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Abstracts from the 25th Meeting of the European Council for Cardiovascular Research (ECCR)

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Oral Communication - Friday 30th September, session 3, 17:45 - 19:05h

0-01

Role of ISG15 in cardiac damage mediated by angiotensinII-induced hypertension

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The immune system and different inflammatory cytokines including TNFα and IFNγ play a role in cardiovascular damage in hypertension. We have recently described that interferon stimulated gene 15 (ISG15) and the posttranslational modification that ISG15 produces (ISGylation), play a role in vascular damage and aneurysms formation in hypertension. However, the role of ISG15 pathway in hypertension-associated cardiac damage is unknown. ISGylation is a reversible process driven by the protease USP18. We used knock-in mice (USP18C61A) which show absent protease activity against ISG15 leading to excessive ISGylation, infused with AngiotensinII (AngII; 1.44 mg/kg/day, for 3 and 14 days). USP18C61A mice showed greater blood pressure levels in response to AngII than wild type mice, even at day 1 of treatment. Hearts from AngII-infused USP18C61A mice showed an increase in cardiac hypertrophy characterized by left ventricular hypertrophy, and enlargement of cardiomyocytes and augmented atrial and brain natriuretic peptides, alpha-actin-2 and osteopontin expression. Additionally, AngII-infused USP18C61A mice showed greater expression of fibrotic (collagen I and III, and TGFβ), angiogenic (VE-cadherin and CD31) and inflammatory markers (NOX1, 2 and 4, Il-1β, Il-6, CCL2, CCL5, IFNγ and TNFα) than wild type mice. Notably, most of these changes were also found even in USP18C61A control mice or after 3 days of AngII infusion. Therefore, ISG15 contributes to cardiac damage in hypertension trough the modulation of hypertrophy, fibrosis, inflammation, angiogenesis, and oxidative stress.

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0-02

Proof of biological activity and exploration of early signaling events of Angiotensin-(1-5)

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Introduction: Recombinant human ACE2 increases the circulating levels of angiotensin-(1-5) [Ang-(1-5)], which is regarded as biologically inert. We hypothesized that Ang-(1-5) is a biologically active peptide within the protective arm of the renin-angiotensin-system. **Methods:** To test for biological activity of Ang-(1-5) and for AT2-receptor (AT2R) involvement, nitric oxide (NO) release was measured in AT2R transfected (AT2R-CHO) or non-transfected (NT-CHO) CHO cells loaded with DAF-FM diacetate and subsequently treated with Ang-(1-5) or C21 (AT2R agonist) (0.1nM to 10μM) for 15 minutes. Ang-(1-5) signaling patterns were evaluated by mass spectrometry-based phosphoproteomics in human aortic endothelial cells (HAEC) treated with Ang-(1-5) (1μM) for 1, 3, 5 or 20 minutes. Vehicle treated cells served as controls. **Results:** Both C21 (standard AT2R agonist) and Ang-(1-5) induced a concentration-dependent increase in NO release from AT2-CHO cells. At 1μM (standard in vitro concentration for C21), the Ang-(1-5)-induced NO release [Δ(F/F0): 72.4%; $p < 0.05$ vs control] was significantly stronger than C21-induced NO-release [Δ(F/F0): 25.7%; $p < 0.05$ vs control]. Ang-(1-5) effects were AT2R-specific, since they were absent in NT-CHO cells. Treatment of HAEC with Ang-(1-5) significantly modified the phosphorylation status of 831 proteins at 1799 residues, the majority of which (1079) were dephosphorylated. Most changes occurred after 20 minutes. Functional bioinformatic analysis revealed a cluster of proteins involved in cell cycle and cell division regulation. **Conclusion:** Ang-(1-5) is an endogenous high-efficacy AT2R agonist. The early signaling phosphorylation patterns resemble those of other protective RAS agonists, such as C21 and Ang-(1-7).

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0-03

Treatment with Ace-Inhibitors and Angiotensin Receptos Blockers (ARBs) has no impact on outcome nor on long term consequences in COVID-19 patients with moderate to severe pneumonia

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Aim: ACE-2 receptor is highly expressed on the surface of cardiac and pulmonary cells, and used by coronaviruses to enter host cells; this makes the role of ACE-inhibitors and ARBs controversial. Moreover, it is still unclear whether these drugs may impact on sequelae. **Methods:** In this retrospective study, we analyzed a group of 244 hypertensive unvaccinated patients (134 on ACE-inhibitors, 110 on ARBs) admitted for moderate-to-severe COVID-19 pneumonia. The two groups where homogeneous for demographic data, vital parameters and biochemical examination at admission. Of these patients, 46 (20 on ACE-I and 26 on ARBs) came to a follow-up visit after a mean of 260 days; they underwent a quality-of-life assessment, laboratory and radiologic tests and spirometry (with DLCO). **Results:** A total of 20 of 110 (18%) patients under treatment with ARBs and 23 of 134 (17%) died during hospitalization ($p = 0.8$, NS). At discharge, biochemical, radiological and respiratory data were not significantly different. We did not find any significant difference in terms of radiologic alterations, lung fibrosis, spirometry data, DLCO, persisting effort dyspnea. Biochemical data were substantially super-imposable in the two groups. **Conclusions:** no difference in outcome nor in complications type or number was detected in ACE-inhibitor and ARBs groups. This result seems to support and to strengthen the idea that ACE-inhibitors and ARBs do not play a significant role in onset, evolution and outcome of moderate to severe COVID-19 pneumoniae. Although the number of follow-up patients is small, we did not find any difference in sequelae as well.

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0-04

Vascular and hormonal interactions in primary aldosteronism

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Introduction: Primary Aldosteronism (PA) is the most frequent form of secondary hypertension. We make the hypothesis that adrenal vascular changes may modify adrenal cortex structure and function, creating a propitious environment for developing somatic mutations and Aldosterone Producing Adenoma (APA). **Methods:** To study the interaction between the vasculature and cell proliferation, zonation and aldosterone production in the adrenal cortex, we crossed a new mouse model expressing the Cre recombinase controlled by the Cyp11b2 promoter with mTmG mice (Cyp11b2-Cre::mTmG) allowing to track the zona glomerulosa (ZG) to zona fasciculata (ZF) transdifferentiation. Adrenal structure and vascularization were characterized under basal conditions, high or low salt diets (HSD/LSD) as well as dexamethasone (DEX) treatment, both followed by a recovery period. **Results:** In Cyp11b2-Cre::mTmG mice at day 1, only a few GFP+ cells are present in the ZG, while at day 14 all ZG-cells are GFP+. Subsequently, GFP+ cells transdifferentiate and colonize the ZF. Under HSD, Cyp11b2 expression was reduced, whereas under LSD, the ZG was expanded, Cyp11b2 expression increased and vessels area grew in the whole adrenal cortex. Additionally, salt diets do not affect ZG-to-ZF transdifferentiation. After 2 weeks of DEX treatment, complete disorganization of the ZF was observed; after three weeks of recovery ZF and vessel regeneration was complete. **Conclusion:** Evaluation of the hormonal influence on the steroidogenic cell lineage and the adrenal vascular network, as well as of vascular changes on hormonal parameters, provides new insight in the mechanisms of APA development.

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0-05

Aged Munich Wistar Frömter Rat as a Model of Cardiorenal Syndrome: From Chronic Kidney Disease to Cardiovascular Impairment

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Introduction: Cardiorenal syndrome (CRS, type IV) is the result of chronic abnormalities in renal function progressing to cardiac dysfunction and cardiovascular disease. The availability of suitable animal models of CRS to study the pathogenesis and progression of CRS is essential in preclinical translational research, useful to test new therapeutic strategies. We aim to determine if kidney damage in aged Munich Wistar Frömter (MWF) rat may translate to cardiovascular alterations of CRS. **Methods:** We compared 22 and 45-week-old (aged) male MWF rats (n = 7-10 per group). Systolic (SBP) and diastolic blood pressure (DBP), pulse wave velocity (PWV) and heart rate were determined in anesthetized rats. Vascular functional and structural studies were assessed in thoracic aorta and mesenteric arteries, respectively. **Results:** Both groups were hypertensive with similar SBP and DBP levels. However, 45-week-old MWF showed an increased PWV and heart weight. Aortic contraction to KCl 75mM or noradrenaline (10-10-10-6M) were significantly higher in aged MWF. Relaxation to acetylcholine was similar between groups due to a higher vascular smooth muscle sensitivity to nitric oxide. Vascular hydrogen peroxide was increased in 45-week-old rats, whereas prostacyclin levels were reduced. An outward hypertrophic remodeling and an increased intrinsic arterial stiffness were confirmed in aged MWF by pressure myography. Thoracic aortas exhibited a higher expression of the profibrotic markers CTGF-1 and Col1-A1, while expression of AT2R was lower in 45-week-old MWF. **Conclusion:** The presence of hypertension, arterial stiffness and cardiac hypertrophy, together with cardiovascular fibrosis, inflammation, and remodeling, make aged-MWF rat an useful model for CRS study.

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0-06

Metabolic Effects of Carvedilol on Control and HFpEF Mouse Heart and Hypertrophied H9c2 Cells: The Potential Contribution of a Biased Agonist Effect

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Introduction: Carvedilol is a third-generation β-blocker and a biased agonist that exhibits agonist-like effects through β-arrestins by activating extracellular signal-regulated kinase (ERK). This study investigates the effects of carvedilol on cardiac energy substrate metabolism in heart failure with preserved ejection fraction (HFpEF) and the contribution of β-arrestin proteins to these effects. **Methods:** Acute effects of carvedilol on glucose and palmitate oxidation rates were evaluated in isolated working hearts from 8-week-old male control C57BL/6J mice. For chronic effects, some mice were subjected to an obesity and hypertension HFpEF protocol ± carvedilol for 10 weeks. Glycolysis and the oxidation of glucose, ketones and palmitate were measured. β-arrestins, phospho-ERK and metabolism-related proteins were evaluated by Western blots. In differentiated H9c2 cells, hypertrophy was induced with phenylephrine. Glucose metabolism was measured in these cells treated with or without carvedilol or prazosin. **Results:** Carvedilol had no acute effect on glucose or palmitate oxidation in control hearts, but significantly increased phospho-ERK and pyruvate dehydrogenase activity. In HFpEF mice hearts, glucose oxidation decreased, with a parallel increase in palmitate oxidation. Carvedilol increased overall energy production resulting in an increase in cardiac work and a slight increase in cardiac efficiency. Carvedilol treatment increased the expression of β-arrestin2 and some mitochondrial proteins. In hypertrophic H9c2 cells, carvedilol reduced glycolysis and slightly increased glucose oxidation, resulting in a significant improvement in glycolysis-glucose oxidation coupling. **Conclusions:** Increased β-arrestin2 is accompanied by mitochondrial and metabolic alterations. This suggests that biased agonism has a role in carvedilol's effect on cardiac energy substrate metabolism.

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0-07

Promoting Mas1R:ETBR interaction improves vascular function and lowers blood pressure in experimental hypertension

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Introduction: We characterized/mapped a physical interaction between Mas1 and ETBR, involved in Ang-(1-7) protective actions in endothelial cells and in a model of pulmonary hypertension. Using high throughput screening of >20,000 drug-like compounds, we identified several compounds that enhance Mas1:ETBR interaction and evaluated the effects of candidate compounds, enhancers 3 and 4 (Enh3 and Enh4), on vascular reactivity and blood pressure regulation in hypertensive (SHRSP) and normotensive (WKY) rats. **Methods:** WKY and SHRSP rats (18-21 weeks) were treated subcutaneously with Enh4 (10mg/kg/day) for 7 days and blood pressure was assessed via radiotelemetry. Vascular reactivity was assessed by wire myography in mesenteric arteries from WKY and SHRSP rats preincubated with Enh3 and Enh4 (10-5M; 30 min). **Results and conclusions:** Enh4 treatment in SHRSP rats reduced daytime systolic (SBP), diastolic (DBP) and mean arterial pressure (MAP) (ΔSBP:4.26 ± 1.98; ΔDBP:1.48 ± 0.92; ΔMAP:2.07 ± 0.99 vs veh; $p < 0.01$) compared to baseline, with no effect in WKY rats. Furthermore, Enh4 treatment reduced U46619 induced contraction in SHRSP rat vessels (Emax (Mn): Veh- 13.16 ± 1.81; Enh4- 9.48 ± 1.31; $p < 0.05$). Enh3 and 4 reduced ET-1-induced contraction (Emax (Mn): Veh- 7.00 ± 0.82; Enh3- 4.43 ± 0.56: Enh4– 4.75 ± 0.58; $p < 0.001$) in WKY, but not SHRSP rats. Enh3 and Enh4 increased NO production in endothelial cells and reduced contractile mechanisms in VSMCs. Taken together, enhancing Mas1:ETBR interaction reduces blood pressure and improves vascular function in hypertensive rats. Hence, Mas1:ETBR enhancers may be of potential therapeutic use as a new vasoprotective strategy to reduce blood pressure and improve vascular function in hypertension.

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0-08

Differential involvement of smooth muscle cells in pro- and antithrombotic activities of abdominal versus ascending aorta aneurysms in human

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Introduction: Aneurysms of the ascending (TAA) and the abdominal aorta (AAA) share the common feature of dilation of the aorta but differ by their respective physiopathology and tissue environment in human. AAA is characterized by associated thrombosis forming an intraluminal clot, whereas thrombotic events are extremely rare in TAA, suggesting different coagulant properties between AAA and TAA. **Objectives:** To compare coagulation capacities at tissue and cellular levels, derived from both AAA and TAA. **Methods and results:** Human healthy aorta, AAA or TAA tissues and primary cultures of aortic smooth muscle cells (SMCs) were used. Thrombin generation was monitored by thrombography in the presence of healthy plasma. AAA tissues and SMCs have a higher ability to promote fibrin formation, to activate prothrombin, and to mobilize the tissue factor (TF) pathway, whereas TAA tissues and derived SMCs express an anti-thrombotic phenotype. Activation of the TF pathway in AAA tissue and SMCs is provoked by oxidative stress, protease-activated receptor 2 (PAR-2) overexpression and nuclear factor-kappa B (NF-κB) mobilization which could be reproduced by SMC efferocytosis of senescent red blood cells. Moreover, the high coherence between what was observed ex vivo in tissue and in passaged SMCs in vitro, demonstrated a procogulant phenotype shift in AAA SMCs, potentially as an imprinting of environmental pro-oxidative conditions of AAA. **Conclusion:** Our data indicate that oxidative stress-induced activation of the PAR-2 – NF-κB axis and leads to an increase in TF activity and prothrombotic properties of SMCs from AAA.

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0-09

Identification of novel ECM proteins associated with early-onset hypertension by deep proteomic mapping of different arterial beds

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Introduction: In hypertension, resistance arteries undergo remodeling that contributes to increased peripheral resistance. Studies have investigated specific proteins and pathways that are altered in resistance arteries during hypertension, however, a comprehensive overview of proteomic changes during onset of the disease is currently lacking. **Aim:** Using a novel mass spectrometry (MS) approach, we aimed to investigate which proteins and pathways are involved in the remodeling process in the spontaneously hypertensive rat (SHR). **Methods:** Small arteries from three different vascular beds (mesenteric, renal and cerebral) were dissected from 12-week-old SHRs and normotensive control rats (Wistar Kyoto) (n = 7-12 rats per group). Samples were analyzed with data-independent acquisition MS (DIA-MS), western blot and immunohistochemistry. **Results:** Histological staining confirmed remodeling of the vascular wall in the early-onset hypertensive rats. The MS analysis identified 286, 281 and 140 proteins in mesenteric, renal and cerebral arteries, respectively, which protein expression were significantly different in SHRs compared to normotensive controls. When comparing to an in silico matrisome database, we identified 38, 17 and 14 extracellular matrix-associated proteins that were significantly altered. Of these, two proteins (Serpina6 and Sparcl1) remained changed across the three vascular beds. **Conclusion:** Our data provides an in-depth analysis of the proteomic architecture of small arteries from SHRs. This study reveals novel extracellular matrix proteins that are associated with early-onset hypertension, thereby providing novel insights into disease progression.

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0-10

REnal Sympathetic DEnervation and Neuro-hormonal evaluation by selective venous Catheterization: the RESiDENCy study

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Introduction: Renal denervation (RD) is effective in reducing blood pressure (BP) in hypertensive patients, but the pathophysiologic mechanisms underlying the variable individual response are unclear and early markers of procedural success are lacking. The aim of the study was to evaluate intra-/peri-operative effects of RD on the renin-angiotensin-aldosterone system (RAAS) either as determinants and markers of efficacy. **Methods:** Patients with resistant hypertension and eGFR >45 ml/min underwent radiofrequency-RD (Medtronic Symplicity-Spyral) with simultaneous sampling of the renal veins (RV), left adrenal vein and pre-renal inferior vena cava (IVC). Samples were collected before (t0) and 15' after RD from each sequentially-approached side. Additional peripheral blood samples collected on the morning before RD and 3h, 24h and 7 days post-procedure were tested for renin, aldosterone and cortisol. During this timeframe, antihypertensive therapy remained unchanged. **Results:** In the 10 recruited patients (61 ± 11yo; 30%F; BP = 149/84 ± 11/12 mmHg on 4 (range 3-6) antihypertensive drugs) peripheral renin concentration showed no significant variations peri-RD and up to 7 days after, although BP decreased (BP 136/78 ± 17/15 mmHg; $p < 0.05$). Similarly, RV renin was unaffected by ipsilateral RD compared to the corresponding t0 values ($p = 0.899$). Aldosterone and cortisol increased in the adrenal vein during RD ($p < 0.001$), but their IVC-corrected ratio and their peripheral plasma concentrations at follow-up did not, likely reflecting a self-limiting stress reaction. **Key conclusions:** These results do not suggest any direct blunting effect of RD on RAAS, and do not support the contention of monitoring acute RAAS changes as proxies for procedural effectiveness.

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0-11

Short-Term Blood Pressure Variability In Young And Old Hypertensive Patients, Compared With Patients With Autonomic Neuropathy

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Introduction: Blood pressure variability (BPV) is an independent cardiovascular risk factor. Increased BPV reflects an altered blood pressure (BP) regulation, including potentially an autonomic imbalance. The objective of this study was assessing the effect of age on BPV in hypertensive patients, using patients with autonomic neuropathy as comparator. **Methods:** The study included three groups: hypertensive subjects aged 40-55 years, hypertensive subjects aged 75-95 years, and patients with autonomic neuropathy. Short-term BPV was assessed with coefficient of variation (CV) of 24h ambulatory blood pressure monitoring (ABPM). **Results:** The study included 616 younger hypertensives, 486 older hypertensives and 46 subjects with autonomic neuropathy. Mean 24h-BP was similar in the three groups. BPV was higher in older compared with younger hypertensive subjects, and even higher in dysautonomic subjects for 24h systolic BP CV (11.0 + -2.5, 13.1 + -2.9, 15.0 + -4.5, $p < 0.001$), daytime systolic and diastolic BP CV. Similar trends were observed for 24h diastolic and night-time systolic BPV, but without significant difference between older hypertensive and dysautonomic subjects. In the older hypertensive group, those in the highest tertile of daytime systolic BPV were more frequently women ($p < 0.001$), older ($p = 0.01$) and had a higher rate of ischemic heart disease ($p = 0.02$). In the younger group, patients with higher daytime BPV were more frequently women ($p = 0.002$) and active smokers ($p = 0.004$). **Key Conclusions:** Short-term BPV is higher in older vs. younger hypertensive patients, irrespective of average BP, though lower than in patients with autonomic neuropathy. Aging appears to be associated with an impairment of BP regulation, at least partly similar to that observed in autonomic neuropathy.

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P-01

The Effect of Remote Ischemic Preconditioning on the Cardioprotective Regulation of the Mitochondrial Proteome

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Mitochondria play an essential role in the processes leading to adaptation and compensation of the myocardial energy deficiency caused by ischemia/reperfusion (IR) injury. In this context, we studied the phenomenon called remote ischemic preconditioning (RPC). Our goal was to expand the available knowledge and contribute to the elucidation of possible initiators of cardioprotective signaling of RPC at the level of proteins identified by quantitative label-free LC-MS/MS proteomic analysis of isolated cardiac mitochondria. Male Wistar rats were subjected to RPC consisting of 3 cycles of 5-min ischemia and reperfusion of the right hind limb femoral artery by inflation of a pressure cuff to 200 mmHg. Extracted hearts were subsequently perfused by a Langendorff protocol (30 min of global ischemia and 40 min of reperfusion). By monitoring the effect of the early phase of RPC on the mitochondrial proteome, we observed stimulation of proteins involved in the β-oxidation pathway of fatty acid, the Krebs cycle, and proteins with antioxidant activity (PRDX3, PRDX5, SODM). One of the most significantly affected proteins by the RPC was peroxiredoxin-5 (PRDX5), a protein with a cytoprotective function. The PRDX5 expression was reduced due to IR injury, but it was significantly increased by the effect of RPC ($p = 0.0002$). Thus, we can consider PRDX5 as a potential marker participating in the provision of cardioprotective signaling induced by RPC. Proteomic analysis revealed a cardioprotective effect of RPC through the regulation of mitochondrial proteins with a function directed to myocardial energy sustainability. This study was supported by APVV 15-0119, APVV 19-0540, APVV 21-0410, APVV 20-0242, VEGA 1/0775/21, VEGA 1/0016/20.

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P-02

Evaluation of chorioretinal vascularization through OCT and angio-OCT in primary aldosteronism patients

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Introduction: Primary aldosteronism (PA), the most common cause of secondary hypertension, is associated with excess cardiovascular risk and is amenable to surgical cure when unilateral. At variance with the established PA-associated peripheral vascular remodelling and endothelial dysfunction, the impact of PA on chorioretinal microvasculature is unknown. **Methods:** Ocular CT-scan (OCT) and angio-OCT automatically-derived images of the superficial and deep retinal plexi were obtained in PA patients at diagnosis in wash-out from confounding drugs (WO), during mineralocorticoid receptor antagonist (MRA) therapy and 4-6 months after surgery. The images were analysed for choroidal thickness and, upon automated ImageJ processing, for indexes of total vascular density (VAD) and number of open vessels (VLF). **Results:** We enrolled 11 patients with unilateral PA (51 ± 10 yo; 27%F, BP = 137/82 ± 14/10 mmHg on RAAS-neutral antihypertensive therapy; sK+ = 3.6 ± 0.3 mmol/L; ARR = 90 [50-130] ng/mIU) who underwent curative surgery (BP = 124/80 ± 16/12; sK+ = 4.5 ± 0.5 mmol/L; ARR = 5 [0-10] ng/mIU; $p < 0.05$ for all). We found that MRA therapy did not affect any of the investigated parameters compared to WO. At variance, after surgery we found a reduction of VAD in both superficial and deep plexi (-6.4% retinal area, $p = 0.017$ and -3.5%, $p = 0.023$, respectively), of VLF in the superficial plexus only (-2.2%, $p = 0.033$), and of choroidal thickness (on average: -21µm, $p = 0.003$ at 2-way ANOVA) vs WO. **Key conclusions:** OCT and angio-OCT are feasible to assess changes in choroid-retinal vascularization. Adrenalectomy in unilateral PA reduces retinal microvascular density and choroidal thickness; the biological significance of these findings, in the context of hypertensive retinopathy, is under investigation.

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P-03

Impact of Transcatheter Aortic Valve Implantation (TAVI) on functional adaptations of resistance arteries after six-months follow-up

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Background and Aim: Early hemodynamic adaptations after TAVI are characterized by vasodilation of resitance arteries. We sought to evaluate whether these adaptations are maintained in mid-term follow-up. **Methods:** Twelve patients (age 81,9 ± 3,6 years, 14% male, 86% female) were studied before TAVI (T0), 48 hours (T1) and 6 months (T6) later. Patients had hypertension (100%), diabetes (57%), dyslipydemia (29%) and on therapy with: beta-blockers (71%), diuretics (86%), RAS-blockers (86%), calcium-channel blockers (57%), statins (29%), oral hypoglycaemic agents (57%). By applanation tonometry (Sphygmocor) we evaluated: 1) ejection duration (ED); 2) aortic stiffness' parameters: pulse wave velocity (PWV), pulse pressure (PP); 3) parameters of functional adaptation of resistance arteries: central (cAI@75%) and peripheral (pAI@75%) Augmentation Index, Reflection Index (RI). Peripheral and central systolic/diastolic blood pressure (BP) were measured by oscillometric device and tonometry respectively. Aortic size, aortic transvalvular gradient (TVG) and parameters of systolic/diastolic left ventricular function were evaluated by echocardiography. **Results:** TVG was reduced at T1 vs T0 (46,6 ± 2,5mmHg-vs-7,3 ± 1,3mmHg, $p < 0,0001$), and maintained reduced at T6 (6,5 ± 0,8mmHg). ED was reduced at T1 vs T0 (292,8 ± 6,8msec-vs-332,3 ± 4,85mmsec, $p < 0,0001$), and increased at T6 (341,5 ± 12,5mmsec, $p < 0,0001$) similarly to T0. At T1 the following parameters were reduced vs T0: cAI@75 (25,8 ± 1,7%-vs-33,9 ± 1,5%, $p < 0,005$), pAI@75 (-13,8 ± 3,4% vs -0,6 ± 1,7%, $p < 0,05$), RI (68,2 ± 3,2%-vs-81,6 ± 2,9%, $p < 0,005$). At T6 these parameters increased similarly to T0, suggesting that the early vasodilation of resistence arteries is not maintained after six months follow-up. **Conclusions:** These findings suggest that the early vasodilation of resistence arteries after TAVI is time dependent and might be influenced by the hemodynamic load variation.

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P-04

Eicosapentanoic Acid Induces Direct Vasodilation Effect In The Abdominal Inferior Vena Cava

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Background: Omega-3 fatty acids, specifically eicosapentaenoic acid (EPA) have been reported to have cardioprotective effects and modulation of various vascular beds, including human veins, and also lower risk for venous thromboembolism, positive impact on chronic venous leg ulcers. There are some reports in which lower dosages do not prevent abdominal Inferior Vena Cava (IVC) stenosis. However, little is known about the effect of the direct effect of EPA on the venous tone, specifically the abdominal Inferior Vena Cava (IVC). **Aims:** We aimed to investigate the direct effect of EPA on the rat isolated Abdominal Inferior Vena Cava (IVC). **Methods:** Abdominal IVC was isolated in small segments from Wistar rats at 350-400 g and prepared for the tissue organ bath apparatus. The direct vasodilation effect of EPA (100 μM) was tested in venous tissues precontracted with Thromboxane A2 agonist (TxA2), u46619 100 nM in the absence or the presence of the non-selective inhibitor for K+ channels, tetraethylammonium chloride (TEA) at concentrations of 10mM. In addition to this, Endothelin-1, 1nM was used as an additional constrictor. **Results:** EPA has given relaxing activity in abdominal IVC with (Emax = 89.73 ± 6.65) with n = 5. The relaxing capacity were reduced significantly in the presence of the TEA 10 mM (Emax = 47.50 ± 4.01, $p < 0.001$). Moreover, EPA has shown vasodilation activity in the veins precontracted with ET-1 as well but with a lower magnitude. **Conclusion:** EPA has shown vasodilation action in isolated abdominal IVC in both thromboxane A2 agonist and Endothelin-1. This effect is likely to involve the K+ channels.

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P-05

Effect of green tea on top of Enzyme Replacement Therapy in patients with Fabry disease. A molecular biology approach

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Background and Aims: Enzymatic replacement therapy (ERT) fails to completely halt the progression of Fabry disease (FD) toward cardiovascular (CV)-renal remodeling, particularly in case of late diagnosis. We have previously shown that FD patients have increased and active Oxidative Stress (OxSt) which plays a primary role in the induction of CV-renal remodeling. We hypothesized that in FD an adjuvant antioxidant treatment on top of ERT might have additive positive effects towards OxSt and related CV-renal complications. **Methods:** 10 naïve Fabry patients were enrolled and the status of OxSt was evaluated *ex vivo* in mononuclear cells before ERT, after 12 months of ERT and after 6 months of the additional treatment with green tea on top of ERT. The oxidative status was evaluated and compared in the tree time periods in terms of protein expression of $p22^{phox}$ and HO-1, phosphorylation state of MYPT-1 and ERK 1/2, MDA and HO-1 levels. **Results:** $p22^{phox}$ was significantly decreased after 12 months of the enzymatic therapy and further decreased after 6 months of green tea treatment, same for MYPT-1 phosphorylation. The phosphorylation of ERK 1/2 remained unchanged after 12 months of ERT, but significantly decreased after 6 months of green tea supplementation, as well as MDA levels. Finally, HO-1 significantly increased with both ERT and green tea supplementation. **Conclusions:** ERT's antioxidant effect is further significantly amplified by green tea treatment. This highlights the fundamental importance of early diagnosis/treatment of FD and may suggest the positive effect of additive antioxidant treatments to prevent/improve cardio-cerebrovascular-renal complications.

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P-06

Cardiovascular events after one year follow-up after moderate to severe COVID-19 pneumonia

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Aim: COVID-19 involves many organs, including cardiovascular system, possibly causing acute or chronic cardiovascular events. Preexisting cardiovascular diseases enhance COVID-19 morbidity, as well. In this retrospective analysis we investigated the onset of cardiovascular events during a time-span of more than one year since hospitalization (384 days). **Methods:** The analysis included 43 patients, hospitalized in our Internal Medicine ward for moderate to severe SARS-CoV2 related pneumonia treated with high-flow oxygen support. Mean age was 63 years, 28% (12/43) were female and 72% (31/43) were male. Thirty-five percent of the patients suffered from heart diseases, 56% of them were hypertensives and 23% had type 2 diabetes; 12% had chronic kidney disease and 5% an active neoplasm; 49% was obese. Nineteen percent took ACE inhibitors and 19% was on ARBs. Statins were taken by 37%; anti-platelet agent by 21%, anticoagulant by 2% **Results:** The follow-up visit included the evaluation of post-covid infection, quality-of-life questionnaire, standard laboratory tests, chest computed tomography, spirometry with evaluation of DLCO. The onset of cardiovascular events during the average period of 384 days was evaluated. None of the 43 patients had major cardiovascular events: coronary heart disease, cerebrovascular disease, peripheral arterial disease, deep vein thrombosis and pulmonary embolism. **Conclusions:** Even if this study failed to demonstrate new-onset CV events, longer follow-up studies on cardiovascular risk following SARS-CoV-1 infection showed persistent hyperlipidemia, cardiovascular system abnormalities, and glucose metabolism disorders in a very high number of patients. A longer-term follow-up may be needed to uncover cardiovascular consequences of SARS-CoV2 infection.

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P-07

Vascular smooth muscle cell phenotype diversity in human hypertension – role of Nox5

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Nox5 is a major ROS-generating enzyme in vascular cells; however the effects of Nox5-derived ROS on VSMC phenotype in hypertension are unknown. We investigated global and oxidative proteome profile of VSMC and the role of Nox5 on VSMC phenotype in human hypertension. VSMC from resistance arteries from normotensive (NT) and hypertensive (HT) subjects were studied. Proteins were labelled with isobaric tandem mass tags and identified by liquid chromatography tandem mass spectrometry. The oxidative proteome was assessed using stable isotope-labelled iodoacetamide to target cysteine thiols. Nox5 silencing was performed by siRNA. Protein expression was detected by western blotting. Pro-inflammatory cytokines (IL-6, IL-8) and pro-collagen I was measured by ELISA. Proteomic analysis identified 207 proteins upregulated in HT subjects (fold change >1.5, $p < 0.05$). Gene ontology enrichment analysis showed proteins upregulated in HT were involved in extracellular matrix (ECM) organization, immune response and cell proliferation. The VSMC oxidative proteome analysis identified 88 cysteine-containing peptides highly oxidized in HT (fold change >1.5, $p < 0.05$), including COL11A1, COL16A1, FBLN1 and FBLN2. VSMCs from HT exhibit increased expression of the proliferation marker, PCNA (0.162 ± 0.3 vs NT:0.051 ± 0.04RFU, $p < 0.05$) and pro-collagen I (23.6 ± 2 vs NT:13.2 ± 0.3ng/ml, $p < 0.05$). Production of IL-6 (501.8 ± 23.6 vs NT:121.7 ± 6.4pg/mL) and IL-8 (373.6 ± 34.1 vs NT:262.5 ± 24.6pg/mL, $p < 0.05$) were increased in HT. Nox5 silencing in HT reduced PCNA expression (43%), pro-collagen I (8%), IL-6 (30% baseline) and IL-8 (21% baseline) release ($p < 0.05$). We provide new insights into the proteomic profile of VSMC in human hypertension and demonstrated that Nox5 is a key player in VSMC phenotypic switching associated with vascular dysfunction in hypertension.

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P-08

A follow-up study of vascular function in Huntington Disease

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Purpose: The aim of the study was to follow microcirculatory response to vascular challenge tests in Huntington disease (HD) mutation carriers and patients and to identify relationships between microvascular involvement and clinical decline. **Methods:** Evaluation of motor functions and total functional capacity was performed in mutation carriers and patients at the start and after 8-10 years. ECG, blood pressure and cutaneous laser Doppler flux were measured at rest, during mental stress and during local cooling tests. Results were compared with age and sex matched healthy controls. **Results:** Eighteen subjects were assorted in 3 groups: 6 HD mutation carriers without motor symptoms who remained so (PHD-PHD); 6 early symptomatic patients who remained so (EHD-EHD); 6 early symptomatic patients who deteriorated to a late symptomatic (EHD-LHD). A group of matched controls was evaluated too. At the start, resting arterial pressure was significantly higher in PHD subjects compared to EHD patients ($p < 0.05$). Furthermore, measurements revealed: 1. reduced pressure response to mental stress in all HD groups ($p < 0.05$), 2. reduced pressure response to local cooling in PHD-PHD group ($p < 0.05$) and 3. absence of pressure response to local cooling in EHD-LHD group after approximately ten years. **Conclusions:** Results of the present longitudinal study confirm the cross-sectional results that initial sympathetic hyperactivity in PHD mutation carriers turns to sympathetic hypofunction during the progression of the disease. Sympathetic hyperactivity normalizes somewhere at the onset of clinical signs of the disease.

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P-09

Polyfluoroalkyl Substances Act as Endocrine Disruptors and Promote Aldosterone Secretion

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Objective: The environmental contamination of drinking water led to a marked increase of the plasma levels of polyfluoroalkyl substances (PFAS), i. e. pentadecafluorooctanoic acid (PFOA) and perfluorooctanesulfonic acid (PFOS), in a large population of Northern Italy, where the prevalence of arterial hypertension and cardiovascular disease is higher than in the surrounding uncontaminated areas. We therefore tested the hypothesis that PFAS can raise blood pressure (BP) by increasing aldosterone biosynthesis. **Design and method:** We exposed the adrenocortical carcinoma cell line HAC15 to PFOA and PFOS alone or in combination at 1 μM or 10 μM concentration for 24, 48 or 72 hours and measured changes in cell viability, aldosterone synthase (CYP11B2) gene expression and aldosterone secretion, mitochondrial reactive oxygen species (ROS) production, in the absence or presence of the superoxide scavenger tempol. Parallel experiments were performed in presence of the aldosterone secretagogue angiotensin II (Ang II) at concentration 10 or 100 nM. **Results:** While not affecting HAC15 viability, PFAS increase CYP11B2 gene expression (by 3-fold), and aldosterone secretion and ROS production (by 2-fold) over baseline ($p < 0.01$ for all). Moreover, PFAS significantly enhanced the effects of Ang II on CYP11B2 gene expression and aldosterone secretion ($p < 0.01$ for all). The ROS scavenger tempol abolished the effect of PFAS on CYP11B2 mRNA. **Conclusions:** This study identifies for the first time PFAS as an environmental pollutant acting as potent endocrine disruptors and possible aetiological factors of arterial hypertension and human primary aldosteronism.

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P-10

Impact of HMGB1 in Experimental Myocardial Infarction

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High mobility group box 1 (HMGB1) is a DNA-binding protein released also during heart ischemia that exerts proinflammatory activity. The aim of the study was to evaluate the effects of anti-HMGB1 protein on biochemical and morphological parameters after myocardial infarction (MI) in 12-week-old male WKY rats. MI was induced by ligation of the left descending coronary artery. Prior to reperfusion anti-HMGB1 protein was administrated. 7 days after MI, nitric oxide synthase (NOS) activity was determined by conversion of 3[H] Arginine to 3[H] Citrulline in the aorta and heart. HMGB1, NFκB, iNOS and eNOS expression was determined by Western blot. TTC-staining procedure was used for morphological analyses. Cytokine levels were investigated using the Bio-Plex Pro Cytokine kit in the plasma. The expression of HMGB1 after MI was significantly upregulated in both tissues. Anti-HMGB1 protein increased NOS activity in both ischemic and border heart zone, as well as in the aorta. The same pattern was found in eNOS expression level. Anti-HMGB-1 protein administration decreased iNOS and NFκB expression in the ischemic zone as well as TNF-alpha and IL-6 level in plasma. Simultaneously, anti-HMGB1 protein decreased area of ischemic part as well as border region of the heart. In conclusion, HMGB1 was upregulated after myocardial infarction, anti-HMGB1 administration could improve inflammatory conditions in rats after MI via inhibiting NF-κB pathways. This was reflected by a reduction in infarct size. Considering the results, HMGB1 protein is a promising molecule for reduction the negative effects of the myocardium infarction. Supported by: VEGA 2/0132/20.

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P-11

Short Dietary intervention improves endothelial dysfunction induced by high-fat feeding through the upregulation of the AMPK-CREB signaling pathway

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Introduction: The activation of the AMPK-CREB-dependent pathway has shown to induce endothelial cell protection through the upregulation of antioxidant and anti-inflammatory gene expression. Because caloric restriction has shown to enhance eNOS activation mediated by AMPK in obese animals, the aim of this study was to elucidate whether the replacement of HF feeding by a standard diet could reverse obesity-induced endothelial dysfunction through the activation of the AMPK-CREB signaling pathway. **Methods:** Five-week-old male C57BL6J mice were fed a standard (Chow) or a very high-fat diet (HF; 61% Kcal from fat) for 8 weeks. For the last 2 weeks, the HF diet was replaced by the Chow diet in half of HF mice, thus generating 3 groups (n = 10/group); Chow, HF and HF-Chow. The thoracic aorta was used for functional studies (organ bath) or western-blot assays. **Results:** The dietary intervention induced a significant reduction in body weight and restored glucose tolerance. Arterial functional studies evidenced that relaxations to both acetylcholine (Ach; Emax: 64.6 ± 4.0) and the AMPK activator (Aicar, Emax: 56.8 ± 4.2) in HF mice were significantly impaired compared to Chow mice (Emax-ACH: 86.2 ± 2.4; Emax-Aicar: 83.0 ± 7.7; $P < 0.01$). These responses were significantly improved in HF-Chow (Emax-ACH: 76.8 ± 1.7; Emax-Aicar: 83.9 ± 4.3; $P < 0.05$). The protein expression of AMPK and phospho-CREB were significantly reduced in HF mice but normalized in HF-Chow mice ($P < 0.05$). **Key conclusions:** Dietary intervention (replacing the HF diet by a standard diet) improves AMPK-mediated endothelial response, probably due to the upregulation of the AMPK-CREB signaling pathway.

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P-12

Enhanced β2-adrenoceptor-mediated relaxation by dynein inhibition rescues β-adrenoceptor-mediated relaxation in hypertensive rat mesenteric arteries

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Background: The voltage-gated Kv7.4 and Kv7.5 channels contribute to the β-adrenoceptor-mediated vasodilatation. In arteries from hypertensive rodents, the Kv7.4 channel is downregulated and function attenuated, which contributes to the reduced β-adrenoceptor-mediated vasodilatation observed in these arteries. Recently, we showed that disruption of the microtubule network, with colchicine, or inhibition of the microtubule motor protein, dynein, with ciliobrevin D, enhanced the membrane abundance and function of Kv7.4 channels in rat mesenteric arteries. This study aimed to determine whether these pharmacological compounds can improve Kv7.4 function in third-order mesenteric arteries from the spontaneously hypertensive rat (SHR), thereby restoring the β-adrenoceptor-mediated vasodilatation. **Methods and Results:** Using both wire myography and intravital microscopy, we show that ciliobrevin D enhanced the β-adrenoceptor-mediated vasodilatation by isoprenaline. This effect was inhibited partially by the Kv7 channel blocker linopirdine and was dependent on an increased functional contribution of the β2-adrenoceptor to the isoprenaline-mediated relaxation. In mesenteric arteries from the SHR, ciliobrevin D and colchicine both improved the isoprenaline-mediated vasorelaxation and relaxation to the Kv7.2-7.5 activator, ML213. Immunostaining of isolated vascular smooth muscle cells, confirmed ciliobrevin D enhanced the membrane abundance of Kv7.4. As well as an increase in the function of Kv7.4, the functional changes were associated with an increase in the contribution of β2-adrenoceptor following isoprenaline treatment. Immunostaining experiments showed ciliobrevin D prevented isoprenaline-mediated internalization of the β2-adrenoceptor. **Conclusions:** Overall, these data show that colchicine and ciliobrevin D can induce a β2-adrenoceptor-mediated vasodilatation in arteries from the SHR as well as reinstating Kv7.4 channel function.

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P-13

Administration of plant-derived mitochondria-like nanoparticles protects against the cardiac consequences of the obesity in rats

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Introduction: Obesity is associated with cardiac alterations in which mitochondrial oxidative stress plays an important role. Lu120819, an aqueous solution containing concentrated mitochondria-like nanoparticles isolated from the plant, shares sequence homology with mitochondrial DNA and expresses mRNAs for mitochondrial specific proteins. Thus, the goal was to evaluate the effect of Lu120819 on cardiac alterations in obesity and define the potential mechanisms involved. **Methods:** Male Wistar rats were fed with a standard diet (3.5% fat; CT) or a high fat diet (35% fat; HFD) and post-treated with vehicle or Lu120819 (7.74mg/Kg/day) in the drinking water for 10 weeks. **Results:** Obese animals showed cardiac hypertrophy, interstitial fibrosis, oxidative stress, high systolic blood pressure ($p < 0.001$) and reduced E/A ratio ($p < 0.01$). These changes were accompanied by an activation of endoplasmic reticulum (ER) stress, characterized by high levels of immunoglobulin heavy chain binding protein (BiP; $p < 0.01$), activating transcription factor 6 alpha (ATF6α; $p < 0.01$) and C/EBP homologous protein (CHOP, $p < 0.001$), along with increased mitochondrial fusion (mitofusin 1) and fission (dynamin-1-like protein). Lu120819 improved all the changes without affecting body weight or insulin resistance. The effects of Lu12089 (0.05-1 mg/ml) on ER stress pathway were also verified in H9c2 cells, where Lu120819 was able to inhibit in a dose-dependent manner the activation of ER stress induced by angiotensin II (10-6 M). **Key Conclusions:** Lu120819 is suggested to be a new potential treatment for the cardiac alterations in the context of obesity due to its beneficial effects on cardiac fibrosis, mitochondrial alterations, oxidative stress and ER stress activation.

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P-14

Immunomodulator fingolimod (FTY-720) induces direct venodilatatory effects

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Background: Sphingolipids are biosynthesized de-novo and also taken with dietary products. They have an important role in cardiovascular diseases and modulation of vascular tone through sphingosine-1-phopshate receptors (S1P). Fingolimod (FTY720) is an approved drug in multiple sclerosis and acts as a potent agonist in S1P1 and S1P3-5 receptors expressed also in various vascular beds. Despite showing some cardioprotective actions, there are still cardiac concerns exhibited mainly with arrhythmia and blood pressure modulation. In addition, it has been studied in the thrombus formation in the inferior vena cava, however, little is known about the direct effect of FTY720 on this vascular wall. **Aims:** We aimed to investigate the direct effect of FTY720 on the rat isolated inferior abdominal vena cava. **Methods:** Isolated abdominal inferior vena cava were obtained from Wistar rats at 350-400 g and dissected carefully under microscopy for mounting in separated segments of the tissue organ bath. The vasodilation effect of FTY720 (1-30 μM) was investigated after precontraction with endothelin-1 at 1 nM in the presence or absence of eNOS and cGMP inhibitors: L-NAME (100 μM) and OQD (10 μM). **Results:** FTY720 has shown vasodilation in the inferior abdominal cava vein tissue with Emax 63.95 ± 5.42 with n = 6 and such effects were diminished after inhibition of NO-dependent pathways. **Conclusion:** FTY720 has shown dose-dependent vasodilatation responses on the inferior abdominal vena cava and such responses were mainly NO-dependent suggesting its impact on the venous tone activity and possible impact on systemic cardiovascular actions or protective effects in inferior vena cava syndrome.

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P-15

In vivo measurement of blood pressure and pulse wave velocity in streptozotocin-induced type 1 diabetes in mice

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Background: In humans, type 1 diabetes (T1D) is associated with arterial stiffening as assessed by carotid-femoral pulse wave velocity (cfPWV). To experimentally study the underlying mechanism of this stiffening, we investigated blood pressure (BP) and cfPWV in streptozotocin (STZ)-induced diabetes in mice. **Methods:** Twenty-four 9-week-old male C57BL/6J mice were divided equally among diabetic (induced through once-daily 50 mg/kg STZ iRnjections for five days) and control (sham injections using citrate buffer) groups, and were kept to an age of 24 weeks. Fasting glucose was measured every 4-5 weeks via tail blood collection with levels of 15 mmol/L and higher considered diabetic. Non-invasive tonometric cfPWV was measured in anaesthetised animals (1% isoflurane) 24h prior to euthanasia; tail-cuff BP was measured directly prior to euthanasia. **Results:** Diabetic mice exhibited higher fasting glucose than controls ($p < 0.0001$, two-way ANOVA with Tukey post-hoc test; Figure A). There was no difference in systolic BP (110 ± 4 vs. 104 ± 3 mmHg, $p = 0.26$, mean ± SE, unpaired t-test) and cfPWV (2.60 ± 0.14 vs. 2.55 ± 0.11 m/s, $p = 0.80$) between diabetic and control mice (Figures B-C). **Discussion:** In the popular animal model of STZ-induced T1D, existing literature on systolic BP is not consistent. Literature about cfPWV is limited: in contrast to our data, one report showed an STZ-induced increase in ultrasound-derived cfPWV. Discrepancies between studies could be due to different methods of measuring BP and cfPWV; the choice of measurement methods therefore needs critical appraisal. **Conclusion:** In the murine model of STZ-induced T1D, we did not find elevated BP or increased arterial stiffness.

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P-16

Vitamin D receptor and its antiproliferative effect in human pulmonary arterial hypertension

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Vitamin D (vitD) deficiency is frequently observed in patients with pulmonary arterial hypertension (PAH) and, in these patients, low levels of vitD correlate with worse prognosis. The aim of this study is to examine the localization, expression and the antiproliferative role of vitD receptor (VDR) and its signaling pathway in the human pulmonary vasculature. VDR presence and expression was analyzed in lungs, pulmonary artery smooth muscle cells (PASMC) and endothelial cells (PAEC) from controls and PAH-patients. VDR expression and localization and VDR-target genes (KNCK3, BIRC5 and BMP signaling pathway) were examined in PASMC treated with calcitriol, the active form of vitD. The antiproliferative effect of 48h-calcitriol was studied in PASMC by MTT and BrdU assays. VDR is expressed in the nucleus and in the cytoplasm of PASMC. It is downregulated in lungs and PASMC from PAH-patients, without changes in PAEC. Calcitriol strongly upregulated VDR expression in PASMC from controls and PAH-patients. Calcitriol also induced the translocation of VDR from the cytosol to the nucleus. The antiproliferative effect of VDR stimulation was similar in PASMC from controls and PAH-patients and was inhibited by silencing or by pharmacological inhibition of survivin or BMPR2, but not of KCNK3. In conclusion, the expression of VDR is low in PAH-patients which may contribute to the pathogenesis of PAH. Low VDR expression can be rescued by calcitriol. VDR exerts an antiproliferative effect in PASMC by opposing the actions of survivin and partially by modulation the BMP signaling pathway.

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T-Cells Autoreactive To Angiotensin Ii Type 1 Receptor In Salt-Dependent Hypertension Due To Primary Aldosteronism

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Objective: High titer of autoantibodies against the angiotensin II receptor 1 (AT1R) were reported in patients with Primary Aldosteronism (PA) suggesting a role of acquired immunity in hypertension and hypertension-mediated organ damage (HMOD). As the pathogenic role of these acquired immunity is still unknown, we investigated if aldosterone affects T-cells clonal proliferation and activation, and the presence of AT1R-autoreactive T-cells in PA patients. **Methods:** The expression of mineralocorticoid receptor (MR) and G Protein-Coupled Estrogen Receptor (GPER) was tested by ddPCR and immunoblotting on T-cells from 7 healthy donors (HD). PBMCs from HD were exposed to different concentrations of aldosterone (from 10-10 M to 10-8 M) with or without MR antagonist (canrenone) and GPER agonist (G1) and antagonist (G36). We evaluated CD8+ proliferation and activation measuring IFNɣ release by flow cytometry. The presence of AT1R-autoreactive T-cells in 11 PA patients was investigated by flow cytometry after stimulation with pool of peptides spanning the full length of AT1R. **Results:** MR and GPER were detected both at mRNA and protein level in CD4+ and CD8+ T-cells. Aldosterone significantly increased IFNɣ release in CD8+ T-cell under clonal proliferation and activation. The proliferation effect was mediated via MR, as it was prevented by canrenone, while the clonal activation was GPER mediated, as it was reduced by G36, and mimicked by G1. CD8+ AT1R-autoreactive T-cells were found in PA patients after activation of PBMC with AT1R peptides. **Conclusions:** These results showed that MR and GPER in CD8+ lymphocytes mediate clonal proliferation and activation respectively. Moreover, they suggest a role of AT1R-autoreactive T-cells in HMOD in PA.

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Bioactive Peptides With Enhanced Inhibitory Activity Towards Angiotensin-Converting Enzyme

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Primary hypertension, the main modifiable risk factor, is potentially caused by higher values of angiotensin II (formed by angiotensin-converting enzyme, ACE). Currently, there is an emergent search for natural compounds with less side effects than the traditionally used ACE-inhibitory drugs while maintaining the therapeutic efficacy. Examples are bioactive peptides, such as those derived from brewing by-products, brewer's spent grain (BSG) and yeast (BSY), which are known to present antioxidant and ACE-inhibitory activities. However, the impact in peptides' bioavailability/effectivity after oral administration have not been assessed. Thus, we have simulated oral administration and evaluated the effective ACE-inhibitory activity of BSG/BSY peptides. Alkaline extracted BSG-proteins and BSY-proteins obtained through mechanical disruption were hydrolysed and subjected to simulated gastrointestinal digestion adapting the INFOGEST protocol. Simulated intestinal absorption (Caco-2 cells) and liver metabolism (HepG2 cells) were performed simultaneously adapting protocols. ACE-inhibitory potential of pre and post oral administrated products (BSY, BSG and a mixture 50:50% - MIX) at identical concentration (0,857mg/mL) were measured. ACE activity in the presence of initial hydrolysates were 0,292, 0,759 and 0,531mU for BSY, BSG and MIX, respectively, whereas after simulated oral administration were 0,269, 0,154 and 0,121mU. Captopril 0,1μM (used as a positive control), dissolved in the same solutions as the initial/final products, exhibited an ACE activity of 0,370 and 0,816mU, respectively. Simulated oral administration seems to greatly increase the ACE-inhibitory capacity of BSG and MIX peptides, presenting a higher inhibition compared to the clinically used ACE inhibitor captopril, thus being promising compounds to manage hypertension.

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TRPM7 deficiency affects cardiac fibroblasts activation phenotype and contributes to cardiovascular fibrosis induced by aldosterone-salt

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Introduction: TRPM7 is a channel permeable to Mg^{2+} and Ca^{2+} bound to alpha-kinase with essential role in cell homeostasis. Hyperaldosteronism is associated with Mg^{2+} wasting. Here, we investigate the importance of TRPM7-Mg^{2+} in hypertension and fibrosis induced by aldosterone-salt. **Methods:** Wild-type (WT) and TRPM7-deficient (M7+/Δ) mice were 4-weeks treated with aldosterone (600µg/Kg/day) plus NaCl (1% in drinking-water). Blood pressure (BP) was evaluated by tail-cuff. Molecular mechanisms were investigated in cardiac fibroblasts (CF). Ca^{2+} influx was assessed by fluorescence microscopy. Intracellular [Mg^{2+}], proliferation and cell size were assessed by FACS. Protein expression was assessed by western-blot. **Results:** M7+/Δ mice exhibited reduced TRPM7 expression(30%), phospho-TRPM7(62%) and tissue [Mg^{2+}] (28%). Levels that were recapitulated in WT-aldo-salt. M7+/Δ mice exhibited increased BP by aldo, salt and aldo-salt (135-140mmHg). In WT, only aldo-salt increased BP(134mmHg). Aldo-salt increased cardiac collagen in M7+/Δ mice (68%) and expression of IL-6, TGFβ, p-Smad3 and p-ERK1/2 (1.5-1.8 fold). CF from M7+/Δ mice exhibit reduced calcium influx induced by aldosterone (peak-response 105 ± 0.7) vs WT:149 ± 10. [Mg^{2+}] was reduced in CF from M7+/Δ mice (fluorescence: 1505 ± 28 vs WT:3428 ± 57), which was further reduced by aldosterone (20%). CF from M7+/Δ exhibited reduced proliferation (30%), increased cell size (25%) and expression of TGFβ, IL-6, p-Smad3 and p-ERK1/2 (1.4-2.0 fold) vs WT. Mg^{2+} supplementation normalized intracellular [Mg^{2+}], cell proliferation, cell size and protein phosphorylation in M7+/Δ CF($p < 0.05$). **Conclusions:** Our findings identify a protective role of TRPM7 in aldosterone induced cardiovascular injury, which when downregulated, facilitates cardiac fibrosis by changing fibroblast activation phenotype through Mg^{2+} -dependent mechanisms.

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Erythropoiesis and Body Iron Homeostasis are Under Influence of the Renin-Angiotensin System

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The renin-angiotensin system (RAS) has a broad set of active peptides and distributed receptor expression influencing several homeostatic physiological processes. In this study, we focused on the role of the brain RAS on erythropoiesis control as well as on the role of the peripheral RAS on iron homeostasis. Erythropoiesis was investigated in transgenic mice with brain-specific angiotensinogen overexpression and transgenic rats with brain-specific angiotensinogen depletion. Iron balance was investigated in mice with genetic deletion of angiotensinogen and renin. Whole blood was collected into EDTA-coated tubes, and erythropoiesis evaluated using glass capillary hematocrit and automated hematology analyzers. Additionally, erythropoiesis was studied in sympathectomized mice treated with 100 mg/Kg 6-hydroxydopamine for five consecutive days. Mice with brain-specific increased angiotensinogen expression presented an increased hematocrit. Complementary analyses revealed increased hemoglobin, red blood cell, and reticulocyte counts in the transgenic mice. All parameters became comparable to controls upon sympathectomy indicating a modulatory role of the brain RAS on erythropoiesis via sympathetic nerve activity. Rats with depleted brain angiotensinogen presented reduced hematocrit, red blood cell, and hemoglobin demonstrating that the brain RAS boosts rodent baseline erythropoiesis. This anemia phenotype was confirmed in mice lacking angiotensinogen and renin. Interestingly, these mice presented microcytic anemia which is an indicator of low levels of circulating iron. Strikingly, plasma levels of iron were reduced in RAS-deficient mice, likewise ferritin, a marker of iron stored in organ, was reduced in blood. Altogether, these studies uncovered two unprecedented RAS-modulated processes, erythropoiesis and iron homeostasis.

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Na+/H+ exchanger-1 (NHE1) activity is downregulated by a critical hypoxic threshold contributing to a decrease in cell proliferation

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Hypoxia and acidosis are the hallmarks tumour microenvironment that can modulate the expression and function of NHE1 via hypoxia inducible factor 1 (Hif) [1,2]. Here, we investigate the severity and time dependent effects of hypoxia on NHE1 activity and its correlation with cell proliferation in mouse atrium tumour derived HL-1 cells. NHE1 activity was recorded using intracellular pH (pHi) sensitive dye cSNARF-1 (Leica SP5). Cell proliferation was assessed by live cell movie analyser (JuLI Br). Our earlier results demonstrated that long term severe hypoxia (1% O2, 48h, Vmax: 4.82 ± 0.47mM/min; control 7.58 ± 0.7 mM/min at pHi:6.75, n:43-82, $p < 0.05$) or DMOG (Hif stabilizer, 1 mM, Vmax: 4.57 ± 0.49 mM/min; control 7.58 ± 0.7mM/min at pHi:6.75, n:26, $p < 0.05$) incubation in normoxia significantly decreased both NHE1 activity and cell proliferation in HL-1 cells [3]. In contrast, milder hypoxia (2% O2, 48h, Vmax: 7.98 ± 1.19 mM/min; control 7.58 ± 0.7mM/min at pHi:6.75, n:19, $p > 0.05$) had no such an effect. Similarly, when hypoxic incubation time was shortened (1% O2, 24 h, Vmax: 8.34 ± 1.23 mM/min; control 7.58 ± 0.7mM/min at pHi:6.75, n:36, $p > 0.05$), the inhibitory effect of hypoxia on NHE-1 activity was also diminished. Moreover, when NHE1 activity was completely blocked in normoxic conditions cell proliferation was supressed correspondingly (zoniporide, 30 uM, proliferation: 66%; control 84%, n:4-12, $p < 0.05$). Thus, NHE-1 activity is an important target in regulating antiproliferative action in heart cancer. 1-Damgacı et al. (2018). Immunology 154(3): 354–3622- Hulikova et. al. (2013). J Cell Physiol 228(4):743-7523- Şimşek et al. (2019). J Cell Physiol 234: 4681-4694.