectrometry and transcriptome analysis, we identified a combined protein, α-Tubulin, that can participate in the process of *DMWD*-mediated social deficits. In autism models, the overexpression of *DMWD* can also exclusively improve social deficits, and functional mutations and potential functional mutations of *DMWD* were also found in population databases.

Conclusions

These studies suggest that *DMWD* deficiency in the mPFC can mediate social deficits via α-Tubulin in mice and that DMWD may be a therapeutic target for ASD and other diseases related to social disorders.

A 17-03 Neuronal Ca^{2+} signaling is regulated by the deubiquitinating enzyme UCH-L1

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Aim

UCH-L1 is the most abundant brain protein and besides being involved in proteasomal degradation as a deubiquitinating enzyme (DUB), the exact role is not yet understood. C57BL/6 mice deficient for UCH-L1 display a dying back axonal degeneration showing progressing motoric deficits and age-dependent accumulation of ubiquitinated proteins. In a first exploratory study, we investigated

whether alteration in neuronal Ca^{2+} signaling could be causative for the motoric phenotype.

Methods & Results

Proteomic, biochemical and histological approaches were used to pre-scan for alterations in neuronal Ca^{2+} homeostasis in constitutive UCH-L1 deficient mice (KO) compared to wildtype (WT) littermates using primary neuronal cultures or total brains from embryonic to adult stages. Cervical dislocation under anesthesia (3.5% isoflurane or ketamine 120 mg/kg, xylazine 16 mg/kg, i.p) was used for brain extraction. Proteomic analysis of P5 brains (n=3) revealed upregulation of important Ca^{2+} signaling regulating proteins, like CamKII, SERCA, AMPAR and the VGCC $Ca_v1.2$, under UCH-L1 deficiency, which was further confirmed by preliminary biochemical and histological analysis (n=2). Further, alteration in the size and morphology of Ca^{2+} storing organelles (ER, mitochondria and lyso/autophagosomes) could be detected by electron microscopy and histology of E15 primary neuronal cultures (pNCs). Further and interestingly, UCH-L1 DUB activity in pNCs is decreased upon intracellular Ca^{2+} rise induced by the application of ionomycin (1µM).

Conclusions

UCH-L1 deficiency leads to alteration in neuronal Ca^{2+} homeostasis thereby influencing the expression of important intracellular Ca^{2+} regulating proteins in the brain. This might contribute to the progressive motoric phenotype of UCH-L1 deficient mice.

A 17-04 Active zone growth during larval development in Drosophila melanogaster

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Larval development in *Drosophila melanogaster* spans from first instar larvae (L1) with a size of about 1 mm to third instar larvae (L3) with a size of 3-4 mm. L3 larvae have evolved into a frequently used model for both electrophysiological and immunohistochemical analysis of neuromuscular synapses. However, little is known about the development of neuronal nanotopology during larval growth. We adopted the standard preparation technique for L3 larvae to fit the minuscule size of L1 larvae. Using superresolution microscopy (*d*STORM, direct Stochastical Optical Reconstruction Microscopy) we find ultrastructural differences between active zones (AZs) of L1 and L3 larvae. Moreover, AZs of L1 larvae seem to lack certain mechanisms of presynaptic homeostatic plasticity, and display ultrastructural differences between distinct types of synapses. We thereby show that larval development of *Drosophila melanogaster* is not just an increase in size.

A 17-05 Microglia-derived exosomal ciRS-7 mediates IL-17A effect of promoting neurodegeneration via miR-7 and SNCA targets in an experimental Parkinson's disease

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The author has objected to a publication of the abstract.

A 17-06 Inhibited follicle stimulting hormone down regulated APOE4 gene expression in aluminum chloride induced-Alzheimer's disease rodent models

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Aim: To investigate APOE4 gene expression in the hippocampus by inhibiting follicle stimulating hormone (FSH) in aluminum chloride induced Alzheimer's disease state.

Methods & Results: A total of 40 Albino wistar rats (250- 300 grams) were used for this study. The rats were randomly assigned to 4 groups with a total of 10 rats per group. Group 1 served as control (water and feed ad libitum); Group 2 ($AlCl_3$ only 100 mg/ kg body weight for 2 days); Group 3 ($AlCl_3$100 mg/kg body weight for 28 days and post treated with Estradiol 3 mg/kg for 21 days); Group 4 (Estradiol only 3 mg/kg body weight for 21 days). AD was confirmed in groups 2 and 3 with neuro-behavioral assessment standards, hormonal and histological analysis. Thereafter, the hippocampi were exercised and homogenized for the analysis of APOE4 gene using reverse-transcriptase polymerase chain reaction (RT-PCR)

Conclusions: The use of $Alcl_3$ as a toxicant to induce AD was achieved as observed in the AD only group which showed an increase in the expression of APOE4 gene which is one of the genetic markers of AD. Administration of estradiol produced negative feedback on APOE4 gene expressed protein; and consequently, downregulated APOE4 gene expressed protein in AD group post treated with estradiol suggesting the importance of investigating FSH levels in AD management.

A 17-07 Structural and functional changes in microglia-parvalbumin interneuron interactions in DISC1 mice

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Microglia, the resident immune cells of the brain, maintain brain homeostasis and are involved in virtually all CNS disorders, with implications ranging from neuroprotection to exacerbation of pathology. While their interactions with excitatory neurons are well-explored, there is only sparse knowledge on how microglia regulate GABAergic inhibition, for which parvalbumin-positive interneurons (PVI) play a crucial role. PVIs are a key component in coordinating neuronal activity underlying memory formation, cognition and sensory information processing, but are also implicated in neuropsychiatric disorders. To investigate microglia-PVI interactions, we used Disrupted-in-Schizophrenia 1 (DISC1) mice as a model system characterized by impairment of PVI-dependent network function. We found that microglia in DISC1 mutant mice exhibit altered morphology, enhanced lysosomal function and phagocytosis, as well as closer proximity to PVI perisomatic regions. Moreover, we identified a compromised number and integrity of perineuronal nets (PNNs) along with larger amounts of incorporated PNN material in microglia in DISC1 mice. Our results indicate dysregulated microglia-PVI interactions in DISC1 mice, which likely contribute to the observed alterations in neuronal network function.

A 17-08 Multifocal two-photon mapping of synaptic activity in vivo

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The author has objected to a publication of the abstract.

A 17-09 Investigating Presynaptic Plasticity via Live Imaging of Glutamate Release and Mitochondrial Dynamics in Hippocampal Neurons

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Aim

Synaptic plasticity is a fundamental mechanism underlying learning and memory, its dysregulation is implicated in various neurodegenerative disorders. The presynaptic plasticity of neurotransmitter release contibutes importantly to the synaptic plasticity. This project aims to better understand the interplay of presynaptic plasticity and mitochondrial localization and mobility in hippocampal neurons.

Methods & Results

We investigated presynaptic glutamate release with the genetically encoded glutamate sensor iGluSnFr.v857. The sensor was expressed in primary neuronal hippocampal mouse cultures via transduction with adeno-associated viral-vectors.

The glutamate sensor, localized to the membrane, allowedthe monitoring of synaptic glutamate release during live imaging. This technique permits to detect release events of individual synaptic vesicles and allows the quantification of the release probabilities of individual presynaptic boutons. We optimized the experimental design and data analysis for stable measurements of the release probability for >30 min. Consistent with previous studies, our results show a highly variable release probability between different boutons. Furthermore, our data indicates that the release probability of individual boutons remains rather stable for >30 min. Additionally, cells were transfected with a mCherryMitoTracker providing the possibility to perform dual imaging to visualize mitochondrial structures alongside glutamate release.

Conclusions

Our live-imaging technique offers a powerful tool to spatially and temporally correlate mitochondrial dynamics with presynaptic function. The combination of glutamate release monitoring and mitochondrial imaging provides novel insights into the interplay between energy supply and synaptic function. Our findings demonstrate the robustness of this method for studying presynaptic function and suggest a potential link between mitochondrial dynamics and presynaptic plasticity.

A 17-10 Ternary Neurexin-T178-PTPR complexes represent a presynaptic core-module of neuronal synapse organization

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The organization of cell-cell contacts is fundamental for multi-cellular life and operation of organs. Synapses, prototypic contact sites for neuronal communication, are key to brain function and work over the last decades identified multiple synaptic cell adhesion molecules (sCAMs) that drive their organization. Whether these sCAMs operate independently or in coordination through yet unknown linker proteins remained elusive. Here, we used a systematic large-scale multi-epitope affinity-purification approach combined with quantitative mass spectrometry and immuno-EM to comprehensively map trans-synaptic protein networks in the mouse brain. We discover a presynaptic core-module assembled from the two major sCAM families, Neurexins1-3 and LAR-type receptor protein tyrosine phosphatases (PTPRD,S,F), and the previously uncharacterized tetraspanin proteins T178A, B. These ternary Neurexin-T178-PTPR complexes form through their trans-membrane domains and assemble during biogenesis in the ER. Loss of T178B results in module dissociation, strong reduction of LAR-PTPRs and re-distribution of synaptic Neurexins. At synapses, the Neurexin-T178-PTPR module recruits stable and extended trans-synaptic protein networks with defined pre- and post-synaptic partners and secreted extracellular linkers. The network architecture robustly interlinks the distinct functional modules/machineries of the presynaptic active zone and establishes tight associations with XKR-type lipid scramblases and postsynaptic GABAergic and glutamatergic neurotransmitter receptors. Our data identify a universal presynaptic core-module for synaptic adhesion and trans-synaptic signaling in the mammalian brain.

A 17-11 The final two nanometers determine release probability

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The precise distance of a tightly docked synaptic vesicle (SV) proximal to the active zone (AZ) membrane and the extension of the electron-dense material (EDM) underneath it, containing the fusion machinery, may determine release probability. Munc13-3enhances release probability at the synapses between cerebellar parallel fibers and Purkinje cells (PF-PC). To probe the ultrastructural correlates of release probability, we performed electron tomography on high-pressure frozen and freeze-substituted acute murine brain slices. For that, mice were decapitated under deep isoflurane anesthesia with the bell jar method. We found that the synaptic clefts of

PF-PC synapses were narrower in controls than in Munc13-3$^{-/-}$. Moreover, tightly docked SVs (0-2 nm) had larger diameters, were on average 0.4 nm closer to the AZ membrane, and were nearly twice as numerous at 0 nm from the AZ membrane in controls compared to Munc13-3$^{-/-}$. Surprisingly, almost no SVs were found between 0 and 0.5 nm from the AZ membrane in the docking process. The EDM underneath tightly docked SVs was 5 nm wider in controls and more extensive at the AZ periphery in controls but not in Munc13-3$^{-/-}$. Thus, we hypothesize that an action potential induces first the release of SVs with wide EDM in the AZ periphery, which we test using zap-and-freeze (Eddings et al., bioRxiv 2024.12.26.630393). Our findings suggest a strong correlation of SV proximity to the AZ membrane and EDM extension with release probability, and lateralization of fusion-ready SVs in PF-PC synapses, with implications for short-term plasticity, vesicle recycling, and AZ integrity.

A 17-12 Modulation of AMPA receptor funtion by the auxiliary subunit CKAMP59

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Aim

Core subunits of ionotropic receptors such as AMPA receptors (AMPARs) interact with auxiliary subunits that control their surface trafficking, localization and function. CKAMP59 (aka shisa7) was identified by sequence similarity to CKAMP44 (aka shisa9), as a potential AMPAR auxiliary subunits. There are contradictory studies. Data from our laboratory suggest that CKAMP59 reduces the number of AMPAR in the synapser. In another study, CKAMP59 influenced the gating of the AMPAR (Schmitz et al. 2017). Surprisingly, Han et al. found no effect on AMPAR function, but an effect on the GABAA receptors. The aim of the study is to investigate whether CKAMP59 functions in the brain as an AMPA and/or GABA receptor auxiliary subunit

Methods & Results We analysed the influence of CKAMP59 on AMPAR and GABAR in heterologous expression systems and 2 CKAMP49 deficient mouse models. Using biochemical methods, we found an effect on AMPAR degradation and the expression at the cell surface. Furthermore, we found a reduction of AMPAR-mediated currents in the temporoamonic pathway synapses of CA1 pyramidal cells and a strong reduction of hippocampal long term potention. In contrast we only found minor effects in GABAergic transmission.

Conclusions

Therefor we conclude that CKAMP59 is AMPA receptor auxilary subunit affecting recpetor insertion into the synapse.

A 17-13 Real-Time Probing of NMDA Receptor Co-agonist Dynamics in the Hippocampus Using Optical FRET Sensors

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D-serine and glycine are essential co-agonists at excitatory glutamatergic NMDA receptors (NMDARs), playing key roles in synaptic plasticity and cognitive function within the hippocampus. Multiple lines of evidence suggest that the release NMDAR co-agonists is mediated by astrocytic activity, thus they are often referred to as gliotransmitters.

Precise, real-time monitoring of extracellular levels of gliotransmitters during various modes of neuronal activity remains a major experimental challenge due to their dynamic and spatial distribution. To address this, we employed optical FRET (Förster Resonance Energy Transfer) sensors for the in situ imaging of extracellular NMDAR co-agonists levels in acute hippocampal slices. These sensors, based on mutated bacterial binding proteins fused to CFP/YFP fluorophores, allow for the monitoring of gliotransmitter fluctuations. Using fluorescence lifetime imaging microscopy (FLIM), we detected activity-dependent changes in extracellular gliotransmitters levels during physiological stimulation.

Our findings support the role of glia-regulated NMDAR co-agonists release, contributing to local NMDAR modulation. The use of FRET-based biosensors

provides a powerful tool for investigating D-serine and glycine dynamics in acute slices and, potentially, in the intact brain, providing insights into neuron-glia communication.

A 18 | PHYSIOLOGY OF TRANSPORT AND ENDOTHELIUM

A 18-01 The Japanese Kampo Medicine Boi-ogi-to (BOT) Promotes Cellular Chloride Excretion via Activation of Volume-Sensitive Outwardly Rectifying (VSOR) Anion Channels

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Boi-ogi-to (BOT), a traditional Japanese Kampo medicine, is recognized for its therapeutic effects on edema and nephrosis through its capacity to enhance fluid excretion. However, scientific validation of these effects remains insufficient. This study investigated the molecular mechanisms underlying BOT's action using clinical and in vitro approaches.

A retrospective analysis of clinical data from 28 patients at Akita University Hospital revealed significantly increased blood sodium and chloride levels following BOT administration. To elucidate the underlying mechanism, we examined the impact of BOT on the cell volume of human embryonic kidney (HEK293T) cells in vitro. BOT reduced cell volume in a concentration-dependent manner (EC_{50} = 686 µg/ml), and this effect was inhibited by Cl^- channel blockers DIDS and DCPIB.

Patch-clamp analyses demonstrated that BOT-activated Cl^- currents exhibited outward rectification and time-dependent inactivation upon depolarization—characteristics consistent with volume-sensitive outward rectifier (VSOR) anion channels. BOT-induced Cl^- currents were suppressed by DIDS, DCPIB, and siRNA targeting LRRC8A, a core component of VSOR channels. Immunofluorescence studies confirmed that BOT facilitated LRRC8A translocation to the plasma membrane.

These findings suggest that BOT promotes Cl^- release and subsequent water excretion by activating VSOR channels via LRRC8A translocation to the plasma membrane. This mechanistic insight provides a molecular basis for BOT's clinical efficacy in fluid regulation, bridging empirical use with scientific evidence.

Ethical Compliance: All procedures involving human subjects were conducted in accordance with the Declaration of Helsinki and approved by the ethics committee of Akita University.

A 18-02 Exploration of the mechanism of Occludin co-polymerisation within Claudins' strands in the Tight junctions.

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Tight junctions (TJs) are important in establishing barriers that are either impermeable or selectively permeable to water, ions or small molecules. While selective permeability of TJs depends on Claudin proteins (Cldns), the function of another tetraspan membrane protein – Occludin (Ocln) – remains unclear.

Ocln has two extracellular loops (ECLs) and a mutation in ECL2 has been linked to brain calcification in humans. Complete knockout of Ocln in mice results in a similar phenotype. Cldns seal the intercellular space through the formation of complex polymerized structures (*i.e.* strands), and Ocln has been shown to contribute to the complexity of these strands, thereby modulating the strength of the paracellular epithelial barrier.

In this research we investigate through which mechanism Ocln, and especially its ECLs, supports the Cldn-based TJ barrier. Using stimulated emission depletion (STED) microscopy, we demonstrate that Ocln attaches to Cldn strands *in vitro* and *in vivo*. Moreover, we show that the amino acid composition of the first and second ECLs, particularly the presence of aromatic amino acids, is important for the proper localization of Ocln to strands formed by Cldn2. Moreover, replacement of ECLs with fragments from an evolutionary unrelated but structurally similar protein preserves the colocalization of Ocln with Cldn2. The functional importance of the ECL residues

was tested in epithelial MDCKII cells through protein reconstitution and permeability measurements.
In this study, we describe how Ocln incorporates into Cldn polymers, and identify a role for aromatic ECL residues in TJ incorporation and barrier formation.

A 18-03 Ultrastructural Mechanisms Governing Blood-Brain Barrier Integrity

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Aim

The blood-brain barrier (BBB) is a dynamic, semi-permeable endothelial interface that insulates the brain's microenvironment from chemical and hormonal fluctuations in the peripheral circulatory system and regulates molecular flux between the circulatory system and the central nervous system (CNS). While transcellular flux is well characterized, the role of paracellular ultrastructures in BBB formation and maintenance remains underexplored. In this study high-resolution scanning electron microscopy was used to visualize ultrastructural dynamics during BBB genesis, revealing for the first time membrane bound exosomes. These nanosized vesicles (30-300nm) were observed facilitating paracrine signalling and forming nanotubular structures, which contribute to endothelial alignments and BBB structural integrity.

Methods & Results

To elucidate their functional role, we inhibited the exosome biogenesis with Tipifarnib, which led to disrupted barrier integrity, underscoring their involvement in intercellular communication. Additionally cytoskeletal analysis using Cytochalasin D and Nocodazole confirmed the roles of F-actin and Tubulin-alpha in the structural integrity of exosome and non-exosome associated nanotube formation as evidence of reduced transendothelial electrical resistance upon cytoskeletal disruption. Comparative studies with primary rat cardiac microvascular endothelial cells further illuminated the BBB's unique structural features.

Conclusions

Given the association of BBB dysfunction to neurodegenerative diseases, our findings suggest that exosome mediated signalling and nanotube formation represent novel therapeutic targets for conditions associated with BBB permeability and provide new insights into molecular mechanisms governing BBB integrity.

A 18-04 An intricate interplay between Na^+/H^+ exchangers NHE1, NHE3, NHE8 and HCO_3^- transport differentially affects single cell migration and invasion of colon cancer cells (HT29-MTX)

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In colorectal cancer (CRC), Na^+/H^+ exchanger 1 (NHE1) hyperactivity is associated with elevated intracellular pH (pH_i), tumor growth, motility and chemoresistance. NHE3 and NHE8 expression, however, is reduced in human CRC, and in mice, their dysfunction increases CRC growth. Their role in CRC metastasis is unknown. The contributions of NHE1, NHE3, NHE8 and HCO_3^- to HT29-MTX cell migration and invasion were compared in an 80% NHE8 knockdown (NHE8-KD) and the corresponding mock control cell line.
In the NHE8-KD, the nominal absence of HCO_3^- decreased migration activity whereas invasion into a Huh7 cell layer was hardly HCO_3^--dependent. In the presence of HCO_3^-, inhibition of NHE1 with BI-9627 or NHE3 with tenapanor reduced migration to the same extent with no difference between the two clones; co-administration of BI-9627 and tenapanor had an additive effect. In both clones, invasion was delayed by BI-9627 alone, while co-administration of tenapanor almost abolished the effect of BI-9627. Tenapanor alone accelerated the invasion of only the NHE8-KD.
Na^+-dependent pH_i recovery from cytosolic acidification, measured in the nominal absence of HCO_3^-, was slightly higher in the NHE8-KD, with no difference in intrinsic

buffering capacity. BI-9627 and its co-application with tenapanor strongly decreased pH_i-recovery in both clones, whereas tenapanor alone increased it in the NHE8-KD. NHE1, NHE3, NHE8 and HCO_3^- contribute to CRC cell motility, with NHE1 being the most potent player that can compensate for NHE8-deficiency. NHE8-deficiency alone does not increase metastatic potential. Chemotherapeutic targeting of individual NHEs or NBCs would not be effective in CRC treatment.

A 18-05 Combined exposure to microplastics and particulate matter leads to intestinal inflammation and loss of barrier integrity- the protective efficacy of kefir peptides

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Aim

Epidemiological studies have linked microplastics (MP) and particulate matter (PM) to the development of inflammatory bowel disease (IBD). However, whether combined exposure to MP and PM exacerbates IBD pathogenesis remains unclear. Moreover, the molecular mechanisms underlying pollutant-induced intestinal inflammation and barrier dysfunction are not well understood. This study also explores whether kefir peptides can mitigate such intestinal damage.

Methods & Results

C2BBe1 intestinal epithelial cells were exposed to MP and PM for 24 hours. DHE staining revealed that combined exposure significantly increased oxidative stress. This co-treatment also elevated ICAM-1 expression and enhanced monocyte adhesion to intestinal cells. Furthermore, MP+PM exposure upregulated the tight junction protein ZO-1 while downregulating MUC2 levels. Mechanistically, MP and PM induced endoplasmic reticulum (ER) stress, marked by increased GRP78 and ATF6 expression, and activated autophagy, as shown by elevated p62 and LC3B levels and confirmed by acridine orange (AO) staining. The interplay between oxidative stress, ER stress, and autophagy was further investigated using specific inhibitors. Notably, pretreatment with kefir peptides significantly reduced oxidative stress, decreased ICAM-1 expression, restored ZO-1 levels, and alleviated both ER stress and autophagy.

Conclusions

MP and PM, common environmental pollutants, pose significant risks to intestinal health. This study provides new insights into how these pollutants induce intestinal inflammation and compromise barrier integrity. Importantly, kefir peptides effectively mitigated these adverse effects, primarily through their antioxidant properties. These findings suggest that kefir peptides hold promise as a potential therapeutic strategy for preventing or managing pollutant-induced IBD.

A 18-06 Connexin26 Hemichannels are Involved in Lipopolysaccharide-induced Alteration of the Barrier Function of Respiratory Airway Epithelial Cells

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Epithelial cells form a barrier against pathogens and initiate inflammation via pattern recognition receptor (PRR) activation by pathogen-associated molecular patterns (PAMPs), like lipopolysaccharides (LPS). Connexin (Cx) hemichannels, which allow exchange of ions and small molecules (< 2 kDa) between the extra- and intracellular milieu, show an enhanced activity in response to PAMPs or damage-associated

molecular patterns (DAMPs). Cx hemichannels can release DAMPs which reinforces inflammation, therefore prolonged channel opening could result in chronic epithelial inflammation. Since barrier dysfunction is linked to chronic inflammatory diseases, Cx hemichannels may directly influence the epithelial barrier. Using Calu-3 cells as model of airway epithelial cells, ethidium bromide (EtdBr) uptake assays showed that LPS enhanced the EtdBr uptake into the cells. siRNA-knockdown experiments identified Cx26 as responsible Cx isoform. Immunofluorescence staining of precision cut lung slices (PCLS) showed higher Cx26 signal in LPS-treated PCLS. Furthermore, CVB4-57, a potential Cx26 hemichannel inhibitor as predicted with molecular docking analyses, suppressed the enhanced EtdBr uptake after LPS treatment to control levels. Transepithelial electrical resistance (TEER) measurements in Calu-3 cells showed a reduced barrier function after LPS application, which was accompanied with a reduced presence of claudin-4 in tight junctions (TJ), the key structure for epithelial barrier function. Finally, CVB4-57 attenuated LPS-induced changes in TEER and TJs, probably by blocking Cx26 hemichannels.

These results indicate a direct link between an increased Cx hemichannel activity and changes in barrier function after PAMP exposure, which may suggest Cx hemichannels as new therapeutic targets for chronic inflammatory diseases of the epithelial systems.

A 18-07 Podocyte exopher formation as a novel pathomechanism of immune complex removal

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Aim

To unravel the mechanism leading to glomerular antigen/autoantibody deposition in the vicinity of podocytes in membranous nephropathy, we studied large vesicles containing aggregated proteins and organelles –called exophers– using a THSD7A^{+}-MN cell culture model.

Methods & Results

Human podocytes were seeded on chamber slides and differentiated for 10 days before exposure to AF-647-labeled anti-THSD7A antibody. Live imaging was performed using CellMask™ to visualize the podocyte plasma membrane, along with THSD7A antibodyand the vesicle marker 14-3-3. Imaging was conducted with an LSM980 Airyscan 2 confocal microscope. Cells were then fixed and stained to assess oxidative stress via CellROX™, as well as vesicular marker proteins such as 14-3-3 and annexin A1, and NCAM1 or annexin A2 as potential contributors to exopher formation.

Autoantibody binding is initiated at the membrane of podocytes, especially at the thin actin processes containing THSD7A, leading to small aggregate formation at peripheral membranes. These aggregates translocate along the cell surface to the juxtanuclear membrane region, fusing to larger aggregates. Membrane budding generates a large membrane-stalked extrusion, containing the targeted antigen and autoantibody destined for removal while maintaining a membranous connection. A mature exopher further contains 14-3-3, annexin A1 and A2, peroxidated lipids, and NCAM1. Live imaging suggests 14-3-3 plays a role in exopher translocation, moving with and towards membrane-bound antibody aggregates.

Conclusions

In human podocytes, exopher formation involves a membrane-bound antigen-autoantibody translocation process. Understanding the dynamics of exopher formation and release opens new avenues for research in podocyte autoimmune injury.

A 18-08 Differential uptake of nanodiamonds in cardiomyoblasts and adult rat cardiomyocytes.

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The author has objected to a publication of the abstract.

A 18-09 Aquaporin-5 as a potential new target in breast cancer treatment

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Aim

Increased level of the water channel aquaporin-5 (AQP5) is correlated with poor prognosis and survival in breast cancer patients, making it a potential therapeutic target. A previous study by Villandre J. *et al*. (2022) identified FDA-approved drugs capable of attenuating AQP5 levels in lung epithelial cells. Our study aims to determine whether these drugs can similarly reduce AQP5 protein levels in a breast cancer cell line to evaluate their potential use in breast cancer treatment.

Methods & Results

Human breast cancer cell line MCF7, stably overexpressing myc-tagged AQP5, was treated with Lenvatinib, Ruxolitinib, Ceritinib, Vemurfenib, Ponatinib, Regorafenib or Sorafenib at various concentrations for 48 hours. AQP5 protein levels were assessed via Western blot using an anti-myc antibody. Drugs showing promising AQP5 reduction were tested in 3-4 independent experiments. Data was normalized to total protein and compared to the DMSO control.

Treatment with the drugs Lenvatinib, Ruxolitinib, Ceritinib, Ponatinib and Regorafenib showed a tendency to reduce AQP5 protein levels in MCF7 cells. In contrast, Vemurfenib and Sorafenib appeared to increase AQP5 levels and were excluded from further analysis.

Conclusions

Several drugs including Lenvatinib, Ruxolitinib, Ceritinib, Ponatinib, and Regorafenib demonstrated promising ability to reduce AQP5 protein levels, suggesting their potential for future use in breast cancer treatment. Further studies are needed to evaluate their impact on breast cancer pathogenesis. We are currently testing the drugs in 3D breast cancer cultures with the aim to progress into in vivo studies.

A 18-10 Lithium prevents the neurotoxic effects of paclitaxel mediated through TRPA1 channels

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Aim

Paclitaxel (PTX) is a drug that is commonly used in cancer chemotherapy despite its neurotoxicity. TRPA1s are essential mediators of sensory transduction and nociception. These cation channels are associated with PTX-induced neurotoxicity, which Li+, a well-known neuroprotective molecule, can prevent. This study aimed to determine the effect of Li+ on the neurotoxicity induced by PTX and its impact on TRPA1 channels.

Methods & Results

We used the SH-SY5Y cell line to assess cell viability via the MTT assay. The intracellular Ca2+ concentration in Fura-2-loaded cells was assessed via spectrofluorometry. TRPA1 channel electrophysiology was evaluated via the whole-cell patch-clamp technique. The effects of PTX, Li+, and TRPA1 agonists and antagonists were tested. PTX (100 nM) significantly decreased cell viability, and Li+ (10 mM) attenuated this effect. AITC (300 µM), a TRPA1-selective agonist, reduced cell viability, and the effect was more pronounced when PTX was present. HC-030031 (50 µM), a selective TRPA1 antagonist, significantly decreased the cytotoxic effect of PTX. Li+ diminished the cytotoxic effects of TRPA1 activation in the absence and presence of PTX. PTX increased TRPA1 currents and intensified the TRPA1-mediated intracellular Ca2+ increase, and Li+ neutralized both effects.

Conclusions

These findings suggest a potential role for Li+ as a neuroprotective agent, preventing neuronal damage caused by PTX through a mechanism involving TRPA1 channel activation.

A 18-11 The HUSH complex sustains endothelial angiogenic activity through epigenetic repression of Zinc finger transcription factors

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Aim

The human silencing hub complex (HUSH) is an important regulator that represses retroelements of the DNA, thereby contributing to innate immunity against genome invaders. Previously, we demonstrated that the long non-coding RNA (lncRNA) *HIF1α-AS1* recruits HUSH to partially repress specific target genes in endothelial cells (EC). We hypothesize that in EC, HUSH has a function beyond silencing viral products of reverse transcription.

Methods & Results

Analysis of FANTOM5 transcriptome data revealed high endothelial expression of the core HUSH components MPP8, TASOR and Periphilin. The central subunit MPP8 interacted with H3K9me3 and dsDNA. RNA-seq of >100 EC datasets showed ubiquitous HUSH expression, with MPP8 being upregulated by high shear stress and downregulated by hypoxia. Proteomic studies identified novel MPP8 interactors, including TASOR2, TAF15, and NUMA1, suggesting expanded regulatory roles for the complex.

MPP8 deletion mutants revealed that its C-terminal domain binds the lncRNA *HIF1α-AS1*, while its N-terminal domain -including the chromodomain- is important for dsDNA binding. Knockdown of MPP8 or the effector histone methyltransferase SETDB1 impaired the angiogenic capacity of EC. CUT&RUN identified MPP8 binding sites at many different gene clusters, predominantly at Zinc finger transcription factors (ZNF) genes. RNA-seq and ATAC-seq following MPP8 or SETDB1 depletion revealed a strong overlap in target genes, among them >100 upregulated C2H2-type ZNFs.

Conclusions

In summary, the HUSH complex has a function beyond silencing of viral products, and supports gene regulation and angiogenesis. This might be possible due to the endothelial HUSH complex containing additional, non-canonical members.

A 18-12 *Can the EZH2-dependent reshaping of the microvascular endothelial epigenome explain the alteration of angiogenic capacity after wildland fire $PM_{2.5}$ exposure?*

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Aim: The frequency and severity of large-scale wildland fires is increasing worldwide. Acute exposure to wildland fire smoke increases the risk for adverse cardiovascular-related events, particularly in individuals with microvascular vulnerability. Wildland fires are a source of combustion-derived noxious particle matter (PM). Of these PM, those with a diameter less than 2.5 micrometers ($PM_{2.5}$) are small enough to pass the alveolar-capillary barrier and enter the bloodstream where they could interact with endothelial cells (ECs) lining blood vessels. Dysfunction of ECs and loss of their angiogenic capacity often precede adverse cardiovascular-related events. Therefore, we investigated how wildland fire $PM_{2.5}$ (WFP) could contribute to the dysfunction of primary human pulmonary microvascular ECs (HPMVECs) *in-vitro*.

Methods & Results: We first characterized a dose-response between WFP concentrations and HPMVEC viability/proliferation. Even at very low doses, we found WFP to significantly impair angiogenic capacity by reducing cell migration and

vascular tube formation. RNA-sequencing revealed WFP to largely alter HPMVEC angiogenic gene expression. Among WFP affected genes we identified Enhancer of Zeste Homolog-2 (EZH2), a histone methyltransferase that supports EC homeostasis and angiogenic activity. Immunoblotting revealed WFP to induce an inactive form of EZH2 (p-EZH2-T311) and to reduce basal EZH2 expression in HPMVECs. To delineate the relevance of these findings we performed chromatin immunoprecipitation-sequencing (ChIP-seq) for the canonical EZH2 histone modification $H3K27^{me3}$. Alignment of our RNA-seq and ChIP-seq datasets identified EZH2-dependent genes that could contribute to HPMVEC dysfunction.

Conclusions: Our future directions are to identify preventive or therapeutic interventions that could mitigate the negative impact of WFP on HPMVEC.

A 18-13 Protective role of *Geranium palustre L.* extract against oxidative tissue damage in gentamicin-induced nephrotoxicity mouse model

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Aim

The present study aimed to investigate the nephroprotective and antioxidant potential of *Geranium palustre* L. extract to reduce acute renal oxidative tissue damage and toxicity of the aminoglycoside antibiotic gentamicin (GN) in an experimental mouse model.

Methods & Results

The study was conducted with adult Balb/c mice divided into four groups (n = 6). GN nephrotoxicity was induced by administration of GN 200 mg/kg i.p. for 10 days. The control group received saline. The other groups received either *Geranium palustre* L. extract (10mg/kg, p.o.) applied alone or together with GN. To monitor renal antioxidant status, activities of certain antioxidant enzymes, parameters for lipid and DNA peroxidation and renal functional damages were investigated by standard commercial kits, ELISA and EPR spectroscopy.

The antioxidant effect of *Geranium palustre* L. was demonstrated by reduced malondialdehyde (4.06±0.23μmol/ml), ROS (1.78±0.13a.u.) and 8-hydroxy-2'-deoxyguanosine (5.07±0.39ng/ml) levels compared to the GN treated group (6.11±0.33μmol/ml, 3.56±0.11a.u. and 7.96±0.42ng/ml, respectively). Additionally, increased activation of superoxide dismutase (3.21±0.16U/gPr vs. 1.03±0.17U/gPr), catalase (3.07±0.15U/gPr vs. 1.56±0.13U/gPr) and glutathione (57.09±3.36nmol/gPr vs. 21.09±2.22nmol/gPr) were observed in kidney homogenates of animals having received GN in combination with plant extract, compared to GN group. Nephroprotection was also exhibited by increased expression of PGC-1α (peroxisome proliferator-activated receptor γ coactivator-1alpha) and decreased levels of KIM-1 (Kidney Injury Molecule-1) after plant extract application.

Conclusions

The preserved kidney antioxidant and functional status in groups treated with plant extract indicates that *Geranium palustre* L. could be clinically applied as means of reducing the toxic effects of GN application.

A 18-14 Torin1 modifies the kinetics the LPS induced activation of NF-κB

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Aim

Autophagy is the degradation of cell intern components and is important for the cellular homeostasis. A possible regulator of autophagy is TLR4, a pattern recognition receptor and a component of the innate immune response. During an infection the activation of TLR4 is related to inflammation and autophagy.

Since both processes are highly dynamic it is of interest to analyse the kinetic of the interactions between the receptor activation and autophagy. For this purpose we

examine the effect of torin1, an effective autophagy inductor, to the TLR4 signaling pathway.

Methods & Results

We generated a NF-κB-sensitive luciferase-reporter construct by linking the NF-κB responsive promoter to the luciferase gene. HEK293 cells were retroviral infected with the NF-κB-sensitive luciferase-reporter, to get a stable cell line. The HEK-Luciferase cell line also stably expresses the TLR4/CD14/MD2 receptor complex. The cells were stimulated with LPS and Torin1 alone or in combination in different concentrations. The luciferase activity was measured in a luminometer, which allows analysing the cells for 24 hours or more.

Torin1 inhibits the LPS induced TLR4 activation pathway by reducing the NF-κB activation signal. We also see a difference in the kinetic of the reaction over the time. Torin1 as an inductor of autophagy also influencees the TLR4 signaling pathway.

Conclusions

It is shown that the luciferase-reporter can be used to analyse the inhibition of NF-κB activation when the autophagy inductor torin1 is added. Therefore, the assay might offer a tool to analyse the dynamic of TLR4 activation with autophagy inductors.

A 19 | MOLECULAR PHYSIOLOGY

A 19-01 HMGB domain associated proteins are potential RNA-DNA triplex binding proteins

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Background: Interaction with DNA is one mechanism by which long non-coding RNAs (lncRNAs) affect gene expression. An important mechanism responsible for this interaction is RNA-DNA triplex formation. Triplex formation ex vivo, however, has been shown to be rather unstable. In line with other concepts of epigenetics, it is therefore attractive to postulate that proteins act as readers, writers and erasers for this interaction.

Methods & Results: To identify RNA-DNA triplex-associated proteins, unbiased screens were performed: In a global approach, antibodies against dsDNA and RNA-DNA hybrids were used in combination with RNase H treatment to digest non-triplex hybrids. Co-precipitated proteins were identified by MS-anaylsis. This data was combined with a computational ChIP-Atlas/TripLexicon pipeline. This revealed enrichment for transcription factors, helicases, and chromatin remodelers like SP1, RUVBL2, and HDAC1. Notably, several of the proteins identified in the pull-down, e.g., HMGB2, TOX4, and SSRP1, exhibited significant enrichment of HMG domains. To identify proteins in the triplex of cardiovascular disease-linked lncRNA MEG3 with TGFBR1, IDAP (Isolation of DNA-associated proteins) was performed using synthetic lncRNA oligos mimicking MEG3s triplex-forming region and the corresponding TGFBR1 DNA target site. After IDAP with HEK cells and HUVECs, more than 100 interacting proteins could be recovered. Interestingly, after IDAP with HEK cells and HUVECs, several high enriched proteins were pulled down, including HMGB2.

Conclusion: Enrichment of triplex-associated proteins is possible from different cells and reveals the HMG domain as potential triplex binder.

A 19-02 Long non-coding RNA-SWI/SNF subcomplex assembly and targeting in iPSC differentiation

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The control of chromatin accessibility is essential for gene expression in various cellular processes. The SWItch/Sucrose Non-Fermentable (SWI/SNF) chromatin remodeling complex actively maintains genome-wide chromatin accessibility and orchestrates cell type-specific gene expression via its dynamic genomic targeting. In addition to tumorigenesis, SWI/SNF dysfunction also drives the pathogenesis of cardiovascular diseases. However, how SWI/SNF activities are regulated remains understudied, especially in the cardiovascular context. Our recent publication elucidated the pivotal roles of trans-acting lncRNAs in recruiting SWI/SNF to endothelial cell-specific enhancers. However, where and how lncRNAs bind and regulate SWI/SNF subcomplexes dynamically remains unknown.

IP-MS and complexome profiling analyses revealed that SWI/SNF interacts with hundreds of accessory proteins in human endothelial cells. Three approaches were employed to identify the accessory protein subunits of SWI/SNF: affinity purification-mass spectrometry (AP-MS), IP-MS, and complexome profiling. Combined with the RNA regulation, we aim to investigate how SWI/SNF accessory proteins cooperate with lncRNAs in rendering functional specificity to SWI/SNF by employing iCLIP to identify regulatory lncRNAs. Subsequently, protein and lncRNA candidates will be selected for perturbation for subsequent CUT&RUN for SWI/SNF genomic localization, ATAC-seq for chromatin accessibility, and RNA-seq for gene expression analysis. Furthermore, we plan to dissect the role of SWI/SNF in cardiovascular development and differentiation by employing iPSC differentiation to cardiomyocytes and endothelial cells. iPSC-derived cells will be harvested at different time points for the aforementioned assays.This project aims to profile the integrative RNA-protein interactome of SWI/SNF in a cardiovascular context-specific manner and to reveal the regulatory roles of RNA-protein crosstalk on SWI/SNF during cardiovascular development and differentiation.

A 19-03 Neddylation stabilizes IκB, which in turn suppresses cell migration mediated by NF-κB

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Aim

Renal cell carcinoma (RCC) is the most common form of kidney cancer, and activation of the nuclear factor kappa B (NF-κB) pathway is known to drive its malignant progression. NF-κB activity is tightly regulated by IκB-α, which sequesters NF-κB in the cytoplasm. Neddylation, a post-translational modification involving the conjugation of NEDD8 to target proteins, plays a critical role in modulating protein stability and function. In this study, we aimed to determine whether inhibition of neddylation by MLN4924 affects RCC progression by disrupting the IKK/IκB-α/NF-κB signaling pathway.

Methods & Results

Human RCC cell lines were treated with MLN4924, a selective neddylation inhibitor. Western blotting was used to assess protein expression and post-translational modifications. Cell migration assays were conducted to evaluate changes in cancer cell motility. The involvement of the PI3K/IKK signaling axis was examined using specific pathway inhibitors. Neddylation status of IκB-α was assessed to determine its role in protein stability and NF-κB regulation.

Conclusions

Our findings demonstrate that IκB-α is neddylated in RCC cells, contributing to its stabilization and suppression of NF-κB activity. MLN4924 treatment activated the PI3K/IKK pathway, promoted IκB-α degradation, and enhanced NF-κB-dependent transcription and cancer cell migration. These results suggest that neddylation serves as a protective mechanism for IκB-α and that its inhibition by MLN4924 may inadvertently promote RCC malignancy through NF-κB activation. Targeting

neddylation in RCC therefore requires careful evaluation due to its dual role in tumor regulation.

A 19-04 Structural insights into the polymerase catalyzed FAD-capping of hepatitis C viral RNA

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The RNA polymerase NS5B of HCV is capable of catalyzing the addition of flavin adenine dinucleotide (FAD) to its RNA as a 5′ cap structure, aiding the virus in evading host immune responses. However, the exact mechanism underlying the 5′-FAD capping process of HCV RNA remains to be elucidated. Here, we determined crystal structures of the HCV NS5B de novo initiation, primed initiation and elongation complexes in presence of FAD. Structural analysis and comparisons showed that residues M447 and Y448 of the β loop in the priming element (PE) of NS5B is the determinant for specific recognition of FAD. The adenine group of FAD is exclusively paired with the uracil base at the 3′ end of the template RNA strand. At the initial elongation stage, the C-terminal linker (residues 530-570) of NS5B is involved in stabilizing the 5′ FAD, which in turn induces sequential conformational changes of the bases in the product strand and creates a unique intermediate state of the RNA duplex, facilitating the translocation of the product strand. Our study offers novel insights for developments of new anti-HCV therapies.

A 19-05 Pemetrexed Enhances γδ T Cell-Mediated Cytotoxicity in NSCLC via the ATM-STING-NF-κB Axis

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The author has objected to a publication of the abstract.

A 19-06 Genetic control of DNA methylation : example of the *CD36* gene

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Aim

[The objective was to determine the control of a CD36 polymorphism on DNA methylation during these conditions.Please replace your content here]

Methods & Results

[we collected women aged at least 18 years (50 healthy controls, 50 obese, 50 type 2 diabetics and 50 obese type 2 diabetics). Determination of the polymorphism (rs3211867) by RT-PCR and of the methylation by MS-PCR was carried out with leukocyte DNA. sCD36 was determined by ELISA test. The protocol was carried out according to the Declaration of Helsinki (1989) then approved by the UCAD ethics committee (Protocole : 027512018/CERruCAD).

Among diabetic subjects of the CC genotype, 63.2% had methylation compared to 33.3% among diabetic subjects of the AA+AC genotype (p=0.04). In obese diabetic subjects, 25% of carriers of the CC genotype had methylation compared to 70.6% of carriers of the AA+CC genotype (p=0.003). Among carriers of the AA+AC genotype, methylation concerned 37.9% of control subjects; 33.3% of diabetic subjects; 55.6% of obese subjects and 70.6% of obese diabetic subjects. The presence of the AA+AC genotype reduces sCD36 (3426.0 ± 831.2 & 1858.7±673.5 p=0.026). This reduction persists when DNA methylation is added (5775.5 ± 1363.9 & 1858.7 ± 673.5 p=0.08).]

Conclusions

[differential genetic variability would have effects on DNA methylation leading to a lifting of inhibition on the expression of the CD36 gene.]

A 19-07 HDAC6 regulates breast cancer stem cells via Akt/Stat3 signaling axis

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Triple-negative breast cancer (TNBC) is the most aggressive breast cancer subtype, characterized by poor prognosis and high recurrence rates. Breast cancer stem cells (BCSCs) are a small subset of cells within breast tumors that play a crucial role in tumor initiation, metastasis, and chemotherapy resistance. They are responsible for the development of chemoresistance and radioresistance. Targeting BCSCs may be an appropriate approach for the treatment of breast cancer. Histone deacetylase (HDAC) 6 has emerged as a key regulator in various oncogenic pathways, but its role in the regulation of BCSCs remains poorly understood. In this study, we investigated the function of HDAC6 in BCSC maintenance using a specific HDAC6 inhibitor, Tubastatin A (Tub A). Treatment with Tub A significantly reduced the BCSC population in MDA-MB-231 cell line, decreased expression of stemness markers ($CD44^+/CD24^-$ and ALDH), impaired tumorsphere formation, and induced downregulation of pluripotency-associated genes (Nanog, Oct4, and Sox2). Furthermore, Tub A treatment suppressed tumor growth and reduced the BCSC population in the xenograft model. Mechanistically, we found that HDAC6 inhibition disrupted the Akt/Stat3 signaling axis and led to increased phosphorylation activity of Stat3 in Ser727. Inhibition of Akt or Stat3 mimicked the effects of HDAC6 inhibition, confirming the importance of this pathway in BCSC regulation. These findings suggest that HDAC6 promotes BCSC maintenance via the Akt/Stat3 signaling pathway and that targeting HDAC6 with Tub A may offer a promising strategy for eliminating BCSCs in TNBC.

A 19-08 Multi-omics Profiling of Serum Exosomes Identifies Potential Biomarkers for Brain Metastasis in Lung Cancer

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Aim

Brain metastasis (BM) is a frequent and devastating complication in lung cancer, occurring in up to 50% of patients and leading to poor clinical outcomes. In this study, we aimed to discover potential biomarkers in serum exosomes for early detection of BM in lung cancer using an integrative multi-omics approach.

Methods & Results

We utilized a lung cancer mouse model that spontaneously develops brain metastases and collected serum samples at distinct disease stages (normal, lung cancer without brain metastasis, and lung cancer with brain metastasis). Serum-derived exosomes were isolated and analyzed by small RNA sequencing and proteomic LC-MS/MS analysis. Our integrated analysis identified exosomal miR-206-3p as a key microRNA upregulated during brain metastasis, with target genes enriched in cancer-associated pathways including Hippo, MAPK, Ras, and PI3K-Akt

signaling. Proteomic profiling further revealed 77 proteins elevated in brain metastatic exosomes, highlighting vinculin (VCL) as a promising biomarker candidate. Notably, VCL was specifically enriched in serum exosomes during brain metastasis, despite its decreased expression in lung tissues and unchanged levels in brain tissues. Clinical data analysis supported the prognostic value of VCL, with higher expression correlating with worse patient survival.

Conclusions

Our study demonstrates that exosomal miR-206-3p and VCL represent potential non-invasive biomarkers for brain metastasis in lung cancer. These findings may contribute to improved early detection and therapeutic monitoring of metastatic progression.

A 19-09 SENP3-governed epigenetic signaling regulates sarcomere organization and cachexia.

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The sarcomere is a highly organized functional unit of myofibrils in striated muscle. Precisely arranged repetitive arrays of the sarcomeres are crucial for muscle cells to generate force and shortening during muscle contraction. The myofibrillar/sarcomere disarray poses an underlying cause of abnormal force generation in human diseases including cachexia. A detailed understanding of how this specialized, coherent sarcomeric structure is modulated and maintained, remains poorly understood. Here, we report that the SUMO isopeptidase SENP3 serves an important role to maintain sarcomere assembly and thereby the normal function of myofibrils. As compare to progenitor myoblasts, SENP3 is upregulated in differentiated myotubes and promotes selective recruitment and deSUMOylation of SETD7 histone methyltransferase specifically on the molecular motor protein coding gene *myosin-II*. Moreover, the same mechanism precludes Suv39h1 histone methyltransferase from *myosin-II*. Thus, SENP3 enhances transcriptionally active histone monomethylation on lysine 4 (H3K4me1) and reduced transcriptionally repressive H3K9me3 from *myosin-II* gene. We showed that perturbation of SENP3 expression results in deficient production of MyHC-IId followed by severe disruption of sarcomeric assembly and contractile potential of myotubes. Importantly, SENP3 was degraded in denervation-induced cachexia. As a consequence, transcription of *myosin-II* was also directly affected. Restoration of the SENP3 level in cell culture model of cachexia could significantly rescue *myosin-II* gene expression, a hallmark of cachexia. Altogether, our findings showed an unanticipated link between SUMOylation pathway and cachexia. This study also present potential therapeutic implications regarding cachexia

A 19-11 An *in vivo* atlas of the proteome and phosphoproteome reveals fundamental principles of cell and tissue organization

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Aim

To uncover how protein abundance and turnover establish tissue-specific cellular function, we created a comprehensive in vivo atlas of proteome and phosphoproteome turnover that allowed the identification of global organizing principles in several tissues.

Methods & Results

We obtained a high-coverage dataset containing over 256,000 turnover measurements derived from in vivo metabolic labeling combined with DIA and TMT-based mass spectrometry. The combined approach ensured accurate quantification of total protein and site-specific phosphorylated peptide turnover. Our work provides a large dataset enabling browsing of protein abundance (PA) and turnover (PT) data across tissues.

Our multi-layered examinations revealed several fundamental principles: (1) Adaptive speed is facilitated by short-lived, high-abundance proteins, while tissue function buffering is exerted by low-abundance, long-lived proteins; (2) Phospho-regulation, altering PT site specifically, modulates stability of major proteins such as

Tau and SNCA, indicating a homeostatic role of phospho-regulation that extends beyond transduction signaling; (3) Protein-protein interaction partners share similar PTs, indicating coordinate degradation through complexes; (4) Inter-tissue comparison stressed that peroxisomal proteins exhibit inverse PA and PT profiles. Notably, tissue-level PT reflects both protein degradation and cell turnover, which underscores differences in proliferative versus post-mitotic tissues.

Conclusions

Our large resource provides a quantitative foundation for understanding how cells can manage proteome homeostasis in complex tissue environments. By integrating protein turnover with abundance, phosphorylation, and interaction networks, the atlas reveals underlying principles of regulation of proteome structure, tissue identity, and functional diversity. It paves the way for additional high-resolution exploration.

A 20 | ION CHANNEL PHYSIOLOGY

A 20-01 Electrophysiological evaluation of neuronal electrical activity in *Clcn4*$^{-/-}$and *Clcn4*^{A549V} rat hippocampal neurons

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CLCN4 encodes the intracellular 2Cl^-/H^+ exchanger ClC-4, an endosomal protein highly expressed within the CNS, including the hippocampus. Variants in *CLCN4* resulted in complex neurological manifestations, such as developmental delay, intellectual disability, behavioural issues and epilepsy. Over 140 individuals with *CLCN4*-related conditions have been identified, with Ala555Val being particularly frequent (Palmer et al., 2023; He et al., 2024; and Li et al., 2023). The disease aetiology remains unknown. Here, we used *Clcn4*$^{-/-}$ and *Clcn4*$^{A549V/0}$ rat models (founded by cureCLCN4) to study the impact of ClC-4 deletion and dysfunction on neuronal excitability and function. p.A549V corresponds to p.A555V in humans, and it reduced to ~40% Cl^-/H^+ transport (Guzman et al., 2022). Using acute hippocampal slices and patch-clamp electrophysiology, we assessed firing patterns and action potential properties of CA1, CA2, and dentate granule cells in *Clcn4*$^{A549V/0}$ and *Clcn4*$^{-/-}$ and compared them to WT slices. WT Pyramidal neurons and dentate granule cells show two distinct firing patterns: regular spiking (RS) and rhythmic bursting (RB). Upon ablation "*Clcn4*$^{-/-}$" or dysfunction "*Clcn4*$^{A549V/0}$" of ClC-4, neuronal firing patterns were substantially altered across different hippocampal regions. The RB neuron population decreased while RS neurons increased, suggesting reduced excitability in mutant cells. The burst-firing defect was rescued by blocking Kv7/KCNQ and Kv1 K^+ channels, linking Cl^-/H^+ exchange activity, these K^+ channel families and neuronal excitability. Pharmacological targeting of Kv7 and/or Kv1 may help alleviate symptoms associated with the *CLCN4*-related conditions. We conclude that homeostasis plasticity of neuronal intrinsic excitability tightly depends on the Cl^-/H^+ exchange function of ClC-4.

A 20-02 Sex differences of Cav1.3 function in DLS-projecting dopamine substantia nigra neurons

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We recently demonstrated that Cav1.3 channels act as full-range linear amplifiers of firing rates in dorsolateral striatum projecting dopamine substantia nigra (DLS-DA SN) neurons in male mice (Shin et al., 2022). However, the role of Cav1.3 in DLS-DA SN neurons in female mice remained unexplored.

To this end, we compared firing rates across the entire dynamic range in both sexes by dynamic clamp, where we stimulated neurons with a virtual NMDA receptor conductance (gNMDA) in the range from 4-32 nS. To identify Cav1.3 function selectively, we recorded from Cav1.2DHP-/- mice with and without 300nM isradipine in the bath solution.

First, we identified a sex difference in baseline excitability with DLS-DA SN neurons from females being significantly less excitable by injecting gNMDA in dynamic clamp (male ctrl: 0.98±0.30Hz/nS, n=16, N=3; female ctrl: 0.66±0.17Hz/nS, n=19, N=4,

p=0.0012). In addition, the excitability of DLS-DA SN neurons from females were – in contrast to males– not affected by 300nM isradipine (male ISR: 0.74±0.22Hz/nS, n=17, N=5; female ISR: 0.75±0.23Hz/nS, n=15, N=4, p=0.9424). Indeed, sex differences were eliminated in the presence of isradipine. Currently, we are investigating further potential sex differences in other calcium channels or in calcium handling and coupling to calcium-activated potassium channels.Our results demonstrate sex differences in excitability and Cav1.3 function in DLS-DA SN neurons. It remains to be shown whether these cellular differences also result in functional dopamine differences on the behavioural level and might contribute to sex differences in vulnerability to neurodegeneration as observed in Parkinson Disease (Baldereschi et al., 2004).

A 20-03 Cellular mechanisms of low repeat spike timing-dependent plasticity (STDP) at hippocampal input synapses to the mPFC of adult mice

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Fear extinction is a complex behavioural mechanism to reshape consolidated fear memories, involving neuronal circuits of hippocampus, mPFC, and amygdala. BDNF is a key regulator of fear extinction and neuronal plasticity alongside the hippocam-pus-mPFC axis. However, whether BDNF-depen-dent STDP in mPFC contributes to fear extinction is unknown.

Here, we used patch-clamp-recordings in layer III/V neurons to record STDP at excitatory inputs in acute mPFC slices from adult mice. Building upon our previous findings that STDP protocols involving postsyn-aptic theta-burst-firing induce BDNF-dependent timing-LTP (t-LTP) at hippocampal CA3-CA1 synapses, we estab-lished a new protocol that elicited t-LTP with the least possible number of repeated pairings (Δt= +10 ms) of 1 presynaptic spike with a theta-burst of 4 postsynaptic spikes (1:4 pairing). We found that 12 repeats (at 0,1Hz) of this protocol were sufficient to elicit t-LTP in IL-mPFC pyramidal cells and GABAergic interneurons. Using specific inhibitors we found that this 12x 1:4 t-LTP could be observed under conditions of intact GABA-ergic inhibition and did not require NMDA-receptor activation.

Next, we applied TrkB-receptor-bodies (TrkB-Fc) to investigate whether endogenous BDNF release from hippo-campal input fibers or postsynaptic mPFC pyramidal cells is involved in 12x 1:4 t-LTP. Moreover, we will investigate whether this possibly BDNF-dependent 12x 1:4 t-LTP is specific for hippocampal inputs or can be observed also at intra-cortical excitatory inputs within the IL-mPFC. These experiments should reveal the role of endogenous BDNF secretion in physiological relevant low repeat t-LTP in the IL-mPFC.

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A 20-04 “Multi-patch clamp analysis of principal neuron-interneuron microcircuits in human neocortex”

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Principal neuron networks in the neocortex are controlled by diverse sub-types of GABAergic interneurons. Recent work showed that this control defines more subtle embedding of these interneurons into the microcircuit architectures in some regions of the rodent neocortex to promote specific computations (Peng et al. 2021). Given the larger proportion of GABAergic interneurons in the human neocortex compared to rodents (Loomba et al. 2022), we hypothesized that in the human neocortex specialization of Gabaergic interneurons has increased. To study the integration of interneurons into micro-circuits in the neocortex of humans, we used multi-patch clamp recordings in acute brain slices (up to 6 simultaneous recordings) of layer 2/3 of human neocortex. To identify interneurons, with a focus on Parvalbumin-positive, fast-spiking interneurons for our cluster recordings, we used either Wisteria Floribunda Lectin fluorescein or H2DCFDA (Gotti et al. 2021). Both labelling strategies worked well in tissue from rodents, but unfortunately was less successful in the human tissue, partially due to the strong autofluorescence in the human

neocortex. Accordingly, the present data set contains only few identified interneurons (n=17) and thus preliminary information with regard to principal neuron-interneuron synaptic interactions (10/90 identified unitary inhibitory IN-PN synapses and 10/90 unitary excitatory PN-IN synapses). We conclude that the rather sparse (~ 11%) connectivity in both directions between principal neurons and interneurons in layer 2/3 of the human neocortex may serve as a first indication of more inhibitory micro-circuit specificity in the human neocortex, which requires further investigations in future research.

A 20-05 Effects of Neuropeptide Y on hippocampal network oscillations in vitro

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Neuropeptide Y (NPY) is an abundant neuromodulator in the mammalian brain, including the hippocampus . It acts through five G-protein-coupled receptors (R), from which Y1 and Y2 R are most widely expressed. NPY is involved in fear reduction, the regulation of stress responses and emotional memory processes. The hippocampus is involved in these actions. At the same time, it exhibits different patterns of oscillatory network activity, which are associated with specific memory processes. Sharp wave-ripples (SPW-R) are associated with memory consolidation, while gamma rhythms with memory encoding.
Combining these findings, we hypothesized that the effects of NPY on anxiety and fear memory may be associated with changes in hippocampal network oscillations. We examined the two dominant patterns (SPW-R and gamma oscillations) in mouse brain slices, containing the ventral-to-intermediate hippocampus. NPY caused a decrease of SPW-R activity, predominantly in the CA1 subregion of the hippocampus. The effect could be replicated with a selective Y2 R agonist, but not by activating Y1 R. In contrast, carbachol-induced gamma oscillations were not strongly affected by NPY. Our results are suggestive of selective Y2R-mediated actions of NPY on network processes supporting memory consolidation, but not memory acquisition.

A 20-06 The role of endosomal Na^+/H^+exchanger NHE6 in iron homeostasis and metabolism in neurons and astrocytes

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Disturbances in iron homeostasis and mitochondrial activity have been implicated in the pathology of neurodegenerative diseases. Mutations in endosomal Na+/H+exchanger NHE6 (SLC9A6) underlie several X-linked neurodevelopmental diseases including the devastating disease Christianson Syndrome, symptoms of which include non-verbal status, seizures and severe cognitive disabilities. We hypothesize that NHE6 loss alters the tight link between iron homeostasis and mitochondrial function leading to oxidative stress, neuronal malfunction and pathology. To test this, we subjected SH-SY5Y human neuroblastoma cells with stable NHE6 knockdown (KD) or overexpression (OE) and used primary cultures of neurons and astrocytes from WT and NHE6 KO mice to analyze iron homeostasis and mitochondrial function. Protein expression of Transferrin receptor and divalent-metal-ion-transporter-1 was downregulated in NHE6 KO astrocytes and SH-SY5Y cells while ferritin levels were upregulated. Interestingly, we did not observe any changes in labile iron pool measurements. Hydrogen peroxide treatment increased reactive oxygen species (ROS) levels in NHE6 KO SH-SY5Y cells compared to WT and NHE6 OE cells. While loss of NHE6 led to an increase in mitochondrial activity in SH-SY5Y cells, we did not observe any changes in astrocytes and neurons. In conclusion, our results are consistent with the notion that dysregulation of iron homeostasis and mitochondrial activity could contribute to the pathology of NHE6 loss but that precise downstream effects differ between cell types. In ongoing work, we study ferroptosis and mitochondrial iron and ROS levels to dissect the link

between iron homeostasis and mitochondrial function in WT and NHE6 KO neurons and astrocytes.

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A 20-07 Serotonin bidirectionally modulates network activity in the developing hippocampus via 5-HT4 and 5-HT1A receptors

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The formation of neural circuits in the hippocampus depends on early patterned neuronal activity, and disturbances during critical periods may contribute to neurodevelopmental disorders. Among these, autism spectrum disorder has been associated with alterations in serotonin (5-HT)-mediated neuromodulation. Serotonin is one of the earliest neurotransmitters expressed in the developing brain and is broadly released throughout the central nervous system. However, whether 5-HT directly affects early hippocampal network activity remains unclear. Here, we investigated the effects of 5-HT on CA1 network dynamics in acute hippocampal slices from mice during the first postnatal week, employing confocal calcium imaging and patch-clamp electrophysiology. Activation of G_s-coupled 5-HT4 receptors increased the frequency of giant depolarizing potentials (GDPs), enhanced neuronal participation during GDPs, and facilitated asynchronous activity. Conversely, activation of $G_{i/o}$-coupled 5-HT1A receptors reduced both synchronous and asynchronous network activity. Preliminary data suggest that 5-HT4 receptors also increased the frequency of spontaneous excitatory postsynaptic currents (sEPSCs), indicating enhanced glutamatergic transmission. Agonists of 5-HT2A and 5-HT7 receptors had no significant effects. Our findings demonstrate that 5-HT modulates early hippocampal activity in a bidirectional manner via distinct receptor pathways. This indicates that altered serotonergic signaling during early development may disrupt activity-dependent synaptogenesis, potentially contributing to the pathogenesis of neurodevelopmental disorders.

A 20-08 Endothelin-1 modulates hippocampal and hypothalamic network activity in mouse primary dissociated cultures and brain slices

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Endothelin-1 (ET-1) has been extensively studied in the past not only as a potent vasoconstrictor, but also as a neuromodulator in circuits processing fear, pain and anxiety. Previous electrophysiological studies report uncertainties about ET-1 effects in brain tissue due to vasoconstriction and tissue movements. We now studied the expression of ET-receptors (EdnrA and EdnrB) and responses to ET-1 in the hippocampus (HPC) and the caudal hypothalamus (cHPT) containing the tuberomamillary nucleus with histaminergic neurons (HN) that promote arousal. Receptor expression and effects on firing frequency and neuronal network activity were measured with single-cell RT-PCR, microelectrode-array (MEA) recordings in a reduced cell culture system with 60 recording electrodes and patch-clamp recordings in mouse brain slices.

Among all MEA electrodes, firing frequency was significantly reduced in HPC and increased in cHPT. Single-channel analysis revealed heterogenous responses after ET-1 application, with inhibition prevailing in HPC and excitation prevailing in cHPT. Synchronicity of neuronal discharge, measured by Cohen's kappa, was significantly increased in cHPT but not in HPC. HN recorded in brain slices responded to ET-1 in a bosentan (EdnrA/EdnrB antagonist)-sensitive way and expressed EdnrB (70% of cells) and EdnrA (3 out of 7 EdnrB-positive cells).

We conclude that in cultures devoid of vasculature, ET-1 differentially modulates the spontaneous network activity of HPC and cHPT. By synchronizing neuronal network activity in the cHPT and exciting HN, ET-1 may influence arousal states. The underlying mechanisms of receptor signalling remain under investigation.

A 20-09 Eight novel variants in *CLCN3* expand our understanding of the molecular and phenotypic spectrum of *CLCN3*-related condition

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Variations in *CLCN3*, the gene encoding the endosomal ClC-3 transporter, have been linked to human disease. A cohort of 11 individuals was initially described (Duncan et al., 2021), followed by two additional cases (Nakashima et al., 2023), and only four *CLCN3* variants have been functionally characterized to date. Systematic studies linking clinical phenotypes with functional data in *CLCN3*-related disorders remain limited.

In this study, we comprehensively reviewed and expanded the clinical and genetic spectrum of *CLCN3*-related neurodevelopmental conditions. We analyzed both newly diagnosed and previously reported cases, identifying eight novel heterozygous missense variants in *CLCN3* (NM_001829.4: p.Tyr208Cys, p.Gly211Asp, p.Thr234Ile, p.Ala247Pro, p.Gly294Val, p.Phe614Leu, p.Val700Ile, and p.Arg749Glu).

The novel variants were functionally tested using HEK293 cells, Western blot, confocal fluorescent microscopy, and patch-clamp electrophysiology. Clinically, all affected individuals exhibited global developmental delay/intellectual disability, delayed speech, and facial dysmorphism. Other common features are behavioral and/or psychiatric disorder (71.43%), ophthalmological abnormalities (66.67%), hypotonia (63.16%), feeding difficulties (57.14%), seizures (42.85%) and hearing impairment (31.58%). Most variants arose *de novo*.

Functionally, the majority of the novel variants significantly reduced ClC-3 transport activity, with p.Gly211Asp and p.Gly294Val completely abolishing function. p.Gly294Val affected ClC-3 protein levels. Variants p.Ala247Pro, p.Thr234Ile, and p.Arg749Glu altered the voltage dependence of ClC-3. Transport efficiency was reduced by p.Thr234Ile, but enhanced by p.Ala247Pro and p.Arg749Glu. None of the variants disrupted the subcellular localization of ClC-3. Our findings expand the phenotypic and genetic spectrum of *CLCN3*-related conditions and demonstrate that loss of function is the predominant pathogenic mechanism of the variants identified in this study.

A 20-10 Calcium-activated chloride channel TMEM16A opens via pi-helical transition in transmembrane segment 4

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TMEM16A (Anoctamin-1) is a Ca^{2+}-activated Cl^- channel that has crucial roles in various physiological and pathological processes. However, the structure of the open state of the channel and the mechanism of Ca^{2+}-induced pore opening have remained elusive. Using extensive molecular dynamics simulations and patch-clamp electrophysiology, we demonstrate that TMEM16A opens a hydrated Cl^--conductive pore via a pi-helical transition in transmembrane segment 4 (TM4). We also describe a coupling mechanism that links pi-helical transition and pore opening to the Ca^{2+}-induced conformational changes in TMEM16A. Finally, we designed a pi-helix-stabilizing mutation (I551P) that facilitates TMEM16A activation, revealing atomistic details of the ion-conduction mechanism.

A 20-11 Analgesic effects of acupuncture on migraine via trigeminal pathway

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Aim

This study explored the pain-relieving effects of acupuncture on nitroglycerin (NTG)-induced rat migraine.

Methods & Results

Twenty-four rats were randomly assigned to control, model and acupuncture groups (n=8). Rats in the control group received saline injections, while in the model group received NTG injections, and in acupuncture group underwent acupuncture treatment. Behavioral assessments and head pain threshold tests were performed. After transcardially perfused, tissues including the trigeminal ganglion (TG), dura mater, and brain were collected. Immunofluorescent staining was conducted to evaluate the expression levels of c-fos, calcitonin gene-related peptide (CGRP), tryptase, and lysosomal-associated membrane protein 1 (LAMP-1). Compared to the control group, rats in the model group displayed heightened irritability and reduced pain thresholds (P<0.05). Acupuncture treatment significantly improved pain thresholds (P<0.05). In the model group, c-fos expression was predominantly observed along the trigeminal pathway. Additionally, CGRP expression in the TG and Sp5, as well as tryptase and tryptase/LAMP-1 co-expressing mast cells in the dura mater, were significantly elevated compared to the control group (P<0.05). These elevated levels were notably reduced after acupuncture treatment (P<0.05).

Conclusions

These findings suggest that acupuncture exerts its therapeutic effects on migraines by targeting the trigeminal nerve system, providing a potential non-pharmacological approach for migraine management.

A 20-12 Kit$^+$ interstitial cells are targets of nitrergic neurotransmission in mouse lower oesophageal but not urethral smooth muscle sphincter

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The lower esophageal sphincter (LES) plays a critical role in preventing reflux of gastric contents into the esophagus, while contractions of urethral smooth muscle (USM) prevents leakage of urine during bladder filling to maintain continence. Both LES and USM generate tonic contractile activity and relax in response to nitric oxide (NO), released from nitrergic neurons. Both organs contain Kit$^+$ interstitial cells of Cajal (ICC in LES) or Kit$^+$ ICC-like cells (ICC-LC in USM). Previous studies on mutant animal models lacking ICC, or lacking post-junctional receptor for NO (soluble guanylate cyclase, sGC) exhibit impaired LES responses to nitrergic innervation. Similarly, spontaneous activity in isolated urethral ICC-LC are inhibited by NO donors. These data suggest that Kit$^+$ interstitial cells in the LES and USM may be involved in transducing inhibitory nitrergic signals to SMC in these sphincters. However, direct visualization of effects of nerve stimulation on these cells *in-situ* is lacking. In mice expressing the genetically encoded Ca^{2+} sensor GCaMP6f, specifically in Kit$^+$ cells (Kit-GCaMP6f mice), we found that electrical field stimulation (EFS) inhibited LES ICC Ca^{2+} signalling under - non-adrenergic, non-cholinergic (NANC) conditions, and this was reversed by L-NNA (NO synthase inhibitor). In contrast, EFS under NANC conditions failed to affect Ca^{2+} signalling in urethral ICC-LC. LES ICC activity was inhibited by the NO donor sodium nitroprusside (SNP 1 mM), but NO donors did not affect urethral ICC-LC. Our data suggest that Kit$^+$ interstitial cells are targets of nitrergic neurotransmission in the lower esophageal but not urethral smooth muscle sphincter.

A 20-13 Differentiation of hippocampal immediate early gene responses to frequency-dependent locus coeruleus stimulation

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Aim

Coincident activation of the locus coeruleus (LC) and hippocampal afferents results in hippocampal synaptic plasticity[1]. Depending on the frequency of activation, the retrieval of recent and remote spatial memory is impaired or facilitated[2]. This raises the question as to whether different LC activation frequencies exert different effects on hippocampal information processing.

Methods & Results

Fluorescence in situ hybridization (FISH), used to detect experience-dependent nuclear immediate early gene (IEG) expression offers a precise localization of neurons that respond to a specific associative experience or the induction of synaptic plasticity[3]. Here, we explored whether electrophysiological stimulation of the LC in freely behaving male Wistar rats results in differences in nuclear IEG expression in the hippocampus. The LC was stimulated at 2Hz and 100Hz frequencies, which promote dopamine D1/D5 receptor, or β-adrenergic receptor-dependent hippocampal long-term depression (LTD), respectively. Following FISH, hippocampal subfields were segregated and analyzed to their putative dorsal and ventral stream inputs[3].

We observed that LC stimulation at 2Hz significantly increased nuclear IEG mRNA expression in the CA1 compared to control conditions. By contrast, no significant differences in IEG expression were found in the CA1 following LC stimulation at 100Hz.

Conclusions

Taken together these data suggest that 2Hz, but not 100Hz LC stimulation triggers hippocampal IEG gene encoding. This suggests that synaptic plasticity resulting from the coincident, frequency-dependent activation of the LC and hippocampus enables LTD by means of mechanistically distinct processes.

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A 20-14 Inter-site synchrony of ripple oscillations as a hallmark of GABAergic function

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Aim

Neuronal oscillations are considered a non-invasive biomarker of intact brain network function and cognitive processes, founded in a delicate balance of neuronal excitation and inhibition ("E/I-balance"). However, particularly the analysis of fast oscillations (> 100 Hertz) is challenged by an overlap of electrophysiological parameters between putatively physiological ("ripple") and pathophysiological, disinhibited events ("high frequency oscillations"). Analyzing the interactions of fast oscillations at several sites may improve on distinguishing these phenomena.

Methods & Results

We re-analyze previously published recordings of ripple oscillations in the rodent hippocampus *in vivo* and identify ripple oscillations by a marked increase of inter-site correlation across all sub-fields of the hippocampus. We further investigate this cross-correlation in an *ex vivo* slice model of mouse hippocampal slices and find it is disproportionately dependent on intact GABAergic, rather than glutamatergic, signaling. Further, this dependency relies on the cell-type specific contribution of of fast-spiking, parvalbumin expressing interneurons (PVIs). Finally, we explore novel avenues targeting PVIs to differentially modulate inter-site synchrony both in the rodent model as well as in acute brain tissue derived from human epilepsy patients.

Conclusions

Assessing the synchronization of fast oscillations at several sites permits a non-invasive discrimination between putatively physiological and pathophysiological brain network states. This capability can be traced to a cell-type specific contribution

of PVI-mediated GABAergic transmission. Leveraging this cell-to-network interaction in brain slices of humans predicts a new approach to developing future therapies.

A 20-15 Investigating the role of I_{Ks} and I_{Kr} in the electrophysiological responses to sympathetic nerve stimulation in an innervated isolated rabbit heart preparation

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The author has objected to a publication of the abstract.

A 21 | PHYSIOLOGY OF POTASSIUM CHANNELS

A 21-01 Thermosensitivity of TREK K2P channels is controlled by a PKA switch and depends on the microtubular network

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The author has objected to a publication of the abstract.

A 21-02 NOVEL MODULATORS OF HUMAN TWIK-RELATED SPINAL CORD K^+ (hTRESK) CHANNELS UNCOVER AN UNSELECTIVE DRUG INTERACTION SITE

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Aim

The hTRESK channel is a member of the two-pore-domain (K_{2P}) family of potassium (K^+)channels, which are responsible for controlling the resting membrane potential in various cells. Modulators of hTRESK are potential drug candidates and useful experimental tools. Here, we describe novel inhibitors and a novel activator of the hTRESK channel and show that these disparate compounds share a similar binding region.

Methods

We identified novel hTRESK modulators by screening different lipids e.g. anandamide (AEA) and small molecules like ML67-33 etc., that are known to modulate other potassium channels, in excised patches from *Xenopus* oocytes. To gain mechanistic insights, we utilized competition experiments with Quaternary Ammonium (QA^+) ions, which are known pore blockers in other K_{2P} channels, and we tested the effects of these modulators on the alanine mutant of hTRESK F352, a phenylalanine residue that is part of a putative constriction below the selectivity filter of TRESK channels.

Results & Conclusions

Our measurements revealed that many potassium channel modulators inhibited TRESK currents, often with very high affinities. All novel modulators competed with QA^+ ions for binding, showing their presence in the pore. Furthermore, they all bound with decreased affinity to the hTRESK F352A mutant. This points to a notably unselective drug interaction site located below the selectivity filter in the pore of TRESK channels that is utilized by very disparate hTRESK modulators. The new

activating substance must bind at this site without blocking permeation and activate TRESK channels by a so far unknown mechanism.

A 21-03 Characterization of the dynamic movement of DII-S4 voltage sensor in Two-pore channel 3

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Aim:

Two-pore channels (TPCs) are a family of voltage-gated cation channels localized on the membranes of intracellular organelles such as endosomes and lysosomes. TPCs consist of two homologous domains, and the S4 helix in domain II (DII) is primarily responsible for voltage sensing. Our previous research has shown that DII-S4 undergoes a unique two-step movement. This study further investigates the details of the voltage-dependent movement of DII-S4 in TPC3 using a combination of multiple techniques.

Method & Results:

We applied cysteine scanning mutagenesis, accessibility analysis of the introduced cysteines with reagent modification and cadmium bridging, and voltage clamp fluorometry (VCF) to TPC3 expressed in *Xenopus* oocytes. The systematic mutations in DII-S4 identified some residues critical for voltage sensitivity, including Gln508, Leu510, Asp511, and Phe514, along with the classical gating charge residues such as Arg517 (the first gating charge in DII-S4). These identified cysteine mutations were further analyzed with cadmium bridging and cysteine-accessibility analysis to pinpoint neighboring positions during the voltage-dependent movement, providing insight into the DII-S4 movement. In addition, VCF analysis with the mutations affecting voltage sensitivity (D511A and R517Q) revealed that Arg517 is essential for the first of the two steps of DII-S4 movement.

Conclusion:

Our study elucidated the unique voltage-dependent movement of DII-S4 in TPC3, expanding our understanding of the complex gating mechanisms in TPCs.

A 21-04 Licorice ingredients modulate GIRK channel activity and atrial rhythms

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Aim

Licorice is a popular sweetener added to food and beverage. Overconsumption of licorice can induce atrial fibrillation. Abnormal activation of G-protein-gated inwardly rectifying K^+ (GIRK) channel is associated to the pathology of atrial fibrillation. Here we aim to clarify whether licorice disrupts atrial rhythms via modulation of GIRK channel activity.

Methods & Results

Using electrophysiological recordings in *Xenopus* oocytes expressing the cardiac-type GIRK channel, we investigated the effects of glycyrrhizic acid (GA), the main ingredient of licorice, and its metabolite, 18β-glycyrrhetinic acid (18β-GA), on GIRK currents. GA decreased the GIRK currents, whereas 18β-GA increased them. To identify structural determinants of drug effect, we performed site-directed mutagenesis and molecular docking analysis. Mutation of a glutamate residue located at the cytoplasmic pore of GIRK channel prohibits the 18β-GA-induced activation, suggesting its involvement in drug binding. Motion analyses using primary cultured rat atrial myocytes demonstrated that 18β-GA suppress spontaneous beating, which effects are reversed by the GIRK channel blocker, tertiapin-Q. Since 18β-GA is also known to activate mineralocorticoid receptor (MR) signaling and potentially induce arrythmias, we tested whether this pathway contributes to the observed effects. Application of the MR antagonist canrenone failed to reverse the 18β-GA-induced suppression of beating, thereby ruling out a role for MR signaling mediating 18β-GA effects.

Conclusions

Our results indicate that licorice metabolite 18β-GA activates cardiac GIRK channel and a glutamate residue at cytoplasmic region is critical for the drug action. 18β-GA down-regulates atrial contraction via activation of GIRK channel, suggesting a potential mechanism of licorice-induced atrial arrythmias.

A 21-05 $K_{Ca}3.1$ channel inhibition as a therapeutic approach in osimertinib-resistant lung cancer

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Aim

Using in vitro models, we investigate the therapeutic potential of pharmacological inhibition of $K_{Ca}3.1$ channels for osimertinib-resistant non-small cell lung cancer (NSCLC). Osimertinib is a third-generation EGFR-tyrosine kinase inhibitor that is a standard of care for EGFR-mutated NSCLC. However, acquired resistance hinders its long-term efficacy, presenting an urgent need for alternative strategies. $K_{Ca}3.1$ channels are known to be overexpressed in NSCLC, promoting cellular aggressiveness and worsening clinical outcome of the patients.

Methods & Results

In this study, we employed atomic force microscopy, live-cell imaging, cell death and viability assays to evaluate the impact of $K_{Ca}3.1$ channel inhibition on osimertinib-resistant PC9ER-AZDR cells. We compare the influence of osimertinib, senicapoc ($K_{Ca}3.1$ channel inhibitor) and a combination of both substances. Our findings revealed that the double treatment is the most effective approach. It reduced NSCLC migration velocity and cell viability by approximately 30%. Furthermore, it led to a threefold increase in the number of dead cells. Interestingly, we observed that $K_{Ca}3.1$ channel activity stiffens NSCLC cells, which may enhance their migratory capabilities. Long-term experiments performed on these cells show, that they retained their sensitivity to the two drugs even after several months of combination treatment.

Conclusions

These results suggest that $K_{Ca}3.1$ channels play an important role for the well-being of osimertinib-resistant NSCLC cells. Targeting these channels together with osimertinib seems to be an interesting therapeutic concept for overcoming acquired resistance.

A 21-06 Crosstalk of *KCNH1* and *KCNH5* Gain-of-Function Mutations Leading to Neurodevelopmental Disorders

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Voltage-gated K^+ channels (Kv, encoded by *KCN* genes) are tetrameric protein complexes. Gain-of-function (GOF) mutations in *KCNH1* (Kv10.1, hEAG1) and *KCNH5* (Kv10.2, hEAG2) lead to developmental disorders, intellectual disability, and epilepsy. Currently, there is no straightforward way to associate clinical symptoms to functional properties of mutated channels. Here we investigated how members of the *KCNH* subfamily might be affected by heteromerization with mutant Kv10.1 or Kv10.2 subunits.

The *de novo* variant *KCNH1*-G496E , which is associated with impaired neurodevelopment and epilepsy (Fukai, J Hum Genet 61, 2016), was expressed alone and with other wild-type subunits in HEK293T cells and characterized using whole-cell patch-clamp. While *KCNH1*-G496E alone did not yield functional K^+ channels, coexpression of *KCNH1* shifted the half-maximum voltage of activation (V_h) from −22.5 ±1.8 mV (*n*=8, wild type) to −82.6 ±1.6 mV (9). *KCNH1*-G496E also induced GOF when coexpressed with *KCNH5*, shifting V_h from −54.1 ±3.4 mV (9) to −81.6 ±1.6 mV (6). Likewise, the homologous mutation *KCNH5*-G465E did not yield functional channels but induced GOF upon coexpression with wild-type *KCNH1* or *KCNH5*. By contrast, the mutants did not affect the function of Kv11.1 (*KCNH2*, hERG1) channels. To infer the relevance of *KCNH1/5* GOF mutations under physiological conditions, we employed the fluorescent genetically encoded voltage

indicator rEstus-mK2 and found that both, *KCNH1* and *KCNH5*, hyperpolarized HEK293T cells, while coexpression of the GOF mutants further augmented hyperpolarization.
Our findings imply that interpretation of clinical symptoms related to *KCNH1/5* GOF mutations requires considering the functional heteromerization of Kv10.1 and Kv10.2 subunits.

A 21-07 A complete genetic loss-of-function in a K_{2P} channel linked to Ivemark syndrome II

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Congenital heart defects with heterotaxia combined with asplenia are known as Ivemark syndrome type II. The presence of families with increased susceptibility to the disease suggests a genetic origin. However, no specific gene has been conclusively linked to the disease and only a few genes have been associated with heterotaxia. Using a trio whole exome sequencing approach, we identified a homozygous deleterious stop variant in a two-pore domain potassium (K_{2P}) channel in a newborn with Ivemark syndrome type II. The developmental phenotypic spectrum included a complex cardiac manifestation with an imbalanced atrio-ventricular septal defect combined with a double outlet right ventricle, aortic valve stenosis/extension, patent ductus arteriosus (PDA), PDA-dependent perfusion of the lower body together with an anomaly of the knuckles and an asplenia. In addition, recurrent infections of the skin and respiratory tract were observed. While cardiac developmental disorders have not been previously reported for K_{2P} channel mutations, developmental phenotypes are consistent with ion channels playing an essential role in embryonic development by regulating cell proliferation, apoptosis, differentiation, migration and intercellular communication. Our electrophysiological studies revealed a complete loss of channel function, so the patient suffers from a complete functional knock-out of this K_{2P} channel. As we also found that this particular K_{2P} channel forms functional heterodimers with other K_{2P} channels of the same or other subfamilies, the patient most likely suffers from a multi-channel dysregulation, which is likely to contribute to this novel channelopathy with a severe developmental and multi-systemic phenotype.

A 21-08 Functional Role of K2P Channels in the Regulation of Tone in Systemic Arteries

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Aim

Organ perfusion is regulated by arterial tone, which is determined by the membrane potential of vascular smooth muscle cells. Potassium (K^+) channels are key modulators of this potential. Notably two-pore domain K^+ (K2P) channels play roles in negative feedback regulation of vasoconstriction and pH-induced vascular tone. While well studied in the pulmonary circulation, their function in the systemic circulation remains largely unexplored. We hypothesized that K2P channels, particularlyTASK-1 or TREK-1 channels, contribute to tone regulation in the systemic circulation.

Methods & Results

Intact segments of a rat systemic artery (Arteria saphena) were studied ex vivo using wire myography. For TREK-1 channels, the selective blocker spadin and the opener ML335 were explored. TASK-1 channel function was examined using the blocker ML365. Due to their small conductance, we aimed to unmask them by blocking major potassium conductances with iberiotoxin (BK_{Ca} channels) and DPO-1 ($K_v1.5$ channels). We did not detect any relevant effect of spadin alone or in the presence of ML335, either in the absence or in the presence of IBTX+DPO-1. ML335 reduced methoxamine-induced contractions, suggesting an unspecific effect at the tested concentration. We did not detect any relevant effect of ML365 in the absence of IBTX+DPO-1,but increased vascular tone in the presence of IBTX+DPO-1, with ML365 being more potent than the previously studied AVE1231.

Conclusions
Our data suggest that in A. saphena TREK-1 channels do not contribute to basal or methoxamine-induced tone. In contrast, TASK-1 channels contribute to negative feedback on vasoconstriction when dominating potassium conductances are suppressed.

A 21-09 The role of K^+ channels for neutrophil migration

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The author has objected to a publication of the abstract.

A 21-10 Channel Pharmacology and Calcium Sensitivity of Small Conductance Calcium-Activated Potassium Channels: Automated Whole-cell vs Inside-Out Patch Clamp Assays

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Aim Small conductance calcium-activated potassium channel subtype 3 (SK3) plays a crucial role in cardiac excitability and endothelial cell regulation. SK3 channels are gated in a voltage-independent manner through calcium/calmodulin interactions. Understanding their pharmacology and Ca^{2+}-dependent function is essential for elucidating their physiological and pathological roles. This study aim to characterize SK3 channel pharmacology and Ca^{2+} sensitivity using automated patch-clamp system and compare its reliability with inside-out patch-clamp electrophysiology.
Methods & Results HEK293 cells transiently transfected with SK3 were studied, using automated whole-cell patch-clamp (QPatch 16X and 48X) assays. SK3 channel response to the specific inhibitor apamin and activator NS309 were characterized. Additionally, SK3 calcium sensitivity were investigated and the results were benchmarked against inside-out manual patch-clamp electrophysiology Ca^{2+} sensitivity was evaluated using different intracellular Ca^{2+} concentrations. In QPatch experiments, SK3 channels were highly sensitive to apamin (IC50 at 1 µM Ca^{2+} = 67.27±13.84 pM, mean±SEM; n=13). NS309 exhibited Ca^{2+}-dependent activation, with an EC50 of 1.32±0.10 µM at 300 nM Ca^{2+} (n=6) and 6.21±0.45 µM at 100 nM Ca^{2+} (n=8). SK3 channel Ca^{2+} sensitivity in whole-cell QPatch and inside-out patch-clamp assays was comparable (EC50 = 622.30±1.81 nM (n=5-8) vs. 694.20±4.81 nM (n=6)).
Conclusions Collectively, our results demonstrate that the high-throughput patch-clamp system QPatch enables both screening of SK3 channel pharmacology and precise control of intracellular Ca^{2+} levels, allowing for accurate measurement of Ca^{2+} sensitivity comparable to the inside-out patch-clamp configuration.

A 21-11 Probing a contribution of erg (KCNH) potassium channels to the electrical response properties of Auditory Outer Hair Cells

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Aim
[Cochlear outer hair cells (OHCs) drive electromotility-based cochlear amplification. The required high-frequency electrical responses of OHCs are supported by a voltage-gated potassium current with unusual voltage dependency, termed $I_{K,n}$. $I_{K,n}$ activates at an atypically negative voltage (half activation around -80 mV), such that it is constitutively activated in OHCs.
KCNQ4 (Kv7.4) K+ channel subunits have been identified as principal molecular components of $I_{K,n}$. Thus, genetic deletion of KCNQ4 abolished $I_{K,n}$ in mouse models, and loss-of-function mutations cause human hereditary hearing loss (DFNA2). Despite this essential role, recombinant KCNQ4 does not reproduce the atypical biophysical characteristics of IK,n. In contrast, KCNQ4 mediates a typical M-type potassium current with half-maximal activation at around -20 mV *in heterologous expression systems*. Therefore, we hypothesized that unidentified hair cell-specific

interaction partners modulate KCNQ4 activity, resulting in a channel complex with $I_{K,n}$ properties.]

Methods & Results

[Single-cell transcriptomics showed the expression of erg channel subunits (*erg1* and *erg2*, KCNH2, and KCNH6, respectively) in OHCs. Using immunohistochemical labeling with specific antibodies, we here confirm expression of both erg channel subunits in mouse OHCs and show subcellular co-localisation with KCNQ4 subunits at the basal pole of OHCs.

Patch-clamp was performed on OHCs from conditional *erg1/erg2* double knockout mice and transgenic mice carrying a dominant negative loss-of-function mutation in the KCNH6 gene (G480S). However, $I_{K,n}$ persisted in both mouse models. Current amplitudes and voltage dependence were not significantly different from $I_{K,n}$ recorded from wild-type OHCs.]

Conclusions

[Thus, we exclude erg1/2 as subunits of the native channels that mediate $I_{K,n}$in OHCs.]

A 21-12 Comparison between the effects of NS1643 and NS3623 on the gating properties of hERG

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The human ether-a-go-go related gene (hERG) encodes a voltage-dependent K^+ channel that terminates the cardiac action potential (AP) by accelerating membrane repolarisation. NS1643 and NS3623 are compounds that increase hERG activity in experimental settings. This study aims to document the differences between the effects of NS1643 and NS3623 on hERG. We used the whole-cell patch clamp technique to study the response of hERG to NS1643 [10 µM] and NS3623 [20 µM] at room temperature in the presence of 4 mM (extracellular) and 130 mM (intracellular) K^+. Data are mean ± SEM of n number of cells, paired Student's t-test. NS1643 had no effect on the maximal amplitude of outward tail currents (p = 0.140, n = 5), whereas NS3623 increased it by 74.5 ± 17.1% (p = 0.02, n = 6). NS1643 induced a leftward shift of the voltage dependence of activation by 19.6 ± 4.2 mV (n = 5, p = 0.01), while NS3623 induced a rightward shift by 2.3 ± 0.87 mV (n = 6; p = 0.047). NS1643 shifted the proportions of the open states to favour slow deactivation mode whereas NS1634 increased the fast and slow deactivation rate constants by 38.2 ± 12.5% and 26.4 ± 7.8%, respectively ($p < 0.05$, n = 4 - 6). Both drugs enhanced the repolarisation currents of hERG measured using the AP clamp technique (n > 6, each). In conclusion, NS1643 and NS3623 are two chemical analogues that have different mechanisms of action on hERG.

A 21-13 A Mechanism for hERG Kv Channel Conductance Increase by its Blocker

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Many drugs that block voltage-gated K^+ channels encoded by the human Ether-à-go-go-Related Gene (hERG), can cause long QT syndrome and life-threatening cardiac arrhythmias. The molecular mechanisms that distinguish deadlier from safer hERG blockers remain elusive. Under certain circumstances, a process called facilitation will increase hERG conductance following application of clinically approved hERG blockers including nifekalant, amiodarone, promethazine, imipramine, nortriptyline, haloperidol, verapamil, carvedilol, metoprolol, propranolol, quinidine, fluoxetine, and chlorpheniramine. The circumstances that cause facilitation occur during contraction of cardiac myocytes and might counteract arrhythmogenic hERG block. Since block and facilitation have opposing effects on hERG conductance, they are proposed to result from distinct modes of drug binding. Here, we propose instead that unblock can produce facilitation. Nifekalant is a Class

III antiarrhythmic drug and exemplar blocker that facilitates. We propose that hERG channels blocked by nifekalant are biased toward their open state, and the supranormal conductance of facilitation is mediated by channels that were opened by nifekalant and then became unblocked. In the presence of nifekalant, similar negative voltages trap hERG in blocked and facilitated states, consistent with occupancy of the blocking site underlying facilitation. Rate-theory kinetics models identify the features necessary to produce facilitation. Atomistic models indicate block of the conduction path and suggest modulation of intracellular gate opening by nifekalant. We conclude that distinct binding modes of block and facilitation are not needed to explain facilitation by nifekalant. Rather, an agonism-while-blocking mechanism is sufficient. We speculate that the agonism-while-blocking mechanism contributes to the relative safety of some hERG blockers.

A 21-14 Heterodimerization leads to a K_{2P} channel complex with altered functional properties, receptor-coupling and pharmacology

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Two-pore domain potassium channels (K_{2P}) are modulated by a variety of different physiological stimuli and therefore play an important role in the regulation of the membrane potential. In the last couple of decades, there has been increasing evidence that K_{2P} channels are not only limited to homomer formation, but also form functional heterodimers with distinct characteristics, thereby increasing the functional and pharmacological heterogeneity within this channel family. In the current study we describe the formation of a novel functional heterodimer by performing two-electrode voltage-clamp experiments and inside-out macropatch-clamp recordings, as well as ELISA-based assays to quantify channel expression at the plasma membrane. Most importantly, the heterodimeric channels that we describe here, are characterized by an altered regulation by physiological stimuli, a unique pharmacology and G-protein-coupled receptor-mediated modulation. Therefore, the formation of this K_{2P} channel heterodimer with its altered reactions to different physiological stimuli as well as its distinct pharmacological profile must be taken into account in electrophysiological studies in native tissues as well as in the development of selective drugs, targeting homo- or heterodimeric K_{2P} channels.

POSTER SESSION B

B 01 | HYPOXIC SIGNALLING

B 01-01 Tumor spheroids effectively model hypoxia's impact on adenoviral replication

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Aim

Previous studies demonstrated that hypoxia negatively affects adenoviral replication. It has been a key factor limiting the success of oncolytic adenoviruses. From the other hand, spheroids have emerged as a physiologically relevant model for the hypoxic microenvironment of solid tumors. Here, we sought to determine whether spheroids could serve as a model to study the impact of hypoxia on adenoviral replication, thereby providing a platform for oncolytic adenoviruses in solid tumors.

Methods & Results

We employed hanging-drop method to generate spheroids and first assessed the suitability of different cell lines for spheroid formation. HEK293A and A549 cells, gold standard in adenoviral research, were used to generate spheroids. They were analyzed for stability, circularity, and density via cryosectioning and microscopy. Although these cell lines are permissive to adenoviral infection, our results indicated that they do not form robust spheroids.

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