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APSET17.8
The Endothelin System in Neurovascular Function: Lessons Learned in Diabetes

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In the US alone, 5.8 million individuals are affected by cognitive impairment/dementia and there is no effective treatment. Endothelial dysfunction and decreased cerebral blood flow are early changes that precede the development of neuropathologies and cognitive deficits. Hence, cardio/cerebrovascular health is critical for brain health and it is believed that any approach to prevent/improve dementia should target neurogliovascular (NGVU) unit rather than neurons alone. The role of endothelin-1 (ET-1), the most potent vasoconstrictor with profibrotic, prooxidative and proinflammatory properties, in brain health is inadequately studied and limited to its vasoactive properties. While it was established early on that ET-3 and ETB are the most abundant isoform and cognate receptor in the brain, the physiological significance and whether this is altered in disease states are poorly understood. While some preclinical studies reported that ETA selective or dual ET receptor blockers improve ischemic stroke outcomes and cognitive impairment, there are also conflicting reports with selective ETB agonism preventing the development of cognitive impairment. The results of an ongoing clinical trial investigating the impact of ETB agonism in ischemic stroke patients are anticipated to provide novel insights. ET-1 is increased in diabetes, a common comorbidity that doubles the risk for stroke and cognitive impairment. Cerebrovascular dysfunction is a common pathology between diabetes and cognitive impairment and ET-1 is a likely candidate that may be involved in many facets of diabetic cerebrovascular disease. We have extensively studied the role of ET-1 in diabetes-associated cerebrovascular dysfunction. Our recent data showed that 1) brain microvascular endothelial cells (BMVECs), which were previously thought to have exclusively ETB receptors, also possess ETA receptors in both sexes, 2) male BMVECs secret 10-fold more ET-1 than female cells, 3) ETA receptors are upregulated to a greater level by hypoxia or diabetes like conditions in male cells, 4) ET-1 inhibition improves prosurvival/proangiogenic mature brain derived neurotrophic factor expression in BMVECs grown in diabetes-like conditions, and 5) there is upregulation of circulating and brain ET-1 in diabetic male animals that later develop cognitive impairment. In light of recent advances in our understanding that neurovascular coupling which is generally referred as functional hyperemia to fine-tune cerebral blood flow by signals from neurons is a bidirectional event and also includes trophic coupling with the NGVU, our current knowledge of the ET system in the NGVU and potential impact on cognitive function will be reviewed in the context of diabetes in both sexes.

APSET17.9
BQ788, an endothelin ETB receptor antagonist alleviates blood-brain barrier disruption and brain edema after traumatic brain injury

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Background: Traumatic brain injury (TBI) is severe damage to the head and referred to as a sudden insult caused by traffic accidents, falls, and sporting activity. TBI drives brain edema resulted from disruption of the blood-brain barrier (BBB). Although these conditions induce unexpected death or serious disabilities, beneficial therapeutic drugs have not been established. Endothelin-1 (ET-1) is closely related with the TBI pathogenesis. In patients with TBI and experimental TBI animals, expression of ET-1 is increased. We investigated the effects of ET receptor antagonists for BBB disruption and brain edema in TBI model mice. Methods: As an experimental TBI model, the fluid percussion injury (FPI) was performed on the mouse dura mater (male ddY, 6 to 7 weeks). The BBB disruption was evaluated by extravasation of Evans blue administered from tail vein into the cerebral parenchyma. Brain edema was evaluated by increased brain water content. Expressions of causative factors (matrix metalloproteinase-9: MMP-9 and vascular endothelial growth factor-A: VEGF-A) and protective factors (angiopoietin-1: Ang-1 and sonic hedgehog: Shh) for BBB disruption were examined by Real-time PCR, enzyme immunoassay and immunohistochemistry. To confirm therapeutic efficiencies of ET receptor antagonists,

intraventricular administration of BQ788, an ETB receptor antagonist (i.c.v., 15 nmol/day) or FR139317, an ETA receptor antagonist (i.c.v., 15 nmol/day) was performed from 2 to 5 days after FPI. Additionally, intravenous administration of BQ788 (i.v., 5 mg/kg/day) was also performed. Results: Expressions of ET-1 and ETB receptor were increased in the mouse cerebrum after FPI. FPI promoted Evans blue extravasation and increased in brain water content. The i.c.v. administration of BQ788 ameliorated Evans blue extravasation and reduced brain water content after FPI. On the other hands, FR139317 did not ameliorate Evans blue extravasation and reduce brain water content. The i.v. administration of BQ788 also alleviated Evans blue extravasation and decreased in brain water content after FPI. FPI increased in expressions of MMP-9 and VEGF-A while BQ788 decreased in these expressions. Additionally, BQ788 increased in expressions of Ang-1 and Shh after FPI. Immunohistochemical observations implied that ETB receptors were mainly distributed in astrocytes. In cultured astrocytes, treatment with ET-1 increased in expressions of MMP-9 and VEGF-A, and decreased in expressions of Ang-1 and Shh. BQ788 inhibited these effects of ET-1. Conclusion: These results suggest that BQ788 alleviates TBI-induced BBB disruption and brain edema by decreases of causative factors and increases of protective factors in astrocytes. Therefore, ETB receptor antagonist is expected to be a novel therapeutic drug for TBI in future. Funding: The Japan Society for the Promotion of Science.

APSET17.10

Serum endothelin-1 level is significantly increased in coronary artery disease patients with diabetes mellitus

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Background: Patients with diabetes mellitus DM) have a higher risk of developing coronary artery disease (CAD) than non-diabetic patients. Increased endothelin-1 (ET-1) expression causes endothelial dysfunction, which may become the link between DM and CAD. This study aimed to investigate the association between serum ET-1 and DM among patients with CAD. Methods: The study design was cross-sectional. Subjects were patients with diagnosis of stable angina pectoris or history of acute myocardial infarction who underwent elective coronary angiography in Dr. Sardjito Hospital, Yogyakarta, Indonesia and had proven CAD. Serum ET-1 was measured by ELISA, with blood serum sampled on the day of coronary angiography. Associations between serum ET-1 level and risk factors of CAD, including DM, hypertension, dyslipidemia, abdominal obesity, and smoking status, were analyzed using Student's t-test, Chi-square, and Pearson's correlation. p-value <0.05 was considered as statistically significant. Results: Among 183 subjects, the mean ET-1 level was 2.44±1.49 pg/mL. Subjects with DM had significantly higher serum ET-1 level as compared to non-DM (2.79±1.63 vs 2.29±1.40 pg/mL, p=0.035). Further analysis showed that the difference was driven by group of patients with history of myocardial infarction compared to stable angina pectoris. Within group of patients with history of acute myocardial infarction (n=124), there was significant difference of serum ET-1 level between DM and non-DM (2.82±1.72 vs. 2.18±1.24 pg/mL, p=0.024). Whereas, within groups of stable angina pectoris without previous myocardial infarction (n=59), there was no significant difference of serum ET-1 level between DM and non-DM (2.75±1.50 vs. 2.54±1.70 pg/mL, p=0.647). Serum ET-1 level positively correlated with non-fasting blood glucose (r=0.152, p=0.042). Other CAD risk factors including hypertension, dyslipidemia, abdominal obesity, and smoking status did not associate with increased serum ET-1 level. Conclusion: Increased serum ET-1 level was significantly associated with CAD patients who had DM.
Keywords: coronary artery disease, endothelin-1, diabetes mellitus, risk factor

APSET17.12

Circulating ANGPTL3 and ANGPTL4 in different obese phenotypes and their relationship with ET-1-dependent vasoconstrictor tone

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Background. Obesity is associated with premature atherosclerosis and increased burden of cardiovascular disease, especially when accompanied by abnormalities of lipid and glucose metabolism. Angiopoietin-like (ANGPTL)3 and ANGPTL4 are metabolic regulators, whose upregulation is associated with dyslipidemia, insulin resistance and atherosclerosis. We analyzed, therefore, changes in circulating ANGPTL3 and ANGPTL4 in obese patients with different metabolic phenotypes and their relationship with endothelin (ET)-1-dependent vasoconstrictor tone, an hemodynamic abnormality commonly associated with obesity. Methods. Obese patients (body mass index > 30 kg/m2) were classified as metabolically healthy, in the absence of any of the metabolic abnormalities (hyperglycemia, hypertriglyceridemia, reduced HDL-cholesterol) of the metabolic syndrome (ATPIII criteria), or metabolically unhealthy, in the presence of at least one of those abnormalities. Circulating ANGPTL3 and ANGPTL4 were measured by Luminex assay. ET-1-dependent vasoconstrictor tone was assessed as forearm blood flow

response (strain-gauge plethysmography) to selective blockade of ETA receptors by BQ-123 (10 nmol/min for 60 minutes). Results. Compared to lean subjects (n=42), circulating ANGPTL3 was elevated (P>0.001) in metabolically unhealthy obesity (MUO; n=87), but not in metabolically healthy obesity (MHO; n=48, P>0.05); circulating ANGPTL4, by contrast, was similarly increased in both obese subgroups compared to lean subjects (both P<0.05). The vasodilator response to blockade of ETA receptors was increased in both MHO and MUO compared to lean subjects (both P<0.001), without significant difference between the 2 obese subgroups (P<0.05). In the whole population, a significant linear relationship (R=0.29; P=0.02) was observed between circulating ANGPTL4 and the vasodilator response to BQ-123, whereas no linear association (R=0.14; P=0.29) was found between circulating ANGPTL3 and the vasodilation elicited by ETA antagonism. Interestingly, both circulating ANGPTL4 and BQ-123-induced vasodilation had a significant linear relationship with BMI (R=0.32 and R=0.29, respectively; both P<0.05) and plasma insulin levels (R=0.23 and R=0.31, respectively; both P<0.05). Conclusions. Circulating concentrations of ANGPTL3 and ANGPTL4 undergo variable changes in obese patients with different metabolic phenotypes. The obesity-related changes in ANGPTL4 are linked to increased ETA-dependent vasoconstrictor tone, probably due to a common pathophysiological background. Further studies are warranted to ascertain whether ANGPTL4 plays a mechanistic role in activating the ET-1 system and may thereby represent an effective therapeutic target in these patients.

APSET17.13

Effect of Endothelin-A receptor antagonist, BQ123, on morphine tolerance in SH-SY5Y human neuroblastoma cells using cAMP-GloSensor™ real-time detection assay

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Purpose: Endothelin-A receptor (ETAR) antagonist, BQ123 potentiates morphine analgesia, reverses tolerance, and reduces withdrawal symptoms. Activation of GPCRs by morphine triggers downstream effects including inhibition of adenylyl cyclase and decrease in 3',5'-cyclic adenosine monophosphate (cAMP). cAMP upregulation is observed in morphine tolerance and withdrawal. However, the signaling mechanisms involved in the effects of BQ123 on morphine response are unknown. The aim of this study was to determine morphine- and BQ123-induced changes in cAMP signaling in SH-SY5Y neuroblastoma cells which endogenously express mu-opioid receptors (MORs) and ETARs. Methods: SH-SY5Y cells (ATCC®) were grown in DMEM/10% FBS and maintained in a humidified incubator at 37°C/5%CO2. Cells were seeded in 96-well white, opaque-bottom plates (5X104 cells/well). Transfection conditions were optimized using ViaFect™ (Promega™) and 30% transfection efficiency was achieved. A stably transfected SH-SY5Y cell line was established using GloSensor™ -23F cAMP plasmid (Promega™) containing hygromycin-resistance gene. Promega's real-time detection system in live cells (cAMP-GloSensorTM assay) was optimized and used to measure cAMP response. Stably transfected cells were incubated with equilibrium buffer [cAMP GloSensor™ reagent (2% (v/v)), 88% CO2 independent medium, 10% FBS]. cAMP levels were determined by changes in luminescence using EnSpireTM multimode microplate reader (PerkinElmer). In acute studies, cells were treated for 2 h with morphine (10–10-5 M) and BQ123 (1μM). In chronic studies, cells were treated for 24 h with morphine (1μM) in the presence and absence of BQ123 (1μM). After induction of tolerance for 24 h, two challenge doses of morphine (10μM and 20μM) were tested. Isoproterenol was used as positive control (1–10-8 M). Each experiment was repeated in triplicate and data was analyzed in GraphPad Prism version 9.00 (GraphPad Software, San Diego, CA). Results: A concentration-dependent increase in cAMP was observed with isoproterenol in SH-SY5Y cells stably transfected with -23F cAMP plasmid. Acute morphine treatment (10–10-5 M for 2 h) produced a concentration-dependent decrease in cAMP, while acute treatment with BQ123 (1–10-5 M) increased cAMP levels. Of interest, BQ123 (1μM) appeared to enhance morphine-induced decrease in cAMP response. Chronic morphine treatment (1μM for 24 h) increased cAMP response, indicative of tolerance. In cells treated chronically (24 h) with a combination of morphine (1μM) and BQ123 (1μM), dose-dependent inhibition of cAMP levels was seen with challenge doses of morphine. Conclusion: To better understand the mechanism by which ETAR antagonists reverse morphine tolerance and reduce withdrawal symptoms, signaling mechanisms were explored in SH-SY5Y cells. We found that BQ123 appears to enhance morphine-induced cAMP signaling through Gi-coupled MORs in both acute and chronic studies, which support our previous work. These findings suggest that cAMP may be involved in the effect of BQ123 on morphine tolerance. We will further explore the involvement of beta-arrestin and GPCR kinases (GRKs) in these phenomena.

APSET17.14

Update on SONAR

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The Study of Diabetic Nephrology With Atrasentan (SONAR) trial demonstrated that the endothelin A receptor antagonist, atrasentan, reduced the rate of chronic kidney disease (CKD) progression in patients with type 2 diabetes mellitus (T2D) taking maximally tolerated renin-angiotensin system (RAS) blockers. The SONAR trial was unique in its use of a 6-week enrichment period during which all subjects received atrasentan; this was followed by stratification by albuminuria reduction during enrichment (≥ 30% or <30% in urine albumin-to-creatinine ratio [UACR]), and randomization to atrasentan or placebo. Since publication of the SONAR trial results in 2019, several post hoc analyses have been conducted that addressed unanswered questions about atrasentan use in T2D patients with CKD: 1) Using a population pharmacokinetic model, individual area under the atrasentan concentration-

time-curve (AUC) was estimated. In multivariate analysis, higher atrasentan AUC associated with greater UACR reduction and greater brain natriuretic peptide (BNP) increase independent of estimated glomerular filtration rate (eGFR) or baseline BNP. Notably, UACR response did not correlate with BNP response (Koomen et al, Diabetes Obes Metab 23:561, 2021). 2) Using the population pharmacokinetic model, mean atrasentan exposure was correlated with time-to-event long-term outcomes. Compare to placebo, a mean atrasentan exposure equated to a hazard ration of 0.76 (0.28-0.85 95% CI) for kidney events and a hazard ration of 1.13 (1.03-2.20 95% CI) for heart failure events (Koomen et al, Clin Pharmacol Ther 109:1631, 2021). 3) The influence of sodium/glucose cotransporter-2 (SGLT2) inhibitor initiation during the enrichment period on fluid retention and UACR reduction was assessed in a small number (N=14) of patients compared to 42 matched (by all measured baseline characteristics) patients on atrasentan alone. Body weight increased in the atrasentan group by 0.6 kg (0.0-1.1 95% CI) and fell by 0.7 kg (-0.3-1.6 95% CI) in the combined group, with a difference of 1.2 kg (p=0.028). The combined therapy was associated with a 27.6% (p=0.028) greater reduction in UACR than atrasentan treatment alone. 4) Data from studies on the association of baseline UACR and eGFR with SONAR kidney and heart outcomes, as well as the predictive effect of early albuminuria reduction with atrasentan on kidney outcomes, will be presented based on availability. Following completion of the SONAR trial, AbbVie licensed atrasentan to Chinook Therapeutics who are developing it for various CKD applications. Relevant to SONAR, the AFFINITY Study, a phase 2, open-label, basket study lasting 1 year evaluating the efficacy and safety of 0.75 mg/day atrasentan in patients with proteinuric glomerular disease at risk for CKD progression will include a small number (approximately N=20) of patients with T2D and CKD taking RAS and SGLT2 inhibitors. In summary, post hoc analyses of the SONAR trial suggest that the dose of atrasentan used was appropriate to maximize renal benefits and minimize fluid retaining risks, that patient variability in atrasentan exposure may affect response, and that combining atrasentan with an SGLT2 inhibitor (on top of RAS inhibition) may achieve superior renal protection than either agent alone while minimizing adverse events related to fluid retention.

APSET17.16

The myeloid endothelin-B receptor: a sex-dependent regulator of blood pressure

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Introduction Hypertension makes an important contribution to cardiovascular disease and chronic kidney disease but its cause is unknown in the majority of cases. Blood pressure (BP) is regulated by multiple mechanisms including the endothelin and immune systems, and dysregulation of these systems can lead to hypertension. Previously, we demonstrated that macrophages, key cells of the innate immune system, provide a clearance mechanism for endothelin-1 (ET-1) through dynamin- and endothelin-B (ETB) receptor-dependent uptake. This protects from the development and progression of hypertension. Cardiovascular disease incidence and risk varies between men and women, with men more likely to develop cardiovascular disease earlier than women. Here, we investigated the differences between male and female mice in the contribution of the myeloid ET system to the development of hypertension. Methods All experiments were performed in 9-12-week-old male and female myeloid-ETB receptor deficient (LysMETB) or littermate control mice. Mice were infused with 2 weeks of angiotensin II (ANG II, 1 µg/kg/min via minipump), a model of hypertension that is recognised to be ET-1-dependent. BP was measured by gold-standard radiotelemetry and vascular function by wire myography. Bone marrow-derived macrophages (BMDM) were isolated from male and female LysMETB and control mice and cultured in vitro. Gene expression was determined using RT-qPCR and ET-1 concentration by specific ELISA. Macrophages were then exposed to 2 ng/mL fluorescently labelled ET-1 (fET-1), and the concentration of ET-1 assessed by fluorescence spectrophotometry after 12 hours. Results Baseline BP (mean arterial pressure, MAP) was no different between male and female control mice; however, baseline MAP was ~8 mmHg lower in female LysMETB mice compared to male LysMETB mice, and ~4 mmHg lower compared to female controls. Two weeks of ANG II increased MAP to a similar extent in male and female control mice (+30 mmHg); male LysMETB mice demonstrated a rise in MAP of +43 mmHg, whereas female LysMETB mice demonstrated a reduced hypertensive response to ANG II (+25 mmHg, $p < 0.05$ vs. all other groups). Whereas cardiac and renal injury was similar between male and female, control and knockout mice, resistance vessels from female LysMETB mice displayed reduced vasoconstriction in response to ET-1 Edn1 expression and tonic ET-1 production were no different between control male and control female BMDM. However, control male BMDM had greater Ednrb expression. In keeping with this, control male BMDM were able to clear fET-1 more efficiently than female BMDM. The clearance of fET-1 was dependent on the ETB receptor with no clearance observed in macrophages isolated from male or female LysMETB mice. Conclusions We have shown that the myeloid-ET system is a sex dependent regulator of BP. Female LysMETB mice have a reduced hypertensive response to ANG II and this may relate to their reduced vasoconstrictor response to ET-1. Our data suggest potential cross talk between myeloid cells and the vasculature. Whereas in males the myeloid ETB receptor is protective it may be deleterious in females.

APSET17.17

The Effects of Physiologic and Experimental Hyperinsulinemia on Vasoactive Flexibility: A Time-Course Comparison between Insulin Sensitive and Insulin Resistant Individuals

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Background: Skeletal muscle arterioles isolated from patients with type 2 diabetes (T2D) following hyperinsulinemic-euglycemic clamp display impaired ex vivo flow-induced dilation relative to basal, which were underlined by reductions in NO metabolites and increased in ET-1 protein expression. Translating these experimental observations to better understand the potential vascular consequences of elevated fasting insulin, and the post-prandial hyperinsulinemia, experienced in insulin resistant individuals is needed. Methods: Our goal was to evaluate the circulating biomarker and vasoactive substance profiles during both a 75-g OGTT (physiologic post-prandial hyperinsulinemia) and a hyperinsulinemic (40mU/m2/min) - euglycemic clamp (experimental hyperinsulinemia) within lean healthy (LH), insulin sensitive individuals (n=14), and patients with T2D (n=17). Metabolic tests were performed on separate days and after a period of standardized metabolic control. Plasma glucose, insulin, NO metabolites, and ET-1 were analyzed at baseline and in 30-minute intervals for 120 minutes within each condition. Group by time comparisons were completed within each metabolic test as well as bivariate correlations and bidirectional stepwise regressions to predict the Δ ET-1. Results: NO metabolites and ET-1 were inversely associated (Rho=-0.596, p=0.007) across all basal samples. During the OGTT, NO:ET-1 ratio fell 42±9% from baseline to 120' within LH (p=0.036) while T2D displayed a lower NO:ET-1 ratio than LH (p=0.041) and higher ET-1 levels than LH (p=0.041). Experimental hyperinsulinemia induced peak NO metabolite levels at 30' in both groups, however NO bioavailability was 86% higher (p=0.518) and NO:ET-1 ratio 335% higher (p=0.552) in LH than T2DM during this time-point. Under prolonged hyperinsulinemia, ET-1 levels increased from baseline at 120' in LH by 333±122% (p=0.002) and in T2D by 205±55% (p<0.0001). Overall, T2D displayed 94% higher ET-1 levels than LH at 120' (p=0.0005). T2D displayed no change in NO or NO:ET-1 throughout the clamp procedure. For prediction of Δ ET-1, under both physiologic and experimental hyperinsulinemia, stepwise regressions selected Δ insulin (std. β = -0.01, -0.04), BMI (std. β=-0.04, -0.06), and group [LH vs T2D] (std. β = -0.75, -2.54) within each model with the addition of sex [F] (std. β = -0.94) in the experimental hyperinsulinemic model. Conclusion: Patients with T2D display inflexible NO production and elevated circulating ET-1 in response to shifts in insulin. Prolonged hyperinsulinemia favors ET-1 production, independent of insulin-sensitivity status, confirming hyperinsulinemic driven vasoconstrictive signaling. Funding: ADA: 1-14-JF-32; NIH: R01DK109948, UL1RR029879, P30DK020572

APSET17.18

Endothelin B Receptors Agonism Augments Neuronal Progenitor Cell Differentiation and Improves Mitochondrial Function in Ischemic Stroke

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Introduction- Our pre-clinical and clinical (NCT04046484) studies have shown that sovateltide (IRL-1620) mediated agonism of endothelin B receptors (ETBR) is effective in treating ischemic stroke. Sovateltide has the potential to be developed as a novel drug for ischemic stroke. Hypothesis- Neuronal progenitor cells (NPCs) in the adult brain can generate new neurons after ischemic stroke. At the same time, mitochondria in neurons play a significant role in the survival and function of neurons. Sovateltide induced stimulation of ETBR in the brain initiates regenerative and function restoration responses after ischemic stroke; however, its effect on the differentiation of NPCs and mitochondrial fate remains elusive. Objective– We evaluated the effect of sovateltide mediated ETBR agonism on NPCs and mitochondrial fate in the adult rat brain after stroke. Methods– Permanent middle cerebral artery occlusion (MCAO) was performed in adult rats to induce ischemic stroke in the right hemisphere of their brains. Intravenous injections of sovateltide (5 µg/kg) or saline (equal volume) were given. Neurological and motor function testing was done at 24 hrs and day 7 post MCAO. One set of rats were sacrificed at 24 hrs and another at day 7 post-MCAO. Brain tissues were analyzed for NPCs differentiation and mitochondrial fate using western blots, immunofluorescence, transmission electron microscopy, and in situ PCR techniques. In vitro hypoxia experiment was carried out to confirm sovateltide mediated neuronal differentiation. Results- Sovateltide treated rats showed significant improvements in neurological and motor functions at 24 hrs and day 7 post MCAO. Upregulation of neuronal differentiation markers Neuro D1 (p=0.00002), as well as HuC/HuD (p=0.0037) along with a neuronal marker Doublecortin (DCX) (p=0.00011) at 24 hrs post MCAO, was observed in ischemic brain tissues. However, on day 7, significant upregulation only in HuC/HuD (p=0.043) was observed. In vitro exposure of sovateltide and hypoxia to cultured NPCs showed higher NeuroD1 and NeuN (a mature neuronal marker) expression confirming NPCs differentiation. Analyses of rat brain tissues by western blots, electron microscopy, and in situ PCR were conducted to study mitochondrial fate and biogenesis. Downregulation of mitochondrial fission marker, DRP1 (p<0.001), increase in fusion marker, MFN2 (p<0.0001), an increase in mitochondrial cross-sectional area x number (p<0.05) as well as mitochondria/tissue area (p<0.05) at 24 hrs and day 7 post MCAO were observed, which indicated better preserved mitochondrial fate in sovateltide treated rat brains. In situ PCR analysis showed increased mitochondrial DNA (p=0.0418) in sovateltide rats, indicating better mitochondrial biogenesis at day 7 post MCAO. Conclusion - Sovateltide mediated ETBR agonism promotes differentiation of NPCs and mitochondrial fusion

and biogenesis and helps in neuronal regeneration and function restoration in adult brains after acute ischemia.

APSET17.19

Endothelin-1/ZEB1/YAP regulatory circuitry governs epithelial-to-mesenchymal transition and metastatic progression in high-grade serous ovarian cancer

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Understanding the mechanistic dynamics sustaining the peculiar malignant traits of high-grade serous ovarian carcinoma (HG-SOC) is pivotal to develop new strategies for the management of this disease. Tumor aggressiveness is regulated by epithelial-to-mesenchymal transition (EMT), critically controlled by EMT-transcription factors (EMT-TF), as reflected by their association with poor clinical outcome in many tumors. Endothelin-1 (ET-1)/endothelin A receptor (ETAR) axis is aberrantly activated in HG-SOC where it promotes EMT, cell motility, and metastasis through the control over multiple determinants, including EMT-TF. Novel evidence in HG-SOC have uncovered the ET-1 signaling ability to promote the activity of ZEB1, a core TF in modulating the fine-tuning of EMT and metastatic behavior. Intriguingly, the cross-talk between ET-1/ETAR axis and the YAP pathway influences OC growth and metastatic progression by establishing DNA-binding platforms with different transcriptional partners. Because it has been recently reported that ZEB1 achieves a combinatorial control of tumor-promoting genes by complexing with YAP and AP-1, a potent oncogenic factor critically involved in the activation of ET-1 transcription, in this study we evaluate whether ZEB1, YAP signaling and AP-1 may interact and cooperate in mediating the ET-1/ETAR axis-driven tumor progression in HG-SOC. Loss-of-function experiments have been performed in patient-derived HG-SOC cells and cell lines and the ZEB1/YAP/AP-1 interaction has been examined through co-immunoprecipitation and in situ proximity ligation (PLA) assays. Promoters' transcriptional activity has been analyzed through luciferase reporter and chromatin immunoprecipitation assays. In vivo experiments have been conducted in HG-SOC xenografts and survival analyses have been performed in OC patients. Here we show that ETAR activation promotes the direct physical interaction of ZEB1 and YAP by inducing their nuclear accumulation in HG-SOC cells. Moreover, ET-1 guides their engagement in a nuclear active complex with TEAD4, a YAP downstream transcription factor, and the AP-1 subunit c-JUN. This complex, in turn, regulates the ET-1 transcription and its release from HG-SOC cells, thereby creating a feed-forward loop that sustains a persistent ET-1/ETAR signaling activity. In addition, the endothelin-1/ZEB1/YAP/AP-1 circuit promotes EMT, cell plasticity, invasiveness and metastatic progression. Of therapeutic interest, the FDA approved ET-1R antagonist macitentan, disaggregating the ZEB1/YAP/AP-1 protein complex, inhibits EMT, cell invasion and cell plasticity in HG-SOC cells and reduces metastatic dissemination in HG-SOC xenografts. High expression of ETAR/ZEB1/YAP/AP-1 gene signature is a strong predictor of poor clinical outcome in OC patients. This study provides novel mechanistic insights of EMT and tumor progression in HG-SOC revealing the functional integration between the ET-1/ETAR axis and the YAP/ZEB1/AP-1 complex, and suggests that blocking this molecular network using an ET-1R antagonist should be a promising treatment option for this disease.

APSET17.20

Capillary pericyte contraction by Aβ-evoked ET-1 release as a therapeutic target for Alzheimer's disease

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The brain requires a constant supply of energy, in the form of oxygen and glucose in the blood, to maintain ion pumping. However cerebral blood flow (CBF) is reduced early in Alzheimer’s disease (AD) because pericytes, contractile cells that surround capillaries, constrict capillaries by a mechanism dependent on Aβ-evoked endothelin-1 (ET-1) release. Here, we investigated the ion channels controlling the ET-1 evoked pericyte contraction, to devise therapeutic strategies to restore cerebral blood flow in AD. In acute brain slices, we found that ET-1 elevated intracellular pericyte Ca2+ ([Ca2+]i) by triggering Cl- exit via TMEM16A Ca2+-gated Cl- channels. This depolarised pericytes and amplified contraction by opening L-type voltage-gated Ca2+ channels (VGCCs). The pericyte [Ca2+]i rise and contraction were reduced by removing extracellular Ca2+, inhibiting TMEM16A channels or removing the depolarising Cl- gradient across the cell membrane. We show that TMEM16A and VGCCs are highly expressed in pericytes. Using combined in vivo two-photon imaging and laser Doppler flowmetry in a mouse model of AD, we found that the VGCC blocker nimodipine reduced pericyte [Ca2+]i, dilated capillaries, and improved cerebral blood flow. Nimodipine also reduced the number of stalled capillary neutrophils, which may become trapped by pericytes constricting capillaries in AD mice. Our data suggest that TMEM16A channels and VGCCs interact to control pericyte contraction and thus constitute novel therapeutic targets to restore energy supply and cognitive function in AD. Supported by the Wellcome Trust, ERC, British Heart Foundation, BBSRC, EMBO and Chulabhorn Royal Academy PhD studentship

APSET17.21

Endothelin-A Receptor Antagonism Increases Cutaneous Microvascular Vasodilation through Nitric Oxide-Independent Mechanisms in Non-Hispanic Blacks

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The purpose of this study was to investigate the contribution of endothelin-A receptor (ETAR) activation to cutaneous endothelium-dependent and nitric oxide (NO)-dependent vasodilation in non-Hispanic Blacks (NHB) and Whites (NHW). Seventeen young, healthy NHB (n=7) and NHW (n=10) individuals participated in this study. Participants were instrumented with two microdialysis fibers, and each site was randomized as control (lactated Ringer's) or ETAR antagonism (500 nM BQ-123). Laser-Doppler flow (LDF) probes and local heaters were used to measure skin blood flow and induce local thermal hyperemia, respectively. Each site was heated from 33°C to 39°C at a rate of 0.1°C/sec. Once a plateau to local heating was established (an index of generalized microvascular endothelial function), 20 mM L-NAME, a non-specific NO synthase (NOS) inhibitor, was infused at each site to specifically quantify NO-dependent vasodilation. Maximal vasodilation was induced by heating the skin to 43°C and infusing 54 mM sodium nitroprusside. Cutaneous vascular conductance (CVC) was calculated (LDF/MAP) and normalized to maximum (%CVCmax). Baseline and maximal skin blood flow were similar at control and BQ-123 sites in NHB and NHW (all comparisons $p > 0.05$). At control sites, NHB displayed attenuated endothelium-dependent (43 ± 13 vs. 77 ± 11 %CVCmax, $p < 0.01$) and NO-dependent (50 ± 2 vs. 70 ± 11 %NO, $p < 0.05$) vasodilation relative to NHW. BQ-123 increased endothelium-dependent vasodilation compared with respective control in NHB (64 ± 17 %CVCmax, $p = 0.03$) but not in NHW (77 ±13 %CVCmax, $p > 0.99$); additionally, this resulted in similar endothelium-dependent vasodilation in NHB and NHW ($p = 0.21$). There was no effect of BQ-123 on calculated NO-dependent vasodilation in NHB (55 ± 18 %NO, $p = 0.93$) or NHW (75 ± 9 %NO, $p=0.88$). There was no effect of ETAR antagonism on any aspect of microvascular vasodilation in NHW. ETAR antagonism did not impact NO-dependent vasodilation in NHB but did improve general endothelium-dependent vasodilation in this group. Therefore, ETAR appears to augment other endothelium-dependent pathways of vasodilation, but not the NO/NOS pathway, in NHB. This work is supported by NIH grant HL141205 to Brett Wong.

APSET17.22

Mathematical modeling of the physiology of endothelin-1 and its receptors A and B

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Background and objectives: Selective ET-1 receptor A (ET-1A) antagonists have been investigated to treat hypertension, chronic kidney disease (CKD), and cancer. However, their use has been limited because ET-1A antagonism increases the risk of fluid retention, which can increase the risk of heart failure. The mechanisms responsible for fluid retention remain incompletely understood, in part because of the complexity of the endothelin system. Mathematical modeling can be used as a tool to integrate information about complex physiological mechanisms into a quantitative framework. The objective of this study is to incorporate the physiology of ET-1, ET-1A, and ET-1B into an existing model of the cardiorenal system [1], as a tool for investigating endothelin receptor antagonism. Methodology: ET-1 concentrations in the systemic and kidney were described via a two-compartment model, where the binding of ET-1 to its receptors ET-1A and ET-1B takes place in the systemic, renal vasculature, and renal tissue according to the distribution of the receptors in the system. Parameters governing ET-1 distribution and clearance, as well as parameters governing each potential effect of ET-1 through ET-1A and ET-1B, were estimated by fitting published experimental studies of ET-1 infusion, with and without ET-1A or ET-1B antagonism. Results: The model was able to reproduce the clinically observed response to ET-1 infusion [2], including changes in glomerular filtration rate, renal blood flow, renal vascular resistance, sodium/water excretion, etc. It was also able to differentiate between the response to ET-1A and ET-1B antagonism [3,4,5]. The model-estimated effects of ET-1 through ET-1A versus ET-1B were consistent with known physiology about the distribution of these receptors. Conclusion: This model has the potential to provide insights into the mechanisms of selective ET-1A-antagonist-induced fluid retention. It would also be beneficial in exploring the possible combinations of ET-1A antagonists with diuretic drugs for treating patients with hypertension and/or CKD. Reference: Yu, H. et al. 2020. PLoS computational biology, 16(8), p.e1008074.Kaasjager, K.A. et al. 1997. Journal of the American Society of Nephrology, 8(1), pp.32-39.Tycho Vuurmans, J.L. et al. 2004. Nephrology Dialysis Transplantation, 19(11), pp.2742-2746.Pedersen, E.B., et al. 2005. American journal of hypertension, 18(12), pp.1578-1585.Böhm, F., et al. 2003. Clinical Science, 104(2), pp.143-151.Contact information: Hongtao Yu, Ph.D. AstraZeneca R&D | Clinical Pharmacology & Safety Sciences Clinical Pharmacology & Quantitative Pharmacology 1 MedImmune Way, Gaithersburg 20878 US

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APSET17.24

Enhanced BMPR1B Expression by Endothelin-1 Mediates Proliferation of Pulmonary Artery Smooth Muscle Cells

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Background: The pathology of pulmonary arterial hypertension (PAH) indicates the abnormal outgrowth of pulmonary artery smooth muscle cells (PASMCs) of the media. An unspecified disturbance in BMP signaling appears to be involved in the development of this disease. Type I receptors (BMPR1A, BMPR1B) combined with type II receptors (BMPR2) transduce signals via two several distinct pathways, i.e. the canonical pathway leading to ID1 induction, and the noncanonical pathway activating p38MAPK. The p38MAPK is implicated in the cell proliferation. Compared to BMPR1A, BMPR1B expression is lower in PASMCs from control individuals, and is enhanced in those from idiopathic PAH patients. BMP15 binds specifically to BMPR1B. Abundant expression of endothelin 1 (ET-1) and activated p38MAPK are observed in PAH. Objective: To assess the effect of ET-1 on BMPR1B expression and cell proliferation. Methods: We stimulated purchased human PASMCs by BMP2, BMP15, ET-1, or the combination of them in vitro. PH-797804 was used as a selective inhibitor of p38MAPK. Quantitative PCR was performed to quantify mRNA expressions. Results: After pretreatment with ET-1, BMP2 increased BMPR1B expression. Although BMP2 alone did not affect, combination of BMP2 with ET-1 pretreatment significantly accelerated the proliferation of PASMCs. PH-797804 (10-100 nM) abrogated this proliferation. Similarly, after ET-1 pretreatment, BMP15 significantly accelerated the proliferation of PASMCs. Stimulation with BMP15 alone did not affect. Conclusions: In PASMCs, ET-1 exposure in pathological condition increases BMPR1B expression, and modifies BMP signaling to activate p38MAPK, resulting in cell proliferation. Since BMP15 is expressed strongly in oocytes in preantral follicles, our findings illustrate the sex difference in the incidence of PAH.

APSET17.25

Low HDL-Cholesterol is Not Associated with Increased ET-1-Mediated Vasoconstrictor Tone

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High-density lipoprotein (HDL) cholesterol is an important anti-atherogenic fraction of total cholesterol and an inverse predictor of coronary heart disease and associated events. Data from the Framingham Heart Study indicate that the risk for myocardial infarction increases by as much as ~25% for every 5 mg/dL reduction in HDL-cholesterol, below median values for males and females. Nearly 20% of the adults in the United States have low HDL-cholesterol levels (< 40 mg/dL for men; < 50 mg/dL for women). The mechanisms involved with the increased vascular risk with low HDL-cholesterol are not completely understood. Endothelin (ET)-1 is a powerful vasoconstrictor peptide that in addition to contributing to the regulation of vascular tone is also involved in the development and progression of atherosclerotic vascular disease. Increased ET-1 vasoconstrictor tone is a common characteristic of numerous cardiovascular conditions and risk factors, including hypercholesterolemia. The experimental aim of this study was to determine whether ET-1-mediated vasoconstrictor tone is elevated in adults with low HDL-cholesterol, independent of other cardiometabolic abnormalities. Forearm blood flow (FBF; via plethysmography) responses to intra-arterial infusion of selective ETA receptor blockade (BQ-123: 100 nmol/min for 60 min) were determined in 30 middle-aged and older men (45-70 years): 15 with optimal HDL-cholesterol (age: 58+2 yr; BMI: 29.4+1.0 kg/m2; HDL-cholesterol: 50.1+1.6 mg/dL) and 15 with low HDL-cholesterol (57+ 2 yr; 28.2+1.0 kg/m2; 34.7+1.6 mg/dL). All men were sedentary, non-smokers, normotensive and free of overt cardiometabolic disease. Aside from HDL-cholesterol there were no differences in plasma total cholesterol (204.4+5.6 vs 201.3+6.5 mg/dL; P=0.76), low-density lipoprotein cholesterol (127.4+7.0 vs 132.9+6.6 mg/dL; P=0.57), triglyceride (133.3+17.4 vs 168.7+17.3 mg/dL; P=0.16), glucose (90.2+2.7 vs 93.0+3.2 mg/dL; P=0.51) or insulin (8.1+4.2 vs 8.9+0.9 IU; P=0.56) concentrations between the optimal and low HDL-cholesterol men. BQ-123 elicited a significant increase in FBF (~20-25%) in each group. However, there were no group differences (P=0.66) in the FBF responses to selective ETA receptor blockade. Moreover there was no significant correlation (r=0.02) between HDL-cholesterol concentrations and maximal FBF response to BQ-123 (60 min of BQ-123 in both groups) in the study population. Isolated low HDL-cholesterol is not associated with increased ET-1-mediated vasoconstrictor tone. ET-1 system activity may not contribute to the increased cardiovascular risk burden with low HDL-cholesterol.

APSET17.26

High salt diet alters circadian behavior and neurophysiology of the suprachiasmatic nucleus: Implications for Endothelin B receptor function in the brain

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Circadian rhythms are 24-hour oscillations in biological processes such as blood pressure and sodium excretion. These rhythms are driven by a series of transcriptional-translational feedback loops known as the molecular clock and exist in all tissues in the body. The central clock located in the suprachiasmatic nucleus (SCN) of the hypothalamus, uses light as an external cue, or Zeitgeber,

to keep the clocks throughout the body synchronous. Recent evidence from our group and others suggests a connection between the molecular clock and the endothelin system in peripheral tissues, however, less is known about this relationship in the SCN, which contains endothelin receptor B (ETB). Outside of the SCN ETB plays a key role in the excretion of salt, therefore our aim was to explore a potential role for ETB in the SCN in response to a high salt diet (HSD; 4% NaCl). Specifically, we hypothesized that ETB mRNA expression varies over the 24-h day in the SCN and that a HSD impairs circadian behavior and neurophysiological activity of SCN neurons. Using RNAscope® fluorescent in situ hybridization, we determined ETB mRNA expression in the SCN of C57BL/6 mice during the late day (zeitgeber time, or ZT 11) and late night (ZT 23). There was a significant day-night difference, with expression being higher at late day, 4.38 ± 0.4 ETB/cell, compared to late night, 2.69 ± 0.4 ETB/cell ($p < 0.05$, Student's t-test). A HSD increases ETB function in the kidney, so we next examined whether HSD impacts circadian behavior and electrophysiology in the SCN. Male C57BL/6 mice, aged 2-3 months, were released into constant darkness for 2 weeks after a one week habituation in a light controlled chamber. Locomotor activity was assessed via ad libitum running wheel in mice fed a HSD or normal chow (0.49% NaCl). We found no significant differences in overall amount of activity. However, HSD-fed animals lacked the late-night siesta (or night-time sleep in nocturnal mice). For the electrophysiology experiment, male and female C57BL/6 mice, aged 4-5 months, neuronal excitability was significantly higher at night, 2.40 ± 0.24 Hz , in SCN neurons from HSD-fed mice compared to neurons from chow-fed controls, 1.05 ± 0.13 Hz ($p < 0.001$, Mann-Whitney U test). This increase in nighttime spike rate was ameliorated when the SCN was treated with an ETB antagonist (A-192621, 1μM). Our data show that the ETB exhibits diurnal variation in the SCN suggesting a connection between the endothelin system and the molecular clock in the master pacemaker of the body. The endothelin system may also mediate the effects of HSD in the SCN.

APSET17.28

Endothelial endothelin-1 induces perivascular stem cell senescence via endothelin receptor type B in skeletal ageing

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Endothelin-1 (ET-1) is a known potent vasoconstrictor, playing a vital role in regulating the blood flow and blood pressure. It is a peptide that works through two types of G-protein coupled receptor, ETAR and ETBR, upregulated during the progression of hypertension, diabetes and ageing (Seccia et al., 2003). Overexpression of ET-1 can cause endothelial dysfunction and senescence. It is also found associated with the development of age-related diseases (Bohm & Pernow, 2007). The bone marrow is enriched with blood vessels, however, the effect of endothelin-1 secretion from endothelial cells on perivascular mesenchymal stem cells (MSCs) function in skeletal ageing is unclear. Previous study showed that ET-1 can stimulate the production of reactive oxygen species and hypertrophic growth in cells (Tang, Li, & Chen, 2020.) In this study, we investigated the role of ET-1 on MSCs senescence in skeletal ageing. We demonstrated that ET-1 expression is upregulated with age and is related to bone loss in aged mice. Endothelium-derived ET-1 not only results in endothelial dysfunction, but also affecting the bone enriched with blood vessels. We showed that ETBR is responsible for ET-1 induced MSCs senescence through ROS accumulation instead of ETAR. When ETBR is selectively blocked using BQ788, ROS accumulation and presence of senescence MSCs is effectively reduced, bone volume is also improved in 20M aged mice. References Bohm, F., & Pernow, J. (2007). The importance of endothelin-1 for vascular dysfunction in cardiovascular disease. Cardiovasc Res, 76(1), 8-18. doi:10.1016/j.cardiores.2007.06.004 Seccia, T. M., Belloni, A. S., Kreutz, R., Paul, M., Nussdorfer, G. G., Pessina, A. C., & Rossi, G. P. (2003). Cardiac fibrosis occurs early and involves endothelin and AT-1 receptors in hypertension due to endogenous angiotensin II. Journal of the American College of Cardiology, 41(4), 666-673. Tang, X., Li, P.-H., & Chen, H.-Z. (2020). Cardiomyocyte Senescence and Cellular Communications Within Myocardial Microenvironments. Frontiers in Endocrinology, 11. doi:10.3389/fendo.2020.00280

APSET17.30

Trans-myocardial extraction of endothelin-1 correlates with increased microvascular resistance following percutaneous coronary intervention

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Introduction Coronary microvascular dysfunction (CMD) can persist following successful percutaneous coronary intervention (PCI). Endothelin-1 (ET-1) is a potent vasoconstrictor and may be an important mediator of CMD. We sought to assess the trans-myocardial gradient (TMG – coronary sinus minus coronary root levels) of ET-1 and its precursor - Big ET-1 and assess the correlation with pressure-wire indices of CMD: coronary flow reserve (CFR) and index of microvascular resistance (IMR). Methods Paired blood samples from the aortic root and coronary sinus were collected before and after pressure wire guided PCI from patients with stable angina. Plasma was then analysed using specific enzyme linked immunosorbent assay (ELISA) for quantification of ET-1 and Big ET-1 and correlated with pressure-wire data. Results Samples were analysed from 66 patients. Both mean ET-1 and Big ET-1 concentrations increased post-PCI in both the aorta (ET-1: 1.0±0.4 pg/ml to 1.4±0.4 pg/ml, $p<0.0001$ and Big ET-1: 2.8±1.3 pg/ml to 3.4±1.6 pg/ml, $p<0.0001$) and coronary sinus (ET-1: 1.0±0.3 pg/ml to 1.2±0.3 pg/ml, $p=0.03$ and Big ET-1: 3.2±1.7 pg/ml to 3.8±1.5 pg/ml, $p=0.01$). TMG extraction

of ET-1 increased following PCI: 0.05±0.25 pg/ml vs.-0.20±0.41 pg/ml, p=0.01. In contrast, there was TMG release of Big ET-1 before and after PCI: 0.46±1.26 pg/ml vs. 0.38±1.03 pg/ml, p=0.52. ET-1 extraction correlated with IMR post-PCI (Pearson's r = 0.293, p=0.02). Patients with CFR<2 post-PCI demonstrated a trend towards numerically greater mean ET-1 extraction than those with preserved CFR post-PCI (0.30±0.51 pg/ml vs. 0.16±0.42 pg/ml, p=0.31) as did those with criteria for Type 4a Myocardial Infarction compared with those without (0.39±0.57 vs. 0.15±0.41, p=0.11). Conclusions ET-1 and Big ET-1 significantly increase post-PCI. Trans-myocardial extraction of ET-1 increases post-PCI and correlates with post-PCI CMD.

APSET17.31

ET-1 promotes renal iron accumulation in murine models of iron overload

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Renal iron deposition correlates with elevated plasma ET-1 in sickle cell disease (SCD) patients, as well as humanized sickle cell (HbSS) mice. Also, excessive renal iron accumulation in HbSS mice is ameliorated by ETA receptor antagonism. Thus, we hypothesized that ET-1 via ETA receptor activity leads to dysfunctional renal iron trafficking, promoting iron accumulation in the kidney. To determine the involvement of ET-1 and ETA receptor in renal iron handling we generated male and female proximal tubule (PT) specific ETA receptor knockout (PT ETA KO) and control floxed mice. At baseline, 16 weeks old PT ETA KO mice (n=6) presented with higher plasma iron concentration (0.98±0.16 vs. 0.52±0.10 µg/ml; p=0.031) and urinary iron excretion (4.52±0.65 vs. 1.74±0.46 µg/24h; p=0.002), whereas renal iron deposition remained unchanged (2.26±0.48 vs. 2.44±0.73 Kpix/ µm; p=0.85). To induce acute iron overload, KO and floxed mice were injected with phenylhydrazine (PhZ, 40 mg/kg, IP; 2 consecutive days) or saline. PhZ significantly reduced hemoglobin, and increased spleen/body weight ratio in both PT ETA KO and littermate controls when compared to saline injected mice. Moreover, PT ETA KO mice with induced hemolysis had increased plasma iron concentration (4.43±0.32 vs. 3.00±0.47 µg/ml; p=0.031) and urinary iron excretion (5.58±0.68 vs. 3.41±0.72 µg/24h; p=0.051) when compared with control mice. To determine the long-term effect of ET-1 on chronic renal iron accumulation we generated HbSS mice lacking ET-1 in vascular endothelial cell mice (HbSS VEET KO) and genetic controls (HbSS VEET flox). There were no differences in anemia status and spleen/body weight ratio between 20 weeks old male HbSS VEET KO and flox mice (n=9 and 14, respectively). However, plasma iron concentration (117.30±4.28 vs. 103.89±4.28 µg/dl, p=0.037) and urinary iron excretion (10.44±1.89 vs. 7.44±1.14 µg/24h; p=0.059) were elevated and renal iron deposition attenuated (24.3±1.29 vs. 33.80±2.26; p=0.008) in HbSS VEET KO mice. These data suggest that ET-1, via ETA receptor activity, contributes to renal iron accumulation in murine models of iron overload. Support provided by NIH-funded U01 HL117684 to D.M.P and J.S.P, NIH-NHLBI K99HL144817 and R00HL144817 to M.K.

APSET17.32

Protective roles of epithelial-derived Endothelin-2 in bleomycin-induced pulmonary fibrosis

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Elevated endothelin-1 (ET-1) is associated with lung fibrosis disease in both patients and mice. It has been shown that human ET-1 transgenic mice showed spontaneous development of pulmonary fibrosis instead of pulmonary hypertension. Notably, the bleomycin-induced lung fibrosis mice model showed marked improvement after bosentan treatment. Despite the strong evidence, several clinical trials of endothelin receptor antagonists yielded disappointing results, even detrimental effects for lung fibrosis patients. In this current study, we tested the hypothesis that ET-2, which acts via the same receptor as ET-1, might play a distinct role with ET-1 in pulmonary fibrosis. We confirmed ET-2 expression in lung epithelial cells, then knocked out ET-2 in epithelial cells under SHH promoter, termed ET-2fl/fl; SHH-Cre+/- mouse. Pulmonary fibrosis was induced by instilling bleomycin (5gr/kg) or normal saline vehicle intratracheally into the lungs of ET-2fl/fl; SHH-Cre+/- and their ET-2fl/fl littermates under general anesthesia (isoflurane inhalation). Fibrotic properties were assessed on day 14 or 21 after treatment. To further explore the mechanism, we utilized A549 as an alveolar epithelial cells model to investigate the role of ET-1 or ET-2 using siRNA-mediated silencing strategy and isolated mouse lung fibroblast to explore the role of exogenous ET-1 or ET-2 peptide (100 nM) upon 10 ng/mL TGF-β-induced fibroblast activation. Our results showed that bleomycin treatment increased collagen deposition in the lung after 14 and 21 days, and it exacerbated in ET-2fl/fl; SHH-Cre+/- mouse, compared to the vehicle-treated group (1.5 fold and 1.9 fold changes in 21 days, respectively). Static compliance of the lungs were decreased (0.057±0.009 vs 0.038±0.008 mL/cmH2O) in ET-2fl/fl; SHH-Cre+/- mice. Consistently, myofibroblast infiltration was increased in ET-2fl/fl; SHH-Cre+/- mice (12.64±3.6 vs 21.71±6.01% αSMA positive area/DAPI). Furthermore, our in vitro results showed that ET-2, but not ET-1, knockdown A549 cells exhibit enhanced apoptosis compared to control after H2O2 stimulation (7.46±0.85, 5.44±0.79, and 2.74±2.03 arbitrary unit of Cleaved Caspase-3/Caspase-3 relative protein expression ratio, respectively). In the murine lung fibroblast, exogenous ET-1 showed

upregulated, yet interestingly, ET-2 treatment suppressed TGF-β-induced fibroblast activation (73.05±30.17, 19.56±5.6, and 31.73±2.67 arbitrary unit of relative ACTA2 expression respectively, compared to vehicle control). Taken together, our results indicated that blocking ET-2 showed detrimental effects in bleomycin-induced pulmonary fibrosis and suggested the distinct roles of ET-1 and ET-2 pulmonary fibrosis pathophysiology.

APSET17.35

Combination approaches of endothelin-1 receptor antagonist and PARP inhibitor sensitizes high-grade serous ovarian cancers targeting HIF-1α/YAP signaling in tumor and microenvironmental elements

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TP53 and BRCA mutations are distinct attributes of high-grade serous ovarian cancer (HG-SOC), characterized by low therapeutic sensitivity. PARP inhibitors (PARPi) have shown promising clinical activities in HG-SOC, however the signals exchange between HG-SOC cells and the tumor microenvironment (TME) appear to limit PARPi efficacy, suggesting that blunting the HG-SOC/TME dialogue may boost PARPi response rate. It is reported that HG-SOC, through the alliance between endothelin-1 (ET-1) and YAP signals, adopts a drug escaping strategy, unveiling the central contribution of ET-1 receptors (ET-1R)/β-arrestin1 (β-arr1) axis in sustaining the YAP/mutp53 transcriptional integration. Despite the identification of such drug adaptation signaling circuit, the mechanisms leading to PARPi response in HG-SOC are partially elucidated. By loss-of-functions approaches patient-derived (PD) HG-SOC cells (PMOV10) and Human Umbilical Vein Cells (HUVEC) were analysed for cell viability, plasticity and response to pharmacological treatments. In vivo efficacy of combination therapies was evaluated in PD xenograft (PDX) model. RNA-seq analysis reveals the Hypoxia and VEGF pathways enrichment in ET-1-treated PMOV10 cells and their down-regulation upon treatment with the dual ET-1R antagonist macitentan. Considering the importance of hypoxia in ET-1 and VEGF regulation, we evaluated in PMOV10 cells and HUVEC the hypoxia-inducible factor-1α (HIF-1α) enrolment in multimeric transcriptional complexes that mediate the ET-1-induced therapy elusion. Mechanistically, ET-1R/β-arr1 axis, to an extent comparable to hypoxia, enhances YAP/HIF-1α nuclear interaction, allowing YAP/mutp53 recruitment on HIF-1α target gene promoters. Among these, EDN1 transcription ensures an autoregulatory cycle that sustains PARPi therapy evasion. As result of YAP/mutp53/HIF-1α-directed gene transcription PMOV10 cells and HUVEC release pro-angiogenic mediators, that regulating in autocrine/paracrine manner the PMOV10/HUVEC signal reciprocity, support PARPi resistance. Macitentan, dismantling the ET-1R-mediated YAP/mutp53/HIF-1α network, increases cleaved-PARP levels, enhances the number of γH2AX and RAD51 foci and interfere with PMOV10/HUVEC mutual cooperation. In vivo macitentan, sensitizing HG-SOC PDX to the PARPi olaparib, reduces their metastatic potential. Clinically relevant, ETAR/YAP/HIF-1α gene signature correlates with a worst prognosis in HG-SOC patients bearing mutp53. These findings recognize in the networking between ET-1R/β-arr1 and YAP/mutp53/HIF-1α a tumor/TME shared escaping strategy from DNA damaging agents. ET-1R blockade by macitentan, interfering with such signaling network, sensitizes HG-SOC to olaparib providing a rational for novel combinatorial treatments for HG-SOC.

APSET17.36

Chlorogenic acid attenuates kidney injury progression in diabetic rat model through the downregulation of glomerulosclerosis, TGF-β1, and Endothelin-1 (ET-1)

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Diabetic nephropathy (DN) is a common complication arising from Diabetes Mellitus (DM), which is characterized by podocytopathy, inflammation, renal architecture damage, and fibrosis. Endothelin-1 plays a role in the mechanism of kidney fibrosis as a pro-fibrotic substance and associates with the mechanism of DN. Chlorogenic acid (CGA), an active compound of green coffee, has been known to have beneficial effects in kidney injury. However, its effect in preventing kidney injury after DM has not been completely understood. This study elucidated the renoprotective effect of CGA in preventing kidney injury after DM. Diabetic model was performed in male Wistar rats (2 months, 150-200 grams) with a single intraperitoneal injection of streptozotocin for 1.5 months (DM 1.5, n = 5) and 2 months (DM 2, n = 5). Three groups of DM were treated with three different doses of CGA intraperitoneally for 2 weeks: 12.5 (CGA 1 group, n = 5), 25 (CGA 2 group, n = 4), and 50 (CGA 3 group, n = 6) mg/kg of body weight. During termination, blood was collected for creatinine and glucose level, and urine was used for proteinuria scoring. The kidney was harvested for histopathological analysis and RNA extraction. RT-PCR was performed to examine the expression of ppET-1, eNOS, TGF-β1, MCP-1, CD-68, podocin and vimentin mRNA expression. Immunostaining was carried out for fibroblast and myofibroblast observation. DM 1.5 and DM 2 groups represented higher blood glucose and proteinuria score, but not serum creatinine level compared to the control group, which was associated with higher tubular injury and

glomerulosclerosis score. RT-PCR analysis also showed higher ppET-1, eNOS, TGF-β1, MCP-1, CD-68, vimentin, and with lower podocin mRNA expression. CGA-treated groups showed different results. CGA 1 group demonstrated attenuation of the injury with lower glucose level, proteinuria score, tubular injury score, and glomerulosclerosis score, but not serum creatinine level. Attenuation of the injury also showed by the downregulation of ppET-1, TGF-β1, MCP-1, CD-68, vimentin, with the upregulation of eNOS and podocin mRNA expression in the CGA groups. Immunostaining revealed a slight expansion of fibroblast and myofibroblast in DM groups, with a reduction in CGA groups. In conclusion, CGA may attenuate kidney injury progression after DM in association with the downregulation of glomerulosclerosis, TGF-β1, and ET-1 signaling. Keywords: Chlorogenic acid, diabetic nephropathy, glomerulosclerosis, ET-1, TGF-β1

APSET17.37

Obesity-Related Endothelial Microvesicles do not Stimulate Endothelial ET-1 Production

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Obesity is associated with increased endothelin (ET)-1 system activity resulting in enhanced vasoconstrictor tone and increased risk of cardiovascular disease (CVD) and events. The mechanisms responsible for increased ET-1 production with obesity are not fully understood. Circulating extracellular vesicles, particularly endothelial cell-derived microvesicles (EMVs), are elevated with obesity and have been linked to endothelial vasomotor dysfunction and CVD risk, severity and outcome. The number, size, antigenic composition and biological effects of EMVs are dictated by pathology and the stimulus for release. Under normal healthy conditions, EMVs aid in cell-to-cell communication, activate repair or defense mechanisms, and/or stimulate immune responses. Under pathologic conditions, however, EMVs are released in much greater number and their functional phenotype is more likely to evoke and perpetuate pathologic cellular effects and responses. We have previously demonstrated that ET-1 increases EMV release and induces a proatherogenic EMV phenotype. It is currently not known whether obesity-related EMVs, in turn, promote endothelial ET-1 production. We tested the hypothesis that obesity-related EMVs increase ET-1 production and release from endothelial cells. As part of an ongoing study, circulating EMVs (CD31+/CD42b-) were isolated (by flow cytometry) from 8 normal weight (2 F/6 M; age: 57±2 yr; BMI: 24.1±0.3 kg/m2) and eight obese (4 F/4 M; 61±3 yr: 31.3±0.5 kg/m2) adults. All subjects were non-smokers, normotensive, normolipidemic and free of overt cardiometabolic disease. Human umbilical vein endothelial cells were cultured and treated with EMVs from each subject. The concentration of EMVs was the same across the subject wells (2:1; EMV: cell ratio) to ensure that any EMV-induced changes in cell protein expression was due to the EMV phenotype rather than a concentration-related phenomenon. Cell protein expression was determined by capillary electrophoresis immunoassay. Circulating EMVs were markedly higher (~180%; P<0.01) in the obese (177±23 EMV/uL) compared with normal weight (63±10 EMV/uL) adults. There were no significant differences in intracellular protein expression of either endothelin converting enzyme (ECE: 204.2±23.0 vs 203.3±21.3 AU; P=0.98) or Big ET-1 (61.9±8.0 vs 67.2±5.1 AU; P=0.59) in cells treated with EMVs from the normal weight or obese adults. Concordantly, ET-1 production was not significant different between cells treated with normal weight- or obesity-related EMVs (585.6±18.1 vs 634.6±21.9 pg/mL; P=0.11). Although circulating EMVs are elevated in obese adults, contrary to our hypothesis, they do not appear to be a causative factor underlying the increase in ET-1 production with obesity.

APSET17.38

Evidence for multifactorial control of endothelin-1 excretion in rats

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Endothelin-1 (ET-1) is a potent vasoactive peptide that is important in the regulation of renal sodium handling. Recent evidence from our group and others has suggested a connection between the endothelin system and the molecular clock in sodium homeostasis, however, the role of timing of food intake in the context of these findings has not yet been established. In order to investigate this gap in knowledge, we utilized a rat model with a whole-body loss of Bmal1, a core component of the molecular clock in conjunction with a time restricted feeding protocol. Male and female Bmal1-KO rats and littermate controls, aged 10 weeks, were placed in metabolic cages and allowed access to food (0.49% NaCl) ad libitum for 2 days after a 2-day acclimation period. Food availability was then restricted to the inactive/light period for 5 days (iTRF). Urine was collected every 12 hours. Urinary ET-1 concentrations were measured by ELISA and urinary Na+ concentrations were measured by ion-sensitive electrode (EasyLyte, Medica). The night-day difference in the rate was calculated by subtracting inactive period excretion from active period excretion. Male control rats (n=8) had an ET-1 night-day difference of 1.2±0.3 pg during ad lib feeding and 0.6±0.3 pg during iTRF. Male Bmal1-KO rats (n=7) had a night-day difference 0.4±0.5 pg during ad lib feeding and a night-day difference of -0.5±0.4 pg during iTRF. Female control rats (n=5) had a night-day difference of 0.66±0.58 pg during ad lib feeding and a night-day difference of 0.8±0.7 pg during iTRF, while female Bmal1 KO rats (n=6) displayed night-day differences of 1.6±0.5and -0.8±0.3 during ad lib and iTRF, respectively. Male control rats had a Na+ excretion night-day difference of 0.2±0.1mmol during ad lib feeding and a difference of -0.04±0.1 during iTRF. Male Bmal1-KO rats had a Na+ excretion night-day difference of 0.01±0.11 mmol during ad lib feeding and a difference of -0.19±0.09 mmol of Na+ during iTRF. Female control rats had a Na+ excretion night-day difference of 0.3±0.05 mmol during ad lib feeding and 0.065±0.064 mmol during iTRF. Female Bmal1-KO rats had a Na+ excretion night-day difference of 0.11±0.07 and -0.28±0.03

mmol during ad lib and iTRF, respectively. In male rats, the diurnal ET-1 excretion was significantly different by genotype (2-way ANOVA, $p<0.05$). In female rats, ET-1 excretion differences were only significant as an interaction effect between genotype and feeding protocol (2-way ANOVA, $p<0.05$). Na+ excretion differences were significantly different by genotype and protocol (2-way ANOVA, $p<0.05$) in both male and female animals. Together, these data suggest that renal ET-1 excretion is likely mediated by a complex array of physiological systems, including but not limited to nutrient availability, sex, and the molecular clock. Funding provided by NIH PO1 HL136267 to DMP and AHA Postdoctoral Fellowship 827566 to MKR

APSET17.39

Common variants associated with hypertension and CAD epigenetically regulate vascular-specific expression of EDNRA

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Introduction: Genome-wide association studies (GWASs) for coronary artery disease have identified over 270 loci associated with disease risk. The majority of these variants are in non-coding regions of the genome, and therefore affect disease through their regulatory effects on gene expression. Though individual variants have small effects on disease, they collectively account for 38.6% of heritable risk. We have previously shown that variants at chromosome 6p24 increase risk for multiple vascular diseases by regulating Endothelin-1 (EDN1) expression. GWAS signals at other ET-1 pathway genes can implicate epigenetic regulation of ET-1 function in the risk of vascular diseases, including CAD. Hypothesis: Epigenetic regulation of the ETA but not ETB is causally associated with CAD and hypertension risk. Methods and Results: The largest GWAS for CAD comprising 181,522 cases and 1,165,690 study participants identifies multiple significant variants at the chromosome 4q21 locus within 200kB of the EDNRA promoter. The lead SNP, rs6841581, is associated with CAD ($p=2.65x10-30$, $\beta=1.07$), systolic blood pressure ($6.01x10-10$, $\beta=0.012$), pulse pressure ($p=1.95x10-19$, $\beta=0.015$). There is no significant association for any vascular phenotype for variants at the EDNRB locus. Analysis of histone acetylation at the locus identifies vascular-specific open chromatin consistent with an enhancer regulating EDNRA expression. Single cell ATAC-seq of human vascular tissue demonstrates that vascular smooth muscles and fibroblasts are the likely relevant cell types, with both having accessible chromatin at the site of the non-coding SNP, rs6841581. Conclusions: Variants at vascular-specific enhancers in proximal to EDNRA are associated with CAD and hypertension risk. This establishes epigenetic regulation of EDNRA, but not EDNRB, as a causal risk factor both CAD and hypertension. Future investigation will identify the tissue-specific mechanism of gene regulation that underlies this human disease association.

APSET17.40

Porphyromonas gingivalis (Pg) Infection Upregulates the Endothelin (ET) System in Brain Microvascular Endothelial Cells: Relevance to Cognitive Impairment

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Alzheimer's Disease and Related Dementias (AD/RD) are devastating diseases with no treatment. It is increasingly recognized that cerebrovascular dysfunction precedes the development of pathologies such as tau/amyloidβ tangles, neuroinflammation and cognitive decline. Endothelin-1 (ET-1), the most potent vasoconstrictor identified to date, contributes to cerebrovascular dysfunction and brain ET-1 levels were shown to be related to AD progression. ET-1 also contributes to neuroinflammation, especially in infections of the central nervous system. Recent studies causally linked chronic periodontal infection with opportunistic anaerobic bacteria Porphyromonas gingivalis (Pg) to AD development. Thus, the goal of the study was to determine the impact of Pg infection on the ET system and cell senescence in brain microvascular endothelial cells (BMVECs) under hypoxia and oxidative stress conditions. Methods: Cells were infected with 50 MOI Porphyromonas gingivalis (Pg) with and without ATP-induced oxidative stress for 24 hours. Oxidative stress and hypoxia were chosen to mimic hypoperfusion conditions observed in ADRD. Serum starved (2% fetal bovine serum) human brain microvascular endothelial cells (HBEC5i) were used for all conditions. For oxidative stress, cells were exposed to 50uM and 100uM hydrogen peroxide for 2 hours. For hypoxic conditions, they were exposed to 3% Oxygen for 72 hours. Cell lysates were collected for Western blot analysis of ETA/ETB receptor as well as p21 and leukemia inhibitory factor-1 (LIF-1) proteins as markers of senescence. ET-1 levels in cell culture media were measured with ELISA. Results: Pg infection increased ET-1 (pg/ml) secretion almost 4-fold with (48.8 ± 9.3) and without (68.7 ± 5.7) ATP as compared to control (13.5 ± 2.7, $p<0.0001$). ETA receptor expression (ETA/actin, arbitrary units) was also increased from 0.5 ± 0.06 to 1.24 ± 0.1 and 1.24 ± 0.2, respectively ($p<0.05$). There was no change in ETB receptors. There was a trend for decreased LIF-1 and p21 levels in Pg-stimulated cells. Under hypoxic conditions, ETA and p21 levels decreased ($p<0.05$) with an increasing trend in LIF-1. Oxidative stress condition had no effect on ET receptors or ET-1 but decreased p21 levels ($p=0.0621$). Conclusions: Disruption of BMVEC integrity leads to increased blood brain barrier (BBB) permeability, an early marker for ADRD. Current findings suggest that Pg infection may contribute to disruption of endothelial integrity via activation of the ET system and represents a novel mechanism by which chronic Pg infection may contribute to ADRD.

APSET17.41

Chlorogenic acid attenuates pulmonary fibrosis in association with TGF-β1 and Endothelin-1/ETAR downregulation and antifibrotic mediator upregulation in diabetic rat model

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Diabetes Mellitus (DM) is a chronic metabolic disease characterized by hyperglycemia, which complications affect several organs, including the lungs. Pulmonary fibrosis is one of the manifestations of diabetes-related lung injury, characterized by scar formation and destruction of the pulmonary epithelium followed by respiratory failure. Endothelin-1(ET-1) acts synergistically with TGF-β1 as the predominant profibrotic mediators in hyperglycemia-induced fibrosis, whereas Smad7 is an important negative regulator of fibrosis. Chlorogenic acid (CGA), a polyphenolic compound in coffee, has been known as a novel insulin sensitizer with antioxidant content, might have an effect in preventing fibrosis progression although the mechanism is still not well understood. The aim of this study is to elucidate the role of CGA in preventing pulmonary fibrosis progression through ET-1/ETAR and TGF-β1 signaling pathway in diabetes rat model. 24 Male Wistar rats (2 months, 150-200 grams) were randomly divided into 5 groups. 1 group received intraperitoneal injection of normal saline as healthy control (C group, n=5) and 4 groups received single dose intraperitoneal injection of Streptozotocin (STZ) 60 mg/kgBW to induce DM. STZ-induced diabetes rats were sacrificed after 6 weeks (DM1 group, n=5) and 8 weeks (DM2 group, n=5) for diabetes groups. CGA 12.5 mg/kgBW (CGA1 group, n=5) and 25 mg/kgBW (CGA2 group, n=4) were administered intraperitoneally for 14 consecutive days after 6 weeks of STZ injection and sacrificed after a fortnight. Serum glucose level were monitored. Histological and immunohistochemistry staining against alpha-SMA were conducted. Reverse Transcriptase-PCR (RT-PCR) was performed to examine mRNA expressions of TGF-β1, ppET-1, ETAR, CTGF, α-SMA, Collagen-1, and Smad7. DM1 and DM2 groups showed successful DM induction by significant high glucose level and aberrant fibrosis compared to control group, with increased of TGF-β1, ppET-1, ETAR, CTGF, α-SMA, and Collagen-1 mRNA expressions and decreased of Smad7 mRNA expression. In the CGA-treated groups, glucose level was significantly lower than the DM2 group. CGA 12.5 mg/kgBW showed significant down-regulatory effects on TGF-β1, ppET-1, CTGF, and Collagen-1 mRNA expression and up-regulatory effect on Smad7 mRNA compared to DM2 group. mRNA expression of ETAR were decreased although not statistically significant. Histopathological staining also showed milder fibrosis in CGA1 group compared to DM control group. Meanwhile, CGA2 group didn't show improvement in fibrosis progression, although not statistically significant compared to DM2 group. CGA 12.5 mg/kgBW reduces fibrosis progression in hyperglycemic induces pulmonary fibrosis through downregulating ET-1 and TGF-β1 signaling and upregulating Smad7. Keywords: chlorogenic acid, diabetes mellitus, pulmonary fibrosis, TGF-β1, ppET-1, ETAR

APSET17.42

Endothelin-1 and YAP alliance in cancer

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The limited clinical response observed in cancer, particularly in high-grade serous ovarian cancers (HG-SOC) characterized by high frequency of TP53 mutations (mutp53; 98%), might be related to mutp53-driven pervasive signaling integrations orchestrating the aberrant cancer transcriptional programs. An expanding body of evidence highlights that, in addition to their cell-intrinsic activities boosting tumor progression and drug resistance, mutp53 enhances the ability of tumor cells to communicate amongst themselves and with the tumor stroma, by affecting the release of different factors fuelling tumor growth and enabling metastatic competence. The endothelin-1 (ET-1) receptors (ET-1R), known as ETA receptor (ET_AR) and ETB receptor (ETBR) affect different hallmarks of cancer in a broad landscape of malignancies. By the cross-talk with multiple signaling pathways, mediated by the scaffold protein β-arrestin1 (β-arr1), ET-1R axis cooperates with an array of molecular determinants, including transcription factors and co-factors, strongly affecting tumor cell fate and behavior. In this scenario, recent findings shed light on the interplay between ET-1 and the Yes-associated protein (YAP) oncogenic pathway featuring a transcriptional network involving YAP and mutp53 protein, which share a common gene signature. Mechanistically, YAP and mutp53 are enrolled in nuclear complexes that turn on a highly selective YAP/mutp53-dependent transcriptional response involved in tumor progression and resistance to therapy. Our recent evidence indicates that β-arr1/YAP/mutp53 complex represents the initial scaffold on which transcriptional regulatory networks could be built. The interaction with different transcription factors, such as the hypoxia-inducible factor-1α (HIF-1α), tethering mutp53 to the DNA, can expand mutp53 agenda to orchestrate specific gene expression. A precise knowledge of the molecular mechanisms guided by ET-1 underlying the interplay between mutp53/YAP and critical transcriptional factors in the dialogue between tumor cells and the tumor microenvironment (TME) is expected to unveil actionable targets to blunt tumor aggressiveness. Thus, ET-1R activation induces the interaction of YAP/mutp53 with selected transcription factor leading to sustained signaling between tumor and stromal elements, thereby conferring selective advantages to primary tumor and metastasis. Therefore, ET-1R blockade by the FDA approved dual ET-1R antagonist macitentan interferes with ET-1R-driven escape pathway in the tumor and TME contexts, through the

simultaneous suppression of YAP, HIF-1α, and mutp53 functions, hampering metastasis and reawaking drug sensitivity, and thereby considering ET-1R antagonist as a potential therapeutic option for mutp53 cancers. The identification of ET-1/YAP-intertwined and bi-directional signaling pathways as targetable vulnerabilities, may open new therapeutic approaches able to disable the ET-1/YAP alliance in both tumor and stromal cells and concurrently sensitizes to high-efficacy combined therapeutics.

APSET17.43

Kidney expression of endothelin system following cisplatin-induced acute kidney injury in male and female Mice

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The cancer treatment drug, cisplatin, is nephrotoxic and accumulates in the proximal tubules of the kidney leading to the development of acute kidney injury (AKI). Previous studies determined that young female mice exhibit greater protection and recovery from cisplatin-AKI compared to young males. The endothelin (ET) system is composed of 3 different peptides ET-1 (Edn1), ET-2 (Edn2), and ET-3 (Edn3) that are the proteolytic cleavage products of the ET converting enzymes (Ece1 and Ece2). The ETs bind to two different G protein coupled-receptors ETA (Ednra) and ETB(Ednrb). Previously it was determined in male mice that 3 days after a cisplatin injection results in an increase in Edn1and no change in whole kidney Ednra or Edrnb expression. It is undetermined if the ET system is significantly affected in the kidney of cisplatin-AKI female mice or within the different regions of the kidney. We hypothesize that cisplatin-AKI male and female mice will have an increase expression of the kidney ET system. Male and female c57Blk/6J mice (n= 5-7/group) were used in the study. At 9 weeks of age, mice were given a single i.p. injection of either saline (vehicle) or cisplatin (15 mg/kg). The mice were euthanized 3 days later and blood and kidneys were collected. Plasma creatinine was measured as an index of kidney function. One kidney was used for histological assessment of damage and the other was dissected into cortex, outer medulla (OM) and inner medulla (IM). RNA was extracted, converted to cDNA for the cortex and OM and real-time quantitative PCR was performed. The IM was used for total RNA-sequencing but analysis is still pending. Cisplatin caused a rise in plasma creatinine in both male (0.25±0.06 mg/dL vs 0.13±0.007, P=0.02) and female mice (0.16± 0.01 vs 0.14± 0.03, P=0.02) compared to vehicle injected mice. Moreover, cisplatin caused kidney damage such as dilated tubules, loss of proximal tubule brush border, and protein casts, regardless of the sex of the mouse. In the cortex, cisplatin lead to an increase in Edn1 (male vehicle (mv) 1.0±0.09, male cisplatin (mc) 4.3±1.5, female vehicle (fv) 0.7±0.09, female cisplatin (fc) 4.2±1.8, P= 0.003), Edn3 (mv=1.0±0.09, mc=1.4±0.02, fv = 0.8±0.07, fc=1.5±0.3, P=0.006), Ece2 (mv=1.0±0.2, mc=2.3±0.9, fv = 1.5±0.4, fc=4.6±2.1, P=0.04), Ednra (mv=1.0±0.1, mc=1.30±0.2, fv = 0.8±0.1, fc=1.3±0.2, P=0.03), and Ednrb (mv=1.0±0.1, mc=1.6±0.3, fv = 0.7±0.1, fc=1.6±0.3, P=0.002). Edn2 was not detected and Ece1 was not significantly different among the groups. In the OM, cisplatin caused a decrease in Ednra (mv=1.0±0.1, mc=0.8±0.08, fv = 1.0±0.1, fc=0.7±0.07, P=0.02). The OM Ednrb was significantly higher in females compared to males regardless of cisplatin (mv=1.0±0.07, mc=1.1±0.2, fv = 1.5±0.2, fc=1.5±0.3, P=0.04). Edn1, Edn3, Ece1, and Ece2 were not statistically different among the OM groups. To conclude, cisplatin-AKI causes changes in mRNA expression of the kidney ET system regardless of sex. Future experiments will determine if these expression changes correlate with kidney injury and whether the ET receptors are good candidates as therapeutic targets to prevent cisplatin-induced AKI. Funding R01DK126664.

APSET17.44

Small calcium-activated potassium channels openers can recover impairment of endothelial and erectile function induced by endothelin-1- NLRP3 activation.

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Introduction: Endothelial function is pivotal for erectile function. Endothelin-1 (ET-1) is an essential mediator of chronic inflammation and is involved in erectile dysfunction. The NLRP3 inflammasome is involved in pro-inflammatory cytokine production, inflammation process, erectile, and vascular dysfunction. Further, we have shown that downregulation of endothelial Kca2.x functionally drives erectile dysfunction. Therefore, we hypothesized that KCa2.x activation counteracts ET-1-induced NLRP3 stimulation and impairment of erectile function. Objective: To determine if ET-1 inhibits KCa2.x and activates NLRP3 impairing erectile function. Methods: All mice were killed by following the APS Guiding Principles for the Care and Use of Animals in Research and Training. The endothelial cells (EC) from erectile tissue were isolated, and the KCa2.x was evaluated by electrophysiology. The intracavernous pressure (ICP) was measured in DOCA/salt or naïve mice before and after intracavernosal injection of ET-1 or vehicle followed by NS13001, apamin, MCC950, or vehicle. After in vivo ICP measurements, the corpus cavernosum (CC) was isolated, and the activity of caspase-1 was evaluated by western blot. Results: ET-1 increases the caspase-1 activity in the CC and decreases KCa2.x-current density in EC. Treatment with bosentan or NS13001 prevents the DOCA/salt-induced erectile dysfunction. Furthermore, the intracavernous injection of ET-1 impairs erectile function. NS13001 or MCC950 reverted all the alterations induced by ET-1. Apamin did not change the ET-1-induced impairment of erectile function. Conclusion: A KCa2.x channel opener reversed impairment of endothelial and erectile function induced by ET-1. Modulation of KCa2.x channels may have the potential to recover erectile function in inflammatory-related diseases.

APSET17.45

Endothelin B receptor agonist, sovateltide, prevents neuronal loss, reduces beta-amyloid plaques, and restores memory deficit in APP/PS1 mouse model of Alzheimer's disease

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Background: Alzheimer's disease (AD) represents a major healthcare burden with no effective treatment. Endothelin B (ETB) receptor agonist, sovateltide, is being investigated as a potential therapeutic agent for AD. However, its causative effects on beta-amyloid (Aβ) pathology, a major histopathological hallmark of AD, remain unclear. In the present study, we have examined the effects of sovateltide on Aβ plaques load and functional recovery in APP/PS1 transgenic AD mice and explored its mechanism(s) of action. Methods: APP/PS1 transgenic mice (exhibit early Aβ accumulation) and C57BL/6 control mice were divided into two groups (vehicle and sovateltide). Sovateltide (5 µg/kg) was intravenously injected three times at 2-hour intervals on days 1, 3, and 6 every month until study endpoints (3, 6, and 12 months age). Control mice received an equal volume of saline. Memory deficit was assessed using the Morris water maze test. Separate sets of mice were sacrificed at the end of 3, 6, and 12 months. Brain tissues were analyzed for beta-amyloid plaque load, NPCs differentiation, and mitochondrial fate using western blots, immunofluorescence, and transmission electron microscopy. Results: APP/PS1 transgenic mice showed significant ($p<0.001$) impairment in spatial memory and higher levels of Aβ deposits in the brain. Sovateltide significantly ($p<0.001$) enhanced cognition and reduced Aβ plaque load in APP/PS1 mice. Sovateltide treatment significantly reduced learning and memory deficit in 6 months (40%) and 12 months (46%) aged transgenic mice. Moreover, sovateltide increased expression of NeuroD1 ($p<0.0001$), DoubleCortin ($p<0.0001$), and NeuN ($p<0.0001$) along with an upregulation of synaptic proteins (synapsin-1, synaptophysin, and postsynaptic density-95) at 6 and 12 months aged mice indicating attenuation of neuronal loss and enhanced neurogenesis. Additionally, sovateltide treatment preserved mitochondrial fate as evident with downregulation of mitochondrial fission marker (Drp1 and Fis1; $p<0.0001$), increase in fusion marker (Mfn1, Mfn2, Opa1; $p<0.0001$), and increase in cross-sectional area x number ($p<0.0332$) as well as mitochondrial/tissue area ($p<0.0015$). There were no changes in the expression of any of these markers at three months. Conclusions: Sovateltide lowers Aβ deposition, promotes neuronal differentiation, synaptogenesis, and mitochondrial activity, and leads to cognitive recovery. These data demonstrate that sovateltide exerts a disease-modifying effect in a mouse AD model and may be developed as a novel drug for the treatment of AD.

APSET17.46

Reduced CBF in adults with Metabolic Syndrome: Role of ET-1

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Endothelin-1 (ET-1) regulates vascular smooth muscle tone by binding to the ETA receptor and inducing a potent vasoconstriction. In healthy humans, blockade of ETA receptors increases blood flow in peripheral vascular beds. Animal studies suggest ET-1 contributes to altered cerebrovascular control in stroke and brain trauma. Furthermore, adults with insulin resistance (IR) demonstrate upregulated ET-1 (~250% higher plasma concentration) and decreased cerebral blood flow (CBF), which may be linked. Despite this, the role of ET-1 in regulating resting CBF and cerebral perfusion (CP) in humans remains unknown. We tested the hypothesis that blockade of ETA receptors would increase CBF and CP in adults with IR, but have no affect in healthy adults. Healthy normal weight adults (Control; n=8-9 (CP); BMI 23±1 kg/m2; age 24±4 yrs) and adults with Metabolic Syndrome (MetSyn; n=4; BMI 33±8 kg/m2; age 22±4 yrs) participated in a single-blind study. Endothelin receptor antagonist (Ambrisentan, 10mg) or placebo (Lactose, 435.53mg) was administered orally. Participants underwent magnetic resonance imaging (MRI) at peak plasma concentration, ~120 minutes after dosing. High resolution 4D flow MRI was used to assess total and regional CBF, while CP was quantified using pseudocontinuous arterial spin labeling normalized to gray matter volume. Total CBF was calculated as the sum of CBF in the bilateral internal carotid arteries (ICA) and basilar artery (BA). Anterior CBF was calculated as the sum of CBF in the bilateral ICA, and posterior CBF was recorded as the CBF in the BA. Heart rate (HR), mean arterial pressure (MAP), and end-tidal CO2 (ETCO2) were monitored. Significance was determined using a general linear mixed effects model or t test and was set at $p<0.05$. Results are mean±SD. At screening, adults with MetSyn displayed obesity, moderate hypertension, dyslipidemia, and higher fasting glucose compared to Control. HR, MAP, and ETCO2 were not different in Control compared to MetSyn or after administration of Ambrisentan. Basal total CBF was ~17% lower in MetSyn (Control:724±79 vs. MetSyn:601±40, mL/min; $p=0.015$), and basal posterior CBF was ~31% lower in MetSyn (Control:175±23 vs. MetSyn:120±29, mL/min; $p=0.003$). Ambrisentan did not alter total or regional CBF (main effect for condition, $p>0.05$). CP in the bilateral frontal lobe was not different in Control compared to MetSyn. In contrast, basal global CP (Control: 41±4 vs. MetSyn: 36±5 mL/100g/min, $p = 0.026$) and CP in the bilateral occipital, parietal and the left temporal lobe were all lower in MetSyn (range 10 to 14 %; main effect of group, $p<0.05$). Ambrisentan did not alter global or regional CP (main effect for condition, $p>0.05$). In conclusion, young unmedicated adults with MetSyn

demonstrate substantially lower CBF and CP compared to healthy controls. Contrary to our hypothesis, Ambrisentan did not increase total or regional CBF or CP in adults with IR. These data suggest adults with MetSyn do not exhibit enhanced ET-1 tone, and thus excessive endothelin signaling does not explain the observed reduced resting CBF in MetSyn. Longer duration, increased severity or IR, or both may be necessary to modify cerebrovascular responses to ET-1 signaling.

APSET17.47

Sovateltide protects neonatal rat brain in a model of hypoxic-ischemic encephalopathy

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Introduction: Neonatal hypoxic-ischemic encephalopathy (HIE) is a major cause of neurological disability requiring newer therapeutic strategies. We have shown that agonism of endothelin B (ETB) receptors mediated through sovateltide provides neuroprotection and enhances neurovascular remodeling in several neuropathological conditions. Therefore, the objective of this study was to investigate the neuroprotective effects of sovateltide in a neonatal rat model of HIE. Methods: Sprague-Dawley male and female rat pups were grouped separately and divided into 5 different subgroups (1) Control; (2) HIE+Vehicle; (3) HIE+Hypothermia; (4) HIE+sovateltide; and (5) HIE+sovateltide+hypothermia. HIE was induced by ligating the right carotid artery on a postnatal day (PND) 7, followed by hypoxia for 120 min and hypothermia (32-34°C) for 5 h. On PND 7, sovateltide (5 µg/kg, ICV) was injected after hypoxic-ischemic injury. Pups were euthanized on PND 10. Brains were collected for evaluation of ETB receptors, VEGF, NGF, cell death, and oxidative stress markers - malondialdehyde (MDA), glutathione (GSH), and superoxide dismutase (SOD). Results: Animals receiving sovateltide showed a significant ($p<0.0001$) upregulation of ETB receptor (135%), VEGF (110%), and NGF (183%) expression in the brain compared to vehicles. Vehicle-treated animals had high oxidative stress levels, as indicated by increased lipid peroxidation, MDA, and decreased antioxidants, SOD, and GSH compared to control. These effects were reversed ($p<0.001$) in sovateltide alone or combined with hypothermia, indicating reduced oxidative stress in these animals. Additionally, sovateltide alone or in combination with hypothermia reduced ($p<0.001$) cell death compared to vehicle or hypothermia alone. Similar findings were obtained in both male and females rats. Conclusion: Altogether, these results show that sovateltide alone or as an adjunct to hypothermia exerts significant neuroprotective effects when given after HI and suggests a potential therapeutic approach of stimulation of ETB receptors in the treatment of neonatal HIE.

APSET17.48

Endothelin Receptor Antagonism Improves Adipose Tissue Inflammation and Glucose Tolerance in an Experimental Model of Autoimmune Disease

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Systemic lupus erythematosus (SLE) is a chronic multisystem autoimmune disorder that primarily affects women and is characterized by high rates of renal involvement, hypertension, and cardiovascular disease. In addition, patients with SLE are more likely to be overweight or obese and develop type II diabetes as compared to age matched controls. Accumulating data demonstrate that high circulating ET-1 is associated with obesity and insulin resistance in both humans and animal models. Similarly, SLE and other autoimmune diseases are associated with elevated ET-1; however, the role of ET-1 in autoimmune-associated obesity and insulin resistance is unknown. The female NZBWF1 mouse is a well-established mouse model of SLE that closely mimics SLE disease in humans and also develops obesity and insulin resistance when fed a normal chow diet. We hypothesized that endothelin receptor antagonism would improve obesity and insulin resistance in SLE mice. To test this hypothesis, 28 week old control (NZW) and SLE (NZBWF1) mice were treated for four weeks with vehicle (0.1% ethanol), atresentan (ETA antagonist, 10 mg/kg/day), or bosentan (ETA/ETB antagonist, 100 mg/kg/day) in drinking water. SLE mice had increased body weight and adiposity as compared to control mice, but neither atresentan nor bosentan treatment had an effect on either parameter. To assess adipose tissue inflammation, we performed flow cytometric analyses on visceral adipose tissue, and SLE-vehicle mice had increased CD3+CD4+ T cells and CD3+CD8+ T cells compared to control-vehicle (CD4: 8.5±0.7% vs. 14.3±1.9%, $p<0.05$; CD8: 1.7±0.4% vs. 3.6±0.5%, $p<0.05$). Treatment with atresentan reduced CD4+ T cells (7.2±1.5%, $p<0.05$ vs. SLE-vehicle), while treatment with bosentan reduced CD8+ T cells (1.8±0.3%, $p<0.05$ vs. SLE-vehicle). SLE mice had elevated fasting insulin as compared to controls (0.43±0.03 vs. 0.82±0.20 ng/mL, $p<0.05$), and treatment with both atresentan and bosentan lowered insulin in SLE mice (SLE-atresentan: 0.43±0.03 ng/mL, SLE-bosentan: 0.45±0.07 ng/mL, $p<0.05$ vs. SLE-vehicle). SLE mice had impaired glucose tolerance as compared to control mice (AUC: 4225±170 vs. 5918±603 arbitrary units, $p<0.01$), and treatment with bosentan improved glucose tolerance in SLE mice (AUC: 3777±450 arbitrary units, $p<0.05$ vs. SLE-vehicle). Taken together, these suggest that treatment with endothelin receptor antagonists may be beneficial in improving adipose tissue inflammation and glucose tolerance in SLE.

APSET17.49

Chlorogenic Acid (CGA) Ameliorates Liver Injury in Association with Upregulation of eNOS and Downregulation of ET-1/ETAR in Diabetic Rat Model

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Background: Diabetes mellitus (DM) induces micro- and macro-vascular injury with increasing Endothelin-1 (ET-1), then leads to organ dysfunction and injury, including the insulin-targeted organ such as liver. Chlorogenic acid (CGA), an active compound of green-coffee bean, is widely known for its anti-oxidant and anti-inflammatory properties. However, the role of CGA in DM-caused liver injury remains unknown. Therefore, this research aims to investigate the effect of CGA administration on liver injury in diabetic model. Materials and methods: DM was induced in Wistar rats through intraperitoneal injection of streptozotocin (STZ; 60 mg/kg body weight (BW)) for 1.5 months (DM1.5) and 2 months (DM2), meanwhile CGA was administered intraperitoneally from 1.5-2 month. The CGA groups were divided into CGA1 (12.5 mg/kg BW), CGA2 (25 mg/kg BW), and CGA3 (50 mg/kg BW). During termination, blood was collected and the liver was harvested for RNA and fibrosis/histological analysis. The mRNA expression of the collagen-1, collagen-3, ppET-1, ETAR, and eNOS were assessed using Reverse Transcriptase-PCR (RT-PCR). Results: DM groups, especially DM2 group demonstrated significantly higher serum glucose, SGOT, and SGPT serum level compared to the control group. DM2 group also showed significantly higher Collagen-1 and Collagen-3 mRNA expression, with upregulation of ppET-1/ETAR and downregulation of eNOS compared to control. CGA1 group demonstrated reducing fibrosis based on SR staining, liver injury attenuation with significant lower SGPT, SGOT and glucose level, with downregulation of Collagen-1 and Collagen-3 mRNA expression. Furthermore, CGA1 group demonstrated no significant difference in ppET-1 and eNOS mRNA compared to DM2 group. However, CGA1 group showed significantly lower ETAR mRNA expression than DM2 group. Conclusion: Lower dose of CGA may ameliorate liver injury as progressive effect of diabetic condition and may be associated with downregulation of ETAR mRNA expression. Keywords: diabetes mellitus, chlorogenic acid, liver injury, ppET-1, ETAR, eNOS

APSET17.50

Chlorogenic acid ameliorates memory dysfunction and hippocampal apoptosis in association with downregulation of ET-1/ETAR expression

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Background: Diabetes mellitus with endothelial dysfunction and stress oxidative propagation induces organ injury, especially to hippocampus which leads to neuron apoptosis and memory dysfunction. Endothelin-1 (ET-1) signaling may play roles in hippocampus patho-mechanism. Chlorogenic acid, an active compound of green coffee has been known to have antioxidant, and neuroprotective effects. However, its role in hippocampus and memory dysfunction as impact of diabetic condition has not been elucidated yet. Objectives: This study aimed to elucidate the role of CGA to ameliorates endothelial dysfunction, apoptosis, and memory dysfunction in type 1 diabetes mellitus rat's hippocampus. Material and methods: Diabetes mellitus model was induced in Wistar rats (2 months, 150-200 grams) by single injection of streptozotocin 60 mg/KgBW (body weight) intraperitoneally for 1,5 months (DM1,5 Group n=5) and 2 months (DM2 Group n=5). Chlorogenic acid was given intraperitoneally from 1.5 to 2 months with three-doses 12,5 (CGA1 Group n=5), 25 (CGA2 Group n=5), and 50 mg/KgBW (CGA3 Group n=5). Control group without STZ injection and CGA administration was added. Morris water maze (MWM) was used to determine memory dysfunction before termination. Blood was collected, hippocampus was harvested then used for RNA and histopathological analysis. They were measured using Reverse Transcription-Polymerase Chain Reaction (RT-PCR) was carried-out for quantifying Bax, p53, ppET-1, ETAR, and eNOS mRNA expression. Immunostaining was done for observing p53 expression in hippocampus. Data were processed with a statistical significance of $p < 0.05$. Results: DM1,5 and DM2 groups showed longer length and time of acquisition test based on MWM analysis, which associated with higher mRNA expression of Bax and p53 compared to Control group. There was upregulation of ppET-1 and ETAR, with downregulation of eNOS mRNA expression in DM2 group than control group. CGA1 group demonstrated amelioration of memory function based on MWM analysis, which showed significantly lower Bax and p53 mRNA expression compared to DM2 group. There was upregulation of eNOS with downregulation of ppET-1 and ETAR mRNA expression in CGA1 group. Immunostaining of p53 revealed apoptosis in dentate gyrus and CA3 area of hippocampus in DM groups, with reducing the signal in CGA treated group. Conclusion: In conclusion, CGA ameliorates memory dysfunction may associate with downregulation of ET-

1/ETAR and apoptosis in hippocampus of rat with diabetic condition. Keywords: diabetes mellitus, chlorogenic acid (CGA), hippocampus, apoptosis, memory dysfunction, endothelin-1 (ET-1), ETAR

APSET17.51

Glomerular cell crosstalk through Et-1/ETAR in glomerular disease pathogenesis

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Podocyte-initiated and secondary segmental glomerulosclerosis with proteinuria underlies most cases of chronic kidney disease, which are characterized by progressive podocyte injury and depletion associated with collapse and sclerosis of glomerular capillary segments. In models of primary podocytopathies in experimental focal segmental glomerulosclerosis, we have demonstrated an essential role for podocyte-initiated mitochondrial oxidative stress and dysfunction of glomerular endothelial cells in segmental glomerulosclerosis via paracrine endothelin-1 (ET-1) – endothelin receptor type A (ETAR) signaling. We found that podocyte-released factors including ET-1 mediated glycocalyx degradation and mitochondrial dysfunction in endothelial cells with increased ETAR expression, and that ETAR antagonism and mitochondrial reactive oxygen species scavenging prevented glycocalyx degradation and albuminuria in mice. Studies confirmed increased heparanase and hyaluronoglucosaminidase gene expression in glomerular endothelial cells in response to ET-1. Finally, endothelial dysfunction was absolutely required for subsequent podocyte depletion and segmental sclerosis. We have also confirmed that a similar stressed endothelial-to podocyte crosstalk underlies segmental lesions in diabetic kidney disease.

APSET17.52

Lack of renoprotective effects of targeting the endothelin A receptor and/or SGLT2 in a mouse model of Type 2 diabetic kidney disease

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Background: Two recent clinical trials, CREDENCE and SONAR, reported the first new efficacious treatments in 18 years to slow progression of kidney disease in patients with Type 2 diabetes. CREDENCE examined the effects of canagliflozin, a sodium glucose cotransporter (SGLT2) inhibitor while SONAR examined the effects of atrasentan, an endothelin-A (ET-A) receptor antagonist and found that both drugs reduced the composite renal outcomes by 30-35%. We hypothesized that combined inhibition of SGLT2 and endothelin-A receptor may confer greater protection against renal injury and disease progression than either agent alone. Methods: Male db/db mice at 8 weeks of age underwent uninephrectomy and were allowed to recover for 1 week. Mice were randomized to 4 groups (vehicle, dapagliflozin (1 mg/kg/day), ABT-627 (atrasentan, 5mg/kg/day) or dual treatment) from 10 weeks until 22 weeks of age (n=7-8 mice/group). Metabolic balance studies were performed at 10, 16 and 22 weeks of age. Glomerular filtration rate (GFR) was measured at 10 and 22 weeks. Mixed effects ANOVA with Tukey's test was used to examine differences among the 4 groups. Results: At 10 weeks of age, no differences were observed in body weight, non-fasting blood glucose levels or urinary albumin excretion among the 4 groups. Body weight in the vehicle group (week 16: 46.8 ± 2.2 gms, week 22: 47.0 ± 3.2 gms) and ABT-627 group (week 16: 42.6 ± 1.2 gms, week 22: 40.4 ± 1.4 gms) was lower than the dapa group (week 16: 53.2 ± 1.6 gms, week 22: 57.9 ± 2.1 gms) and dual treated group (week 16: 52.8 ± 0.5, week 22: 56.2 ± 1.4 gms, p value <0.001). Blood glucose levels were higher in the vehicle and ABT-627 groups (>650 mg/dl at week 16 and 22) compared to the dapa group (week 16: 450 ± 36 mg/dl, week 22: 350 ± 51.4 mg/dl) or the dual treated group (week 16: 462 ± 40 g/dl, week 22: 417 ± 73 mg/dl, p value <0.01). Urine albumin increased from week 10 to week 22 in the vehicle group (week 10: 154 ± 29, week 16: 529 ± 130, week 22: 773 ± 170 µg/day), ABT-627 group (week 10: 211 ± 45, week 16: 370 ± 63, week 22: 595 ± 155 µg/day), dapa group (week 10: 197 ± 40, week 16: 575 ± 113, week 22: 890 ± 190 µg/day) and dual treated group (week 10: 216 ± 51, week 16: 538 ± 88, week 22: 609 ± 40 µg/day) although no statistical differences were noted between the 4 groups. GFR increased in the vehicle (week 10: 637 ± 47, week 22: 859 ± 94), dapa (week 10: 732 ± 78, week 22: 878 ± 100) and dual treated groups (week 10: 747 ± 117, week 22: 840 ± 80) and decreased in the ABT-627 group (week 10: 785 ± 81, week 22: 588 ± 54) although differences between groups was not statistically significant. Histological analysis showed very mild tubular injury in all 4 groups and mild focal glomerulosclerosis (4% of glomeruli in the vehicle vs 2% in the ABT-627 group, 3% in the Dapa group and 1% in the dual treated group). Conclusion: Individual or combined treatment with an SGLT2 inhibitor and/or an ET-A antagonist did not confer renoprotective effects in this model of uninephrectomized db/db mice.

APSET17.53

Development of sparsentan, a dual antagonist of endothelin and angiotensin II receptors, in rare glomerular diseases.

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Inhibition of the renin-angiotensin-aldosterone (RAAS) or endothelin (ET) systems has been demonstrated to be nephroprotective in a spectrum of clinical and experimental studies. Until recently, clinical evidence regarding the beneficial effects of dual inhibition of these systems relied on studies using a combination of two monoblockers. Sparsentan, a single molecule which acts as a dual endothelin type A and angiotensin II AT1 receptor antagonist (DEARA) represents a new approach. Supported by preclinical evidence showing a greater nephroprotective potential compared with monoblockers of RAAS or ET in a spectrum of models of glomerular diseases, sparsentan is

being developed as a treatment for rare glomerular diseases, such a focal segmental glomerulosclerosis (FSGS) and IgA nephropathy (IgAN). This presentation will review the current state of development of sparsentan at Travere, Inc. in the aforementioned indications. The phase 2 DUET study in patients with FSGS, initiated in 2014, demonstrated greater short-term (8 weeks) antiproteinuric effect of sparsentan compared to AT1 receptor blocker irbesartan, which was used an active control. This antiproteinuric effect was sustained over a prolonged, still ongoing, open-label extension period. Promising results of the DUET study inspired the design of the phase 3 DUPLEX trial in FSGS, which compares long-term (2 year) nephroprotective potential of sparsentan with irbesartan. DUPLEX achieved its enrollment goal at the end of 2020 and recruited 371 patients globally. A recent interim analysis of DUPLEX at week 36 has demonstrated that the proportion of patients who achieved FSGS partial remission endpoint (treatment-associated reduction of urinary protein/creatinine ratio <1.5 g/g and >40% reduction from baseline) was markedly higher in the sparsentan treatment arm as compared to patients treated with irbesartan (42 vs. 26%; p<0.01). This beneficial effect was associated with a favorable safety profile comparable to irbesartan. Primary confirmatory endpoint, i.e. effect of study treatments on eGFR slope, will be analyzed at week 108 after completion of double-blind period. In parallel, Travere, Inc. is conducting a phase 3 trial called PROTECT in patients with IgAN at risk of progression to end-stage renal disease (urinary protein excretion >1 g/day despite maximized RAAS inhibition). Similar to DUPLEX, this double-blind randomized global multicentric study compares nephroprotective potential of sparsentan with irbesartan over a period of 2 years, with an interim analysis of change in proteinuria from baseline at week 36, and analysis of eGFR slopes after completion of double-blind period. The study enrollment has been completed and topline data of the interim analysis are expected in the third quarter of 2021. In conclusion, upcoming analyses of major trials will generate data that will help elucidate the position of sparsentan and dual inhibition of ET and angiotensin II receptors among treatments for FSGS and IgAN.

APSET17.54

Vascular Endothelial-derived ET-1 mediates enhanced aortic vasorelaxation in mice exposed to early life stress

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Early life stress (ELS) is an under-appreciated risk factor for cardiovascular disease (CVD) that has a potentially significant longitudinal impact on health outcomes. In rat models of ELS, we have shown increased circulating ET-1 and downregulation of both ETA and ETB receptors in the aortic vasculature, suggesting that the ET pathway plays a role in ELS-mediated vascular responses. We hypothesized that vascular endothelial-derived ET-1 is critical for vascular responses after exposure to ELS. To test this, we employed a mouse model of ELS known as Maternal Separation with Early Weaning (MSEW). We additionally utilized vascular endothelial-specific ET-1 knockout (VEETKO) and flox mice exposed to normal rearing (NR) or MSEW. MSEW involves maternal separation for 4h/day (postnatal (PD) 2 to 5), 8h/day (PD6 to 16), and weaned at PD17. NR litters weaned at PD21 were used as controls. Aortic vasorelaxation experiments were performed on male and female (10 weeks old) NR and MSEW mice to study endothelium-dependent and -independent vasorelaxation. Aortae from MSEW flox mice showed significantly greater maximal acetylcholine (Ach)-mediated vasorelaxation compared to NR flox mice (Flox-NR 74.7+4.2%, Flox-MSEW 93.7+1.6%; p=0.0023), while no differences were found between MSEW and NR VEETKOs (VEETKO-NR 78.0+6.8%, VEETKO-MSEW 86.9+2.1%; p=0.2485). EC50 responses to Ach were not significantly different (Flox-NR -6.2+0.3, Flox-MSEW -6.9+0.1, VEETKO-NR -6.7+0.1, VEETKO-MSEW -6.9+0.3; F(1,14)=3.71 p=0.074). All endothelium-independent responses as determined with sodium nitroprusside were similar. These results indicate that endothelium-derived ET-1 mediates the exaggerated aortic endothelium-dependent vasorelaxation seen in the flox mice exposed to MSEW. We speculate that the MSEW-induced vasorelaxation response is mediated via the endothelium-derived ET-1/ETB/NO pathway.

APSET17.57

Sex-specific response to Endothelin-1 overexpression mediates thoracic aortic aneurysm development

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Introduction: Thoracic aortic aneurysms (TAAs) are common diseases associated with high morbidity and mortality. Although TAAs are more common in men, women have worse outcomes and dissect at smaller aortic diameters. Endothelin-1 (ET-1) is the most potent vasoconstrictor in humans, and an important mediator of vascular stiffness and tone. ET-1 levels are increased in patients with TAA, and genetic variants that affect ET-1 production and receptor expression are associated with vascular diseases. Although ET-1 levels do not differ between men and women with TAA, it is unknown if response to ET-1 mediates sex-specific differences. Hypothesis: ET-1 has different effects on vascular stiffness and aortic dissection risk in men and women. Methods: Angiotensin-II (AngII) was delivered subcutaneously at 1 ug/kg/min dose and rate using osmotic pumps for 28 days in endothelial cell-specific ET-1 transgenic (eET-1) and matched WT control mice. We measured blood pressure, aortic stiffness, and aortic size at the end of the study. Results: Non-coding variants within 2Kb of the EDNRA promoter are associated with CAD at a genome-wide significant level (CardiogramplusC4D consortium). This locus also demonstrates sex-specific effects, with the minor allele conferring greater risk in male subjects Interestingly,

genome wide association studies for coronary artery disease risk identified a common variant near the EDNRA gene with significant evidence for sex differences (p= 6.6 x 10-9; male b= .203 and female b= .036). This sex dimorphic effect is also seen in mouse models of vascular disease. Despite elevated blood pressure in all animals that received AngII (Fig1A), we observed increased aortic stiffness in male eET-1 mice (+1.5 m/s eET-1 vs. +0.74 m/s WT) but decreased aortic stiffness in female eET-1 mice (-1.9 m/s eET-1 vs. +0.69 m/s WT), measured by pulse wave velocity (Fig1B). Consistent with these results, aortic diameter was only increased in male eET-1 mice (+0.61 mm eET-1 vs. +0.11 mm WT) but not in female eET-1 mice (+0.06 mm eET-1 vs. +0.11 mm WT) (Fig1C). Significantly, 2 of 3 male eET-1 animals died before 28 days from dissected TAAs, whereas all females survived the length of the study. Conclusions: In the AngII-TAA model, there is a sex dimorphic effect of ET-1 overexpression. Male mice have greater vascular stiffness and develop TAA in response to the same level of ET-1 as in matched female mice. This difference may explain the increased incidence of TAA in men.

APSET17.58

Mast cell degranulation specifically enhances the chymase-dependent conversion and pressor properties of big-endothelin-1 in the mouse model.

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Endothelin-1 (ET-1), a 21-amino acid peptide, is a potent vasoconstrictor. We have previously reported that administration of the ET-1 precursor, big endothelin-1 (big-ET-1), induces an increase in blood pressure following its conversion to ET-1 (1-31) by mast cell protease type 4 (mMCP-4), the murine functional analogue of the human chymase (Houde et al., 2013). mMCP-4 is a serine protease exclusively synthesized and stored in mast cell secretory granules. The aim of this study was to investigate the impact of mast cell degranulation or stabilization on mMCP-4-dependent pressor responses following the administration of big ET-1 or Angiotensin I (Ang I) in the mouse model. In anesthetized mice, mast cell degranulation was induced by Compound 48/80 (C48/80) or stabilized by cromolyn, which respectively enhanced or repressed the dose-dependent vasopressor responses to intravenously administered big ET-1 in wild-type (WT) mice. This was not observed in the presence of a chymase inhibitor (TY 51469) nor in mMCP-4 knockout (mMCP-4 KO) mice. Vasopressor responses to ET-1 (1-31) and ET-1 were not modulated by neither C48/80 or cromolyn. In peritoneal mast cells isolated from C48/80 or cromolyn-treated WT mice, mMCP-4-dependent hydrolysis of its specific fluorogenic substrate, Suc-Leu-Leu-Val-Tyr-7-amino-4-methylcoumarin, was respectively depleted ($p<0.001$, n=6) or enhanced ($p<0.05$, n=10), as measured by spectrofluorometric assays in vitro. Furthermore, mast cells isolated from 48/80 or cromolyn-treated mice, respectively increased ($p<0.001$, n=5) or abolished ($p<0.05$, n=5) ET-1 (1-31) conversion from exogenous big ET-1 in vitro. Finally, while the vasopressor responses to Ang I were unaffected by mast cell activation or stabilization, the pressor responses induced by the Angiotensin-converting enzyme-resistant Ang I analogue, [Pro11, D-Ala12] Ang I, were sharply potentiated by C48/80 ($p<0.01$, n=7-8). Altogether, the present study shows that mast cell activation enhances the mMCP-4-dependent vasoactive properties of big ET-1 but not Ang I in the mouse model. The current work demonstrates, in the murine systemic circulation, a significant role for mast cell stability in the cardiovascular pharmacology of big ET-1 but not Ang I. This study also demonstrates for the first time that an ACE-resistant Ang I analogue is susceptible to chymase-dependent activation in the mouse model. This project was financially supported by the Canadian Institutes for Health Research (MOP-57883) and le Réseau Québecois de Recherche sur le Médicament (Fonds de Recherche Santé, Québec).

APSET17.59

Vascular Endothelium-derived Endothelin 1 Mediates the Renal Innate and Adaptive Inflammatory Response in Male but not Female Mice on High Salt Diet

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High salt consumption results in elevated levels of endothelin-1 (ET-1) and inflammation in the kidney; however, whether a sex difference exists in the endothelium-derived ET-1-mediated inflammatory response to high salt intake is unknown. Thus, we utilized male and female vascular endothelial cell ET-1 knockout (VEET KO) and control floxed ET-1 (VEETfl/fl) mice fed 4.0% NaCl diet (HSD). After 3 weeks of HSD, kidney myeloid and lymphoid cell populations and their functional status were examined by flow cytometry. In response to HSD, male VEETfl/fl mice showed significantly elevated kidney TH17 cell numbers compared to female VEETfl/fl mice (IL-17A+CD4+ cell absolute count, male vs. female: 229.6±27.7 vs. 84.9±17.2, $p=0.0003$, n=6-10/group), demonstrating a substantial sex-dependent difference in kidney TH17 cell abundance. Lack of endothelium-derived ET-1 resulted in a 72% reduction in renal TH17 cell number in males (VEETfl/fl vs. VEET KO: 229.6±27.7 vs 64.0±64, $p=0.0002$, n=6-10/group), but not in females ($p>0.05$, n=7-9). The frequency of kidney IL-17A+CD4+ cells was not different between genotypes in females, nor between male and female VEETfl/fl mice. However, male VEET KO mice displayed 57% less renal IL-17A+CD4+ cells than male VEETfl/fl (respectively, 0.6±0.071 vs. 1.4±0.2, $p=0.0055$), and a 59% reduction compared to female VEET KO mice (1.4±0.2, $p=0.0052$). Moreover, we also examined the role of vascular endothelium-derived ET-1 in regulating the expression of IL-17A by γΔ T cells, which bridge the innate and adaptive arms of the immune system.

The numbers of kidney IL-17A+ γΔ T cells were 3.5 fold greater in male than female VEETfl/fl mice (respectively: 228.3±24.9 vs. 68.8±16.7, p=0.0003), and knockout of endothelial ET-1 resulted in reduced IL-17A+ γΔ T cells in male VEET KO mice, similar to the levels found in female VEET KO mice (male vs. female VEET KO: 100±41.5 vs 86.8±20.7 p=0.98). We next evaluated whether endothelial ET-1 may regulate the available pool of general antigen-experienced (CD44hi) T cells in the kidney. Knockout of endothelial ET-1 decreased the number of CD44hi CD4+ T cells in male mice (VEETfl/fl vs. VEET KO: 3333±1109 vs. 1947±412, p=0.47). We also examined the influence of endothelial ET-1 on the innate immune response and found that activated kidney resident macrophages (F4/80hiCD11b+CD64+) were increased in male vs. female VEETfl/fl (median fluorescence intensity, MFI, respectively, 2080±105 vs. 1672±25, p=0.0002). Lack of endothelium-derived ET-1 significantly reduced CD64+ cell abundance in males (p=0.012), but not females (p>0.05), when compared to VEETfl/fl. In conclusion, endothelium-derived ET-1 is critical in promoting renal innate and adaptive inflammatory responses in males on high salt diet, and important male-female differences exist in the manner by which vascular ET-1 regulates kidney inflammation during high salt intake. Funded by NIH F31 HL151264-0 to PAM, P01HL136267 to JSP, and K01HL145324 to CDM.

APSET17.60

Why aprocitentan in resistant hypertension?

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Endothelin has emerged as a target for therapeutic intervention in systemic hypertension. As a vasoconstrictor, co-mitogenic agent, mediator between pulse pressure and vascular remodeling, and mediator of aldosterone and catecholamine release, endothelin could be a key player in hypertension and end-organ damage. In 10 to 20% of the hypertensive population, the high blood pressure (BP) is resistant to administration of anti-hypertensive drugs of different classes in combination. This led us to speculate that in these patients, hypertension is dependent upon endothelin, as endothelin is not targeted by current anti-hypertensive drugs. Our hypothesis is supported by the observation that this form of hypertension is often salt-sensitive, and that the endothelin system is activated in subjects at risk of developing a resistant hypertension: in overweight patients, a T allele is associated with high BP. In diabetic patients, African-Americans, and post-menopausal women with hypertension, endothelin levels are elevated, and these forms of hypertension are often difficult to treat. Aprocitentan is a novel, potent, orally active investigational dual endothelin receptor antagonist. Because vascular endothelin (ET)-1 expression is increased in models of salt-dependent hypertension more than in spontaneously hypertensive rats (SHR), we studied the effect of aprocitentan in DOCA-salt rats (low-renin/high-salt model) and SHR (normal-renin model). Aprocitentan was superior to valsartan in decreasing BP in DOCA-salt rats but not in SHR, and in combination with RAS blockers was synergistic, and safer than a combination of spironolactone with RAS blockers. In a phase 1 study to evaluate the effect of aprocitentan on salt balance in healthy subjects on a high-salt diet, aprocitentan at 50 mg did not cause salt retention, and significantly decreased concentrations of aldosterone, copeptin and pro-BNP. In a phase 2 in subjects with essential hypertension, aprocitentan at daily doses of 10, 25 and 50 mg reduced unattended automated BP at 8 weeks at trough in a dose-dependent fashion, with a plateau at 25 mg. At that dose, sitting BP reduction was of 9.9/7.0 mm Hg (systolic/diastolic), whereas lisinopril 20 mg decreased sitting BP by 4.8/3.8 mg Hg. Aprocitentan produced dose-dependent decreases in hemoglobin and increases in estimated plasma volume, but no change in body weight versus placebo, and no liver enzyme changes. All these data are encouraging for the possible treatment of hypertension when endothelin plays a role and needs to be tackled. The results of the aprocitentan phase 3 study in patients with true resistant hypertension are expected for the second half of 2022.

APSET17.61

Adrenal endothelin axis genes in global Per1 knockout Dahl salt-sensitive rats

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Introduction: The adrenal hormone aldosterone induces the transcription of the endothelin-1 (Edn1) gene and increases endothelin-1 (ET-1) protein expression in the kidney collecting duct. The clock protein PERIOD1 (PER1) is a known negative regulator of Edn1. Global knock out of PER1 in male Dahl salt-sensitive (SS) rats (Per1KO) exacerbated the salt-sensitive phenotype of the SS rat1. Furthermore, male Per1KO rats have increased plasma aldosterone and renal Edn1 expression on a normal salt diet (0.4% NaCl), with a greater rise following 3 weeks high salt diet (4% NaCl) compared with SS control rats. PER1 regulates expression of endothelin axis genes in not just the mouse kidney, but also the heart, liver, and lung. However, little is known about its regulation in the adrenal gland, where aldosterone is synthesized. Hypothesis: I hypothesize that knocking out PER1 increases synthesis of aldosterone in the adrenal gland, increasing adrenal Edn1 gene expression. Methods: To test this hypothesis, adrenal glands were collected in the morning from four groups: male Per1KO SS rats and SS control rats on a normal salt or 3 weeks high salt (4% NaCl) diet (n=4-7). Genes involved in the synthesis of aldosterone and adrenal endothelin axis gene expression were measured by quantitative real time RT-PCR. Effects of genotype and diet were determined using 2-way ANOVA. Results: Per1KO rats exhibit decreased Cyp11b2, the gene encoding aldosterone synthase, on a normal salt diet compared with SS control rats, with significant downregulation following a high salt diet in both genotypes (ANOVA interaction between diet and genotype, p = 0.0315). High salt diet caused a significant decrease in Edn1 in both Per1KO and SS rats

(ANOVA main effect of diet, p = 0.0057), with no genotype effect (ANOVA main effect of diet, p = 0.9520). ET-1 receptors, ETA and ETB, have been reported to be present in the adrenal gland. Adrenal Ednrb, encoding ETB, expression was significantly higher in Per1KO rats both on normal and high salt diets compared with controls (ANOVA main effect of genotype, p = 0.0127), with reduced overall expression in both genotypes following high salt diet (ANOVA main effect of diet, p = 0.0328). There was no change in Ednra (encoding ETA) expression. Conclusion: Knocking out PER1 increased plasma aldosterone but the reduction in aldosterone synthase gene expression suggests this is not due to increased synthesis. Although adrenal Edn1 was unchanged, gene expression of ETB receptor was increased in Per1KO rats. Future work will focus on assessing the protein expression of ETB receptor in the adrenal gland of Per1KO rats. Studies investigating the role of ETB receptor in the adrenal gland are limited to date. However, these data suggest for the first time that PER1 regulates adrenal ETB receptor expression at the level of mRNA. Supported by the National Institutes of Health Grant 1R01DK109570-01A1. References 1 Spires DR, Zietara A, Levchenko V, Gumz ML, Staruschenko A. Knockout of Per1 Exacerbates the Hypertensive Phenotype of the Dahl Salt Sensitive Rat. FASEB J. 2020;34(S1):1.

APSET17.62

The Medical Research Council Precision medicine with Zibotentan in microvascular angina (PRIZE) trial

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Background Microvascular angina is an under-recognized condition associated with reduced quality of life, frequent hospital admissions and long-term clinic attendance. There are currently no specific treatments for MVA and new medicines are urgently required for these patients with an unmet clinical need. Recent studies implicate dysregulation of the endothelin system. We propose the potent, selective inhibitor of the ETA receptor zibotentan as a potential disease-modifying therapy for patients with microvascular angina. The minor G allele (minor allele; population prevalence ~36%) of the non-coding SNP rs9349379 enhances expression of the endothelin-1 gene, in turn increasing plasma concentrations of ET-1. The G allele appears to be more common in patients with microvascular angina than is observed in age and sex-matched controls. The Precision medicine with Zibotentan in microvascular angina (PRIZE) trial aims to test the efficacy and safety of zibotentan in patients with microvascular angina and assess whether the rs9349379 SNP acts as a theragnostic biomarker of the response to treatment with zibotentan. Methods PRIZE is a prospective, randomized, double-blind, placebo-controlled, sequential cross-over trial. The study population will be enriched to ensure at least half of participants have the G-allele of the rs9349379 SNP. The participants will receive a single-blind placebo run-in followed by treatment with either 10 mg of zibotentan daily for 12 weeks then placebo for 12 weeks, or vice versa, in random order. The primary outcome is treadmill exercise duration using the Bruce protocol. The primary analysis will assess the within-subject difference in exercise duration following treatment with zibotentan versus placebo. Conclusion The trial has been underway since October 2019 and recruitment continues at 9 sites across the United Kingdom. Should our hypotheses be confirmed, this developmental trial will inform the rationale and design for undertaking a larger multicenter trial.

APSET17.63

Differential Effects of Endothelin 2 and Endothelin 1 Silencing in Human Lung Adenocarcinoma A549 Cells

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Lung cancer is the leading cause of cancer death worldwide and adenocarcinoma is the most common type of lung cancer. Overexpressed Endothelin 1 (ET-1) has been known in lung adenocarcinoma and its pathophysiological role in cancer has been elucidated. However, the clinical trials using ET receptor antagonists did not show satisfying results. In the present study, we aimed to investigate the pathophysiological functions of ET-2 compared with ET-1 in human lung adenocarcinoma. We transfected human lung adenocarcinoma A549 cells with either siRNA ET-1 or siRNA ET-2, and measured their effects on cell growth, migration, and apoptosis under normoxia and hypoxia (1% O2). We observed a significant increase in ET-2 mRNA levels (> 2 folds, p = 0.0001) and a decrease in ET-1 mRNA levels (> 1.5 folds, p < 0.01) under 24 hours of hypoxia in A549 cells. Furthermore, silencing of ET-2 reduced proliferation (> 2 folds, p < 0.05), migration (3 folds, p < 0.0001), and enhanced apoptosis (> 1.2 folds, p < 0.05) which was not shown by silencing of ET-1 or control under both conditions. Mechanistically, it was found that silencing of ET-2 elevated Prolyl hydroxylase domain (PHD2) mRNA (> 1.5 folds, p < 0.005) and protein levels (> 2 folds, p < 0.01) which induced Hypoxia-inducible factors (HIF1) alpha protein degradation under hypoxia (> 2.5 folds, p = 0.0001 compared to control and > 2 folds, p < 0.005 compared to ET-1 silencing), and a concomitant decrease in its target genes. Moreover, ET-2 silencing reduced mRNA and protein levels of X-linked inhibitor of apoptosis protein (XIAP), the most potent anti-apoptosis IAP family member (mRNA > 2.5 folds, p < 0.005 and protein > 4 folds, p = 0.0001). Both HIF-1 alpha and XIAP, which are involved in cancer progression, were downregulated by ET-2 silencing but not by ET-1 silencing. Taken together, this study showed that ET-2 silencing significantly reduced proliferation, migration, and increased apoptosis of A549 cells via upregulation of PHD2-mediated HIF-1 alpha degradation and reduction of XIAP. Therefore, specific targeting ET-2 might be a potential treatment for lung adenocarcinoma.

APSET17.66

A central role of Endothelin A (ETA) Receptor Activation in Mesangial Cell – Podocyte Crosstalk in IgA Nephropathy and Other Mesangio-Proliferative Glomerulopathies

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Background: Mesangial cell (MC) activation by IgA-immune complexes is the initiating intra-renal event in the pathogenesis of IgAN. Subsequent MC-podocyte crosstalk results in proteinuria, the strongest predictor of progression. However, the molecular mechanisms responsible have not been well defined. Objective: The objective of these studies was to determine the role of the ETA receptor in mesangial cell activation and proteinuria in IgAN and other mesangio-proliferative glomerulopathies, using the potent and selective ETA antagonist atrasentan. Methods: The effect of atrasentan was assessed on ET-1 or IgA-immune complex activation MCs and in the gddY and anti-Thy-1.1 models. Results: ET-1 directly stimulated proliferation and IL-6 secretion in primary human MCs, which was blocked by atrasentan in a concentration-dependent manner. RNA sequencing and gene set enrichment analysis revealed hallmarks of MC activation with ET-1 treatment including up-regulation of cell proliferation, pro-inflammatory and pro-fibrotic networks, which were blocked by atrasentan. MCs exposed to IgA-immune complexes purified from the serum of IgAN patients demonstrated hyper-proliferation relative to complexes from healthy controls, which was significantly attenuated by atrasentan. In the gddY mouse model of spontaneous IgAN, atrasentan rapidly and significantly reduced albuminuria and down-regulated intra-renal proliferative, inflammatory, and fibrotic transcriptional networks. In the rat anti-Thy-1.1 antibody induced model of mesangio-proliferative glomerulopathy, atrasentan attenuated glomerular injury histologically, including mesangial hypercellularity and matrix expansion, segmental mesangiolysis and glomerulosclerosis. In addition, atrasentan significantly reduced proteinuria and tubulointerstitial injury score. Conclusion: These studies suggest an important role of the ETA receptor in MC activation and subsequent proteinuria in IgAN and other mesangio-proliferative glomerulopathies.

APSET17.67

A Phase 3, Randomized, Double-Blind, Placebo Controlled Study of Atrasentan in Patients With IgA Nephropathy (THE ALIGN STUDY)

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Background: IgA nephropathy (IgAN) is the most common primary glomerulonephritis globally and an important cause of chronic kidney disease (CKD). Up to 40% of IgAN patients are at risk of progressing to end-stage kidney disease (ESKD) and proteinuria is the strongest predictor of progression. There are no approved therapies for IgAN, leaving an important need for new strategies to lower proteinuria and preserve kidney function in high-risk patients. Endothelin A (ETA) receptor activation drives proteinuria, along with kidney inflammation and fibrosis. Atrasentan, a potent and selective ETA antagonist, has been studied extensively in >5,000 patients with type 2 diabetes and kidney disease (DKD), demonstrating clinically significant and sustained reductions in proteinuria when administered on top of a maximum tolerated dose of RAS inhibitor (RASi). In a global Phase 3 outcome study in DKD (SONAR), atrasentan demonstrated a 35% reduced risk of the primary composite outcome of doubling of serum creatinine or end stage kidney disease (95% CI: 0.49, 0.88; P = 0.005). The most common adverse event was fluid retention. Selective ETA blockade represents a promising approach to reduce proteinuria and preserve kidney function in high risk IgAN patients. Objective: This is a presentation of a global, phase 3, double-blind, placebo-controlled trial (currently in progress) to determine the effect of atrasentan in IgAN patients at high risk of kidney function loss. Method: Approximately 320 patients across North America, South America, Europe, and Asia-Pacific with biopsy-proven IgAN will be randomized to receive 0.75 mg atrasentan or placebo daily for 132 weeks. Patients will continue receiving a maximally tolerated and stable dose of a RAS inhibitor as standard of care. The study will also include patients that are unable to tolerate RAS inhibitor therapy. Additional eligibility criteria include urine protein creatinine ratio (UPCR) ≥1 g/g and eGFR ≥30 mL/min/1.73 m2. Participants will have study assessments over two and a half years with options for remote study visits using telemedicine and home health visits. The primary objective is to evaluate the effect of atrasentan versus placebo on proteinuria at Week 24. Secondary

objectives include evaluating the change from baseline in eGFR, safety, and tolerability, and quality of life.

APSET17.68

Brain Microvascular Endothelial Endothelin A (ETA) Receptors Contribute to Senescence

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Introduction- Cellular senescence plays a pivotal role in ageing and progression of neurodegenerative diseases including vascular cognitive impairment and dementia (VCID). It is well established that cerebrovascular dysfunction occurs long before cognitive deficits emerge. In postmortem brains from individuals with VCID, endothelin -1 (ET-1) levels closely correlate with blood barrier breakdown and cerebral hypoperfusion. It is now established that brain microvascular endothelial cells (BMVECs), previously thought to have exclusively ETB receptors, also possess ETA receptors in both sexes, however, functional significance of this receptor in BMVEC is not known. Current study was designed to test the hypothesis that activation of the ETA receptors mediate BMVEC senescence. Methods- Serum starved (2% fetal bovine serum) human BMVECs (HBEC5i) were incubated with ET-1 (1μM) in the presence/absence of ETA receptor antagonist BQ123 (20 μM; cells were treated 30 minutes prior to ET-1 treatment) for 16 hours. Cells were collected for Western blot and RT-qPCR analyses of ETA/ETB receptors and senescence marker proteins (p21, p16, LIF1). Results- Treatment of BMVECs with ET-1 increased both the gene and protein expression of ETA receptor while presence of ETA receptor antagonist inhibited ET-1 expression. Expression of senescence marker genes like p21, LIF1 and LIF receptor were upregulated with ET-1 treatment. Additionally, the protein levels of p21, p16 and LIF1 were also increased with ET-1 treatment, which was prevented in the presence of ETA blockade. Conclusions- ET-1 upregulates the ETA receptors in BMVECs in an autocrine manner and triggers the activation of senescence. These preliminary in-vitro findings need to be further studied in-vivo to establish the role of ETA receptors in the progression of endothelial senescence and blood brain barrier disruption in progressive neurogenerative disorders like VCID.

APSET17.70

Contribution of endothelin B receptor (ETB) in post stroke recovery and post stroke cognitive impairment (PSCI) in diabetes

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Introduction: Activation of the endothelin (ET) system contributes to cerebrovascular dysfunction and acute neurovascular injury after an ischemic stroke, especially in diabetes. While some studies suggest that inhibition of ET receptors prevent poststroke cognitive impairment (PSCI), recent advances also suggest that systemic ETB receptor agonism improves cognitive deficits after stroke and other neurodegenerative diseases. Accordingly, this study aimed to investigate the role of the brain ETB receptors in post stroke recovery in physiological and disease states by selectively silencing cerebral ETB receptors in control and diabetic rats. We hypothesized that ETB receptors are protective and molecular inhibition of ETB receptors will worsen PSCI. Methods: ETB receptors were knocked down by intra-cerebroventricular injections of GFP tagged lentiviral vectors with scrambled (sc-shRNA) or ETB-shRNA particles in male Wistar control and diabetic rats. Middle cerebral artery occlusion (MCAO; 30 minutes) was performed 10 days post lentiviral transfection. Animals were monitored for sensory-motor functions with adhesive removal time (ART) and composite score. Cognitive functions were measured with novel object recognition (recognition RI and discrimination d2 index), 2-trial Y-maze (Novel arm entries, spontaneous alternations) and open field (OF, distance moved, velocity). Results: In optimization studies, shRNA injections achieved significant KD (50-60 %). Animals of all the groups showed significant deficits in ART and composite score, indicating consistent ischemic injury. In control animals, there was a trend for lower RI and d2 in sc-RNA group (n=3), which was prevented by ETB KD (n=5). By Day 14, exploration time decreased in both groups. In diabetic animals, both RI and d2 were highly variable in the sc-RNA group (n=3) and ETB receptor KD (n=3) improved both indices. % change (baseline to D14) in novel arm entries and forced alternation scores were similar in all control groups but there was high variability and a trend for lower scores in diabetic ETB-shRNA group as compared to controls, suggesting that ETB silencing worsened working memory in diabetic rats. While both control and diabetic animals in sc and ET-shRNA groups moved around the same amount of time, diabetic animals were slower and hence moved less distance in OF. Conclusions: NOR scores suggest improved working memory in ETB-silenced control and diabetic rats. Y-maze scores, on the other hand, are suggestive of ETB receptors being protective in diabetic rats as ETB silencing worsened deficits. However, this may be due to diabetic animals moving slower as Y-maze test requires more movement compared to NOR. Additional animals and longer monitoring of animals in ongoing studies will provide more definitive answers with respect to the role of ETB receptors in stroke recovery and PSCI in control and disease models.

APSET17.71

Atrasentan in Patients with Proteinuric Glomerular Diseases (The AFFINITY Study)

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Background: Glomerular diseases are the leading cause of end stage kidney disease (ESKD) worldwide. Immunoglobulin A nephropathy (IgAN), focal segmental glomerulosclerosis (FSGS), Alport syndrome, and diabetic kidney disease (DKD) are all characterized by proteinuria, which is a strong predictor of disease progression and ESKD. Currently, there are no approved therapies for IgAN, FSGS, or Alport syndrome and despite the recent approval of sodium glucose co-transporter 2 inhibitors (SGLT2i), residual risk in DKD remains high, leaving an important unmet need for new therapies to lower proteinuria and preserve kidney function in high-risk patients. Endothelin A (ETA) receptor activation drives proteinuria, along with kidney inflammation and fibrosis. Atrasentan, a potent and selective ETA antagonist, targets a key pathogenic pathway common to the progression of proteinuric glomerular disease of different underlying etiologies. Atrasentan has been studied in over 5,300 patients with DKD, showing clinically significant and sustained reductions in proteinuria when administered on top of a maximum tolerated dose of RAS inhibitor (RASi). It was overall well tolerated, and the most common adverse event was fluid retention. Selective ETA blockade represents a potential approach to reduce proteinuria and preserve kidney function in proteinuric glomerular diseases. Objective: A global, phase 2, open-label basket study is currently being conducted to study efficacy and safety of atrasentan in IgAN, FSGS, Alport syndrome and DKD patients at risk of progressive loss of kidney function. Methods: Approximately 80 patients in the United States, Australia, South Korea, Spain, Italy, and the United Kingdom with proteinuric glomerular diseases will be enrolled in a basket study to receive 0.75 mg atrasentan orally for 52 weeks. The study has 4 cohorts, each consisting of 20 patients with the following diseases: IgAN, Alport syndrome, FSGS, and DKD. Patients must be receiving a maximally tolerated and stable dose of RASi and patients with DKD must also be on a stable dose of a SGLT2i. Proteinuria must be present in all patients: IgAN (urine protein creatinine ratio (UPCR) between 0.5 and < 1.0 g/g), FSGS (UPCR > 1.5 g/g), Alport syndrome (UPCR > 0.5 g/g), and DKD (urine albumin creatinine ratio (UACR) ≥ 0.5 g/g)). Patients must also have an eGFR ≥ 30 mL/min/1.73 m2; for DKD patients, ≥ 45 mL/min/1.73 m2. Participants will have study assessments over 1 year with options for remote study visits using telemedicine and home health visits. The primary objective is to evaluate the effect of atrasentan on change in proteinuria (IgAN, FSGS, AS) or albuminuria (DKD) from baseline at Week 12. Key exploratory objectives include changes in eGFR from Baseline to Week 52 and changes in audiology assessments in patients with Alport syndrome.

APSET17.72

Selective Afferent Renal Denervation and Blood Pressure in ETB-deficient Rats

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Defining the precise role of endothelin in autonomic control of blood pressure remains elusive. Using the endothelin B receptor deficient rat (ETB-def), we have previously demonstrated that total renal denervation was able to lower blood pressure but did not affect the salt-sensitive hypertension of the model. Markers of global sympathetic tone were also reduced following total denervation, suggesting a potential role for renal afferent (sensory) nerves in the hypertension of this model. Recent evidence in our lab also indicated increased afferent nerve activity in ETB-def rats compared to transgenic controls (TG), which is exaggerated following high salt diet. We hypothesize that selective afferent renal denervation lowers blood pressure in ETB-def rats. Using both males and females, we instrumented ETB-def (n = 4) and TG (n = 6) rats with telemetry. Following baseline blood pressure recording and measurement of vasomotor sympathetic tone by ganglionic blockade using chlorisondamine, we completed bilateral selective afferent renal denervation by periaxonal application of capsaicin (33 mM). Successful afferent denervation was confirmed by a reduction in the level of the sensory peptide CGRP in the renal pelvis (-141.7 ± 39.8 pg/mg tissue vs Sham, p = 0.01) and no reduction in cortical norepinephrine (p = 0.4 vs sham). At baseline ETB-def were hypertensive compared to TG (131.9 ± 3.3 vs 108.0 ± 4.9 mmHg, p < 0.0001); however, selective afferent renal denervation did not significantly affect blood pressure in either ETB-def or TG (ΔMAP from baseline, 1.0 ± 2.2 vs 1.8 ± 2.2 mmHg, p = 0.62). At baseline, ETB-def rats had greater vasomotor sympathetic tone compared to TG as evidenced by a greater reduction in MAP following ganglionic blockade (-45.7 ± 1.5 vs. -29.6 ± 2.2 mmHg, p < 0.001). Similar to the impact on MAP, afferent denervation did not significantly affect vasomotor sympathetic tone (-43.1 ± 0.8 vs. 32.1 ± 1.6 mmHg, p = 0.33 ETB-def denervated vs baseline). In order to further evaluate the influence of the ETB receptor on renal afferent nerves, we performed a similar experiment utilizing Sprague-Dawley rats that underwent either sham (n = 4), total (n = 3), or selective afferent (n = 3) renal denervation followed by subsequent treatment with the selective ETB receptor antagonist, A192621, in the drinking water (10 mg/kg/day). Sham and afferent denervated rats had similar levels of increased blood pressure following 3 days of ETB receptor blockade (+21.6 ± 0.7 and 20.5 ± 2.7 mmHg compared to baseline) whereas total denervation tended to attenuate this increase (+16.9 ± 1.0 mmHg, p = 0.15 vs. sham). One observation from a comparison between studies was a much greater ability of capsaicin to achieve afferent denervation in Sprague-Dawley rats vs both ETB-def and

TG (63.5 ± 11.8 vs. 269.3 ± 20.3 pg/mg CGRP, p = 0.01). This warrants further investigation into potential expression profile differences in afferent nerves of ETB-def rats. Thus far, we are unable to conclude that the increased renal afferent nerve activity in ETB-def rats contributes to the hypertension and increased vasomotor sympathetic tone observed in this model. Further clarification is needed of the role of endothelin receptors, including action of the ETA receptor.

APSET17.73

Progenitor-derived endothelin controls dermal sheath contraction for hair follicle regression

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Progenitor pruning and dermal papilla relocation during hair follicle regression is essential for stem cell activation and follicle regeneration in the hair growth cycle. This stem cell niche remodeling is coordinated by dermal sheath (DS) smooth muscle contraction, but how DS-generated forces are regulated is unknown. We identify spatiotemporally controlled endothelin signaling as the key activating mechanism of DS contraction. Pharmacological blocking or genetic ablation of both endothelin receptors, ETA and ETB, impedes DS contraction and follicles fail to regress. Epithelial progenitors of regressing follicles at the club hair/strand bottleneck – the site of active DS contraction – are the main source of ET-1 endothelin ligand, and ET-1 is absolutely required for follicle regression. Dissecting the Ca2+-dependent and -independent pathway branches of ET signaling, we identify dynamically-regulated cytoplasmic Ca2+ levels as a main regulatory mechanism of myosin light chain phosphorylation and contraction. Together, these findings illuminate an epithelial-mesenchymal-interaction paradigm in which progenitors control the contraction of surrounding cells to orchestrate homeostatic tissue regression and stem cell niche reorganization.

APSET17.74

Knockout of the endothelin B receptor in adipocytes improves insulin sensitivity and the metabolic profile of male mice fed a high fat diet

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Endothelin-1 (ET-1) is elevated in patients who are overweight and obese; however, its contribution to the pathophysiology of obesity is not fully understood. Blockade of ET_B receptors improves insulin sensitivity in rats, leads to increased PPARγ levels in visceral adipose and increases circulating adiponectin, suggesting a role for ETB activation on adipocytes in pathophysiology of obesity. We hypothesized that elevated ET-1 in obesity, acting through ETB receptors, promotes insulin resistance by reducing adipose PPARγ, causing a reduction in the secretion of insulin sensitizing adipokines. Adipocyte-specific ET_B receptor knockout mice ($adET_BKO$) or wild type littermates were fed either normal diet (NMD) or high fat diet (HFD) for 8 weeks. RNA-Sequencing of epididymal adipose (eWAT) indicated that, compared to HFD mice, HFD $adET_BKO$ mice exhibited an attenuation of ~500 genes enriched within insulin signaling and fatty acid metabolism pathways, while gene expression in eWAT showed significant increases in adipokine expression. HFD $adET_BKO$ mice had significantly improved glucose tolerance and insulin tolerance, in addition to significantly improved plasma adiponectin, insulin and leptin compared to HFD mice. HFD $adET_BKO$ mice also had significantly reduced dyslipidemia compared to HFD mice. These data indicate that loss of the ETB receptor in adipocytes improves peripheral glucose homeostasis, dyslipidemia, and the metabolic/cholesterol profile in HFD-fed mice, which suggest a role for the adipocyte ET_B receptor in the development of insulin resistance exhibited by obese individuals.

APSET17.77

Chlorogenic acid ameliorate muscle wasting and apoptosis in association with Calcineurin/PGC-1α upregulation and Endothelin-1/ETAR downregulation in diabetic rat model

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Diabetes mellitus (DM) induces reactive oxygen species (ROS) and inflammation, then leads to muscle wasting with protein degradation. Some signaling contributes in muscle wasting, such as NFkB and apoptosis activation, Calcineurin/PGC-1α downregulation and Endothelin-1 (ET-1) upregulation. An active compound of coffee, chlorogenic acid (CGA), was elucidated in this study in preventing muscle wasting as progression of DM in relation with those signaling. Diabetic model was carried out in Sprague-dawley rats for 1.5 (DM1.5 group) and 2 (DM2 group) months. Three doses of CGA 12.5 (CGA1), 25 (CGA2) and 50 (CGA3) mg/Kg body weight for 2 weeks (form month 1.5-2) was added. Blood was taken for biochemical analysis. The soleus muscle was harvested for RNA analysis of Calcineurin/PGC-1α, Caspase-3, ppET-1 and ETAR mRNA. Meanwhile, paraffin section was used for HE staining and calcineurin immunostaining. Blood glucose level demonstrated higher than 250 mg/dL in DM groups, meanwhile CGA1 had lower glucose level than DM groups. Muscle wasting occurred I DM groups, which associated with downregulation of SOD-1, SOD-2, Calcineurin and PGC-1α mRNA expression compared to control group.

CGA1 group demonstrated attenuation of wasting with higher SOD-1, SOD-2, Calcineurin and PGC-1α mRNA expression compared to DM2 group. Immunostaining revealed reduction of signal in DM2 group, and preservation of calcineurin signal in CGA1 group. Furthermore, DM2 group also showed upregulation of ppET-1/ETAR mRNA expression, however CGA treated group, especially CGA1 revealed downregulation of ppET-1/ETAR mRNA expression. Low-dose of CGA may attenuate muscle wasting and apoptosis with contribution of Calcineurin/PGC-1α upregulation and ET-1/ETAR downregulation. Keywords: Diabetes mellitus (DM), Muscle wasting, Calcineurin, PGC-1α, SOD-1, SOD-2, Endothelin-1/ETAR, Caspase 3

APSET17.78

Calcitriol supplementation ameliorates vascular remodeling, inflammation, and fibrosis in acute and chronic phases of kidney ischemia-reperfusion injury in mice

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Background Kidney ischemia-reperfusion injury (IRI) is a common cause of acute kidney injury, which often develops further into chronic kidney disease (CKD). Endothelin-1 (ET-1) contributes to pathophysiology of acute and chronic kidney injury, which includes vascular remodeling, inflammation and fibrosis. Calcitriol is not only known for regulating calcium, but also has protective effects on CKD and kidney fibrosis. However, renoprotective roles of Calcitriol in vascular remodeling, inflammation and fibrosis in acute and chronic episodes of kidney IRI have not been confirmed. Therefore, we aimed to investigate potential effects of Calcitriol on kidney injury during acute and chronic phases of kidney IRI in mice. Methods We performed renal bilateral clamping in male Swiss background mice (3-4 month old, 30-40 g) for 30 minute, then reperfused and terminated the mice in day-3 (IR3 group, n=5) and day-12 (IR12 group, n=5). Sham operation (SO group, n=5) was used for control. We injected 0.5 mcg/kgBW/day Calcitriol intraperitoneally for 3 days (IRD3 group, n=5) and 12 days (IRD12 group, n=5) in kidney IRI mice. Serum creatinine level was quantified, and kidney was harvested and used for histology and mRNA analysis. Sirius Red staining was used for fibrosis and vascular remodeling assessment. Reverse Transcriptase-PCR (RT-PCR) was performed to examine mRNA expression level of NF-κB, TGF-β1, SOD-1, ppET-1, ETAR and eNOS. $p < 0.05$ was considered statistically significant. Results Kidney IRI-only groups (IR3 and IR12) showed higher creatinine level, upregulation of NF-κB and TGF-β1, with downregulation of SOD-1 mRNA expression compared to SO. Meanwhile, Calcitriol-treated groups (IRD3 and IRD12) showed reduction of creatinine level, NF-κB, and TGF-β1, with SOD-1 upregulation compared to IRI-only groups. Quantification of small intrarenal arteries in IR3 and IR12 demonstrated higher wall thickness and lower LWAR than SO. Meanwhile, Calcitriol groups showed decreased wall thickness and increased LWAR compared to their IRI-only counterparts. IRI increased mRNA expression of ppET-1 and ETAR in both IR3 and IR12 ($p < 0.05$), and decreased eNOS mRNA expression in IR12 ($p = 0.001$) compared to SO. Calcitriol groups showed lower ppET-1 ($p = 0.037$) and higher eNOS ($p = 0.020$) mRNA expression in chronic phase, as well as lower ETAR mRNA expression in both acute and chronic phases ($p < 0.05$) compared to their respective IRI-only groups. Conclusions Calcitriol may ameliorate vascular remodeling, inflammation, and fibrosis during acute and chronic phases of kidney ischemia-reperfusion injury in mice. Keywords: Calcitriol, vascular remodeling, inflammation, fibrosis, kidney ischemia-reperfusion injury

APSET17.80

ETB Receptors and Vascular Function in Postmenopausal Women

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Women experience an increased risk for cardiovascular disease (CVD) during menopause. Endothelial function – a biomarker for cardiovascular health – declines with aging, but this process is accelerated with menopause. Endothelin-1 regulates endothelial function and has been shown to contribute to the age-related declines in endothelial function in men. However, there are known sex differences in the ET-1 system, and the ET-1 system is modulated by sex hormones. Our laboratory has conducted a series of experiments to understand the impact of menopause as well as fluctuating levels of sex hormones on endothelial function, with a specific focus on the endothelin-B receptor (ETBR). We have demonstrated that the ETBR mediates vasodilation in young women, but that this effect is lost after menopause. Furthermore, expression of the ETBR on endothelial cells is lower in postmenopausal women compared to young women. Endothelial cell expression of ETBR is positively correlated with flow-mediated dilation, a measure of endothelial function. Taken together, declines in endothelial function in postmenopausal women occur, in part, due to a loss off ETBR-mediated dilation as a result of reduced ETBR expression on endothelial cells. Finally, we utilized a controlled hormone intervention in young women to regulate hormone production, and then selectively added back estradiol to isolate the impact of estradiol on ETBR function. We demonstrate ETBR mediate vasodilation in the presence of estradiol, but this effect is absent when estradiol production is suppressed. Collectively, the declines in estradiol that occur with menopause alter ETBR function and expression which contribute to endothelial dysfunction.

APSET17.81

Chlorogenic Acid Influence the Endothelin-1/ETAR Signaling and Reduces the Occurrence of Vascular Remodeling and Inflammation on Rat (Rattus norvegicus) Heart Diabetes Mellitus Model

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Background: Diabetes Mellitus (DM) with hyperglycemia induces Reactive Oxygen Species (ROS) triggered oxidative damage to cardiac cells producing microvascular damage, myocyte injury, the formation of extracellular matrix, activation of pro-inflammatory mediators, endothelial dysfunction, and vascular remodelling. Endothelin-1 (ET-1) and eNOS, vasoactive substances, have been known play role in cardiac injury and fibrosis mechanism. Meanwhile, Chlorogenic acid (CGA), an active component of coffee, functions as an antioxidant, anti-inflammatory, antihypertensive and anti-cancer. However, effect of CGA in preventing heart injury progression in diabetic condition never been analyzed. Purpose: This study aims to elucidate role of CGA on cardiac injury as DM progression in association with vascular remodelling, inflammation and ET-1/eNOS signalling. Method: This research used a quasi-experimental research design with post-test only with controlled group design. The subjects used were rats (Rattus norvegicus) strains Wistar with a weight of 150-200 grams divided into 6 groups, namely: control group by saline administration (NaCl) without STZ and without CGA; DM group 1 (DM for 1.5 months); DM group 2 (DM for 2 months); CGA group 1 (DM 1.5 months + CGA injection dose 12.5 mg/kgBW); group CGA 2 (DM 1.5 months + injection CGA dose 25 mg/kgBW); CGA group 3 (DM 1.5 months+ injection CGA dose 50 mg/kgBW). mRNA expression was quantified by Reverse Transcriptase-PCR. Vascular remodeling examination assessed by lumen area parameters and wall/lumen area ratio (WLAR) quantification based on Sirius red staining. Results: CGA dose 12,5 mg/kgBW decrease the blood sugar levels and significant ($p<0.05$) compared to the DM 1.5 and DM 2 groups. The ratio of the weight of heart organs/length of tibia CGA group 1 is significantly higher than the DM group of 1.5 and DM 2 ($p<0.05$). Diabetes Mellitus influences the modulate of ppET-1/ETAR and JAK/NFATc1 signaling. The mRNA expression of ppET-1 and eNOS in the CGA group dose 50 mg/kgBW was higher and significant than DM groups. Vascular remodeling occurs through the upregulation of mRNA expression (JAK2 and NFATc1), the narrowness of the lumen area, and the higher of WLAR in the DM group compared to the control group ($p<0.05$). CGA inhibits the vascular remodeling through the downregulate of JAK2 and NFATc1 mRNA expression, expand the lumen area and restrict the WLAR vasa resistance. The mRNA expression of NFATc1 in the CGA group dose 50 mg/kgBW was lower and significant compared to the control, DM 1.5, and DM 2 groups ($p<0.05$). CGA dose 12.5 mg/kgBW had a significant effect in expanding the lumen area compared to the DM group. CGA dose 50 mg/kgBW had affected of lowering the WLAR ratio. CGA decreases the inflammatory mediators through downregulation of IL-1 and IL-6 mRNA expression. There was no significant effect on the mRNA expression of SOD1 after administration of CGA. Conclusion: The administration of CGA doses of 50 mg/kgBW affects the increase of ppET-1/ETAR and eNOS mRNA expression, decreases the JAK2/NFATc1 and IL-6 mRNA expression, expands the lumen area vasa resistance, and restrict the wall/lumen area ratio (WLAR) compared to the diabetes mellitus group. The variation of the CGA dose did not affect the SOD1 mRNA expression.
Keywords: Antioxidant, CGA, Endothelial Dysfunction, DM, Vascular Remodeling

APSET17.82

Global PER1 knockout Dahl Salt Sensitive rats show increased expression of renal EDN1 mRNA and Endothelin-1 peptide

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Previously, we showed that global knockout of the circadian clock protein PERIOD1 (PER1) in Dahl Salt Sensitive (SS) rats resulted in worsening of the SS hypertensive phenotype (1). PER1 KO rats also exhibited evidence of renal injury after three weeks of a high salt diet (4% NaCl). The mechanism relating PER1 KO with a hypertensive phenotype may be through PER1's negative regulation of the vasoconstrictor peptide Endothelin-1 (ET-1) via transcriptional control of the ET-1-encoding gene Edn1. Given that ET-1 signaling can be pro-hypertensive and profibrotic in the kidney, we hypothesized that ET-1 peptide levels in the kidney may contribute to the phenotype of the PER1 KO SS rats. Protein and total RNA were isolated from the kidney samples of control and PER1 KO SS rats on normal diet (0.4% NaCl) or after three weeks on a high salt diet (4% NaCl). Renal ET-1 protein levels were measured by ELISA and quantitative PCR was performed to quantify the mRNA levels of ET-1 and its receptors ETA and ETB. Data were evaluated for genotype or diet differences using ANOVA. Renal ET-1 peptide levels were significantly higher in PER1 KO rats on high salt diet when compared to control SS rats ($p = 0.0003$). There was also a significant genotype difference in Edn1 mRNA levels, with increased levels in PER1 KO rats on a high salt diet ($p=0.0389$). In contrast, significant differences were not in the mRNA levels of ET-1 receptors Ednra and Ednrb between genotypes or diets. These data suggest that PER1 KO results in increased ET-1 in the kidney, due to increased transcription of the Edn1 gene. This apparent negative regulation of ET-1 by PER1 is likely to contribute to the hypertensive and renal injury phenotype of PER1 KO SS rats on a high salt diet. Together these data show an important role for PER1 and its target

ET-1 in salt-sensitive hypertension and the accompanying renal injury. Reference: 1 Spires DR, Zietara A, Levchenko V, Gumz ML, Staruschenko A. Knockout of Per1 Exacerbates the Hypertensive Phenotype of the Dahl Salt Sensitive Rat. FASEB J. 2020;34(S1):1. Funding: 1R01DK109570-01A1

APSET17.84

The Gut microbiota and the ET system

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Endothelins and their receptors are expressed in intestinal cells including epithelial cells. Previous pre-clinical studies indicate that concurrent treatment with endothelin receptor antagonists can prevent gut inflammation in animal models. Considering the critical role of the gut microbiota in the development and progression of intestinal inflammatory diseases, we hypothesized that expression and function of endothelin pathway genes in the intestines are regulated by the gut microbiota. We found that, in germ-free mice completely devoid of intestinal microbes, Ednra and Ednrb transcripts are readily detectable in epithelial cells in the large intestine. By comparison, the levels of both transcripts were reduced in epithelial cells from mice with a complete microbiota. However, expression of Edn1 and Edn2 in the large intestine was not significantly impacted by the microbiota. Endothelin receptor antagonism reduced intestinal permeability in mice, suggesting a basal role for endothelin signaling in negatively regulating epithelial barrier integrity even at homeostasis. In support of this, prophylactic use of endothelin receptor antagonists efficiently prevented the development of colitis in mice subsequently administered dextran sodium sulfate. Altogether, these data implicate endothelin receptor signaling in the negative regulation of gut barrier integrity. In addition, our findings support a role for the gut microbiota in actively regulating this pathway in part via negative regulation of endothelin receptor expression in epithelial cells.

APSET17.85

Upregulation of ppET-1 and downregulation of eNOS associate with vascular remodeling and inflammation in the heart and kidney of rats with high fat diet

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Background: High Fat Diet (HFD) modifies several metabolic activities leading to cardiovascular diseases. The insulin resistance induced by high fat diet alters the expression of vasodilator and vasoconstrictor agent, resulting in endothelial dysfunction Objectives: We investigated the effect of high fat diet (HFD) to the heart and kidney vasculature, focusing on inflammatory mediators associated with endothelial dysfunction. Methods:Rats (3 months-old, weight 200 g) were divided into control (n=6), and rats fed on high-fat diet (HFD) for 1 month (n=6, HFD1), 2 months (n=6, HFD2), and 4 months (n=6, HFD4). Then, rats were terminated, and the heart and kidney were harvested for histological quantification and the quantification of mRNA expression of inflammatory mediators, eNOS and ppET-1 using RT-PCR. Sirius Red staining was done to assess vascular remodeling and the immunohistochemistry staining of CD68 protein expression was performed to assess the localization of macrophage. Results: HFD group body weight were higher compared to the control group. It was followed by increase of mRNA expression of NF-κB, MCP-1 and CD68 in the heart of HFD groups compared to the control group, particularly in HFD4 group. Immunostaining revealed positive staining of macrophage in the heart of HFD groups. Histological staining of the kidney showed slight tubular injury and glomerulosclerosis in HFD4 group. Long-term HFD administration promoted vascular remodeling with increased wall in the heart of HFD4 group. HFD4 group demonstrated upregulation of ppET-1 with downregulation of eNOS mRNA expression in the heart and kidney. Conclusion: HFD induce the upregulation of ET-1 and downregulation of eNOS and promote cardiac vascular remodeling, kidney injury and inflammation in both organs. Keywords: high fat diet, inflammation, vascular remodeling, eNOS, ppET-1.

APSET17.86

In-vivo and in-vitro progression of kidney fibrosis and myofibroblast formation associate with Endothelin-1 from Endothelial cells and Endothelin A Receptor from myofibroblast

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Endothelin-1, which is mostly secreted by endothelial cells (EC), is a potent profibrotic factor in many organs, which promotes mesenchymal transition and myofibroblast formation. Endothelial cells-derived ET-1 deletion and Endothelin-A receptor blocker from myofibroblast may give perspective in attenuating fibrogenesis in kidney. This study used myofibroblast culture from fibrotic kidney induced by ureteral ligation. Endothelin blockers was added with ETAR blocker using BQ-123 and dual ETAR and ETBR Blockers (BQ-788+BQ-123). Westernblot of αSMA, PKC and ERK-1 was performed to examin myofibroblast formation and its signaling. In-vivo study using unilateral ureteral obstruction (UUO) model was performed in vascular endothelial cells-ET-1 knock-out (VEETKO) mice

and its WT littermate to induce kidney fibrosis. Immunostaining of PDGFR-β and αSMA was performed to examine fibroblast to myofibroblast transition. Westernblot and immunostaining was carried-out for examining fibroblast, myofibroblast, EC markers and ETAR protein expression. Doppler imaging was also performed to observe renal blood flow. Myofibroblast culture was confirmed with positive signal of αSMA, vimentin, CD75 and PDGFR-β staining, with negative signal of CD-45 and CD31. Both ETAR and dual ET blockers downregulated αSMA, PKC and ERK-1 protein expression. UUO induced fibrosis with fibroblast activation and myofibroblast formation in VEETKO and WT mice. However, VEETKO mice demonstrated reduction of fibroblast activation using westerblot and area fraction of PDGF-β and NG2 immunostaining in VEETKO-UUO compare to WT-UUO group. These findings associated with increase of renal blood flow and VE-cadherin protein expression in VEETKO after UUO. UUO also induced upregulation of ETAR which also expressed by myofibroblast based on IF staining. Furthermore, ET-1 deletion from EC reduced ETAR protein expression, improved renal blood flow and tubular injury compared to WT after UUO. ET-1 from EC and ETAR from myofibroblast may interact and promotes fibrogenesis in kidney.

APSET17.87

Endothelial ETB Receptor Expression in Young and Older Men

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Cardiovascular disease (CVD) is the leading cause of death in men. Changes in endothelial function precede the development of CVD. We have previously shown that age-related declines in endothelial function in women are due in part to a reduction in endothelin-B receptor (ETBR) expression on endothelial cells. However, it is not known if ETBR expression changes with aging in men. Therefore, the purpose of this study was to test the hypothesis that endothelial cells would express fewer ETBR in older men (OM) compared to younger men (YM). METHODS. Primary endothelial cells were harvested from the antecubital vein of 6 OM (age 65±2 yrs.) and 9 YM (age 25±1 yrs.) using a J-wire. Cells were stained with 4',6-diamidino-2-phenylindole and vascular endothelial cadherin to identify intact endothelial cells. ETBR was quantified using immunocytochemistry and fluorescence intensity was measured in 30 cells, which was averaged for each participant. Endothelial function was assessed using brachial artery flow-mediated dilation (FMD). Data are expressed as mean±SE. RESULTS. As expected, OM had higher total cholesterol (OM: 205±11 vs. YM: 133±10 mg/dL; P<0.01) and LDL cholesterol (OM: 128±6 vs. YM: 72±9 mg/dL; P<0.01), fasting plasma glucose (OM: 97±3 vs. YM: 85±2 mg/dL; P<0.01), and BMI (OM: 27±1 vs. YM: 23±1 kg/m2; P=0.02). OM tended to have higher systolic BP (OM: 124±10 vs. YM: 107±4 mmHg; P=0.08) whereas diastolic BP was similar between groups (OM: 77±4 vs. YM: 73±3 mmHg; P=0.31). ETBR expression was similar between OM and YM (OM: 250±49 vs. YM: 233±27 AU; P=0.76). Although FMD was not different between groups (OM: 5.48±1.54 vs. YM: 5.46±0.99%; P=0.99), there was a significant correlation between ETBR and FMD (r2=0.31, P=0.04). However, no significant correlations between ETBR expression and total cholesterol, LDL cholesterol, plasma glucose, or BMI were observed (all P>0.46). CONCLUSION. These preliminary data suggest that ETBR expression is not altered with age in men. These findings are in contrast to data in women, and further support sex differences in the endothelin system. This work was supported by NIH R01 HL 146558.

APSET17.88

Research on the relationship between endothelin-1 and Henoch-Schonlein purpura in children

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Object: To explore the relationship between endothelin-1 (ET-1) gene polymorphisms in locus rs5370, rs1630736 and the morbidity of Henoch-Schonlein purpura, and the changes and significance of the level of urinary endothelin -1 in children with Henoch Schonlein purpura (HSP). Method: Blood samples of thirty seven children with HSP were collected to detect gene polymorphisms of endothelin-1,urine samples of 22 patients at the acute phase were collected to detect the levels of urinary endothelin -1 . 100 healthy children were enrolled as gene control group, The urine of twenty cured children with pnumonia was collected and the urinary ET-1 was detected as control. ET-1 gene polymorphisms was detected by polymerase chain reaction and gene sequencing. And the level of urinary endothelin -1 was detected by radioimmunoassay. Results: 1. There were no significant differences in the gene frequencies and allele frequency distribution of ET-1 gene polymorphisms in locus rs5370 and rs1630736 between HSP group and control group (P > 0.05). 2. The difference of the level of urinary ET-1 between HSP group at acute phase and control group has statistical significant. (z=2.75, P <0.01).3.In locus rs5370, the difference between GG genotype and GT genotype in case group on the level of urinary ET-1 has no statistical significant (P > 0.05). 4. In locus rs1630736, the differences among CC genotype, CT genotype and TT genotype in case group on the level of urinary ET-1 have no statistical significant (P > 0.05). Conclusion: (1) It can not be proved that the endothelin-1 gene polymorphisms in locus rs5370 and rs1630736 has relationship with morbidity of Henoch - Schonlein purpura. (2) The level of urinary ET-1 in children with Henoch Schonlein purpura increased significantly at the acute phase. It is suggested that endothelin-1 plays an important role in the pathogenesis of Henoch Schonlein purpura.

APSET17.89

Endothelin receptor antagonism reduces gut permeability to prevent DSS-induced colitis in mice

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The role of the endothelin (ET) system in regulating blood pressure, renal, and cardiovascular health and disease has been extensively studied, but there is a gap in our knowledge about the potential role of the endothelin system in mediating intestinal homeostasis. Although there have been some publications observing protection from chemically induced colitis with the use of endothelin receptor antagonists (ETRAs), little has been done to assess the mechanism by which these drugs are providing this protection. ET receptor activation has been implicated in glomerular permeability, as well as blood brain barrier permeability in a mouse model of epilepsy. Thus, we sought to investigate the mechanism by which ETRAs convey protection from dextran sulfate sodium (DSS) induced colitis in C57/BL6 mice. We also sought to determine if antagonism of both endothelin receptors A and B (ETAR and ETBR), or selective antagonism of ETAR alone is required for protection from colitis. We hypothesized that ET receptor blockade is protective against DSS-induced colitis by maintaining the epithelial barrier. Six days prior to DSS-colitis induction, mice were treated with either the selective ETAR antagonist, atrasentan, or dual ETRA treatment, atrasentan and A-192621, in the drinking water at a concentration of 10 mg/kg body weight. We assessed body weight, spleen and colon weight, colon length, and histology in mice that received ETRA treatment prior to DSS administration with and without continued antagonism during DSS exposure. Interestingly, we found that ETRA treatment prior to colitis induction was enough to convey protection against colitis development (dual ETRA pre-treatment + DSS (n=9) vs DSS only (n=12); colon length: $p<0.0001$; colon weight normalized to final body weight: $p<0.0001$; spleen weight normalized to final body weight: $p<0.0001$; histology score: $p<0.0001$). Additionally, selective ETAR treatment was sufficient to convey protection from colitis development (ETAR pre-treatment + DSS (n=12) vs DSS only (n=12); colon length: $p=0.0097$; colon weight normalized to final body weight: $p=0.0133$; spleen weight normalized to final body weight: $p<0.0001$; histology score: $p<0.0001$). Given the lack of histological epithelial damage in mice that received ETRAs and DSS, we assessed colonic epithelial barrier permeability using the FITC-dextran assay. Mice that were exposed to 6 days of dual ETRA treatment prior to 3 days of DSS administration (n=4), exhibited significantly less intestinal permeability relative to the DSS only mice (n=4; $p=0.0484$). Taken together, this suggests that ET receptor blockade reduces translocation of luminal substances to convey protection from colitis development.

APSET17.90

Adipocyte specific ETB receptor overexpression induces obesity, insulin resistance and dyslipidemia in a female mouse model of obesity.

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High fat diet (HFD) rapidly induces obesity in male mice, leading to insulin resistance, dyslipidemia, and inflammation. On the other hand, female mice have delayed onset of obesity related pathophysiology. Our lab demonstrated that ET-1 is elevated in adipose tissue in a mouse model of diet induced obesity, and blockade of ET-1 receptors improves metabolic profiles and inflammation. One potential mechanism by which ET-1 promotes insulin resistance and dyslipidemia is through activation of the ET-1 type B receptor (ETB) on adipocytes. Our laboratory demonstrated that male mice have higher expression of ET-1 and ET-1 receptors in adipose tissue compared to females, a potential mechanism by which females are afforded protection against obesity related pathophysiology. Therefore, this study tested the hypothesis that overexpression of ETB receptors on adipocytes of female mice will cause a loss of protection from insulin resistance and obesity in response to HFD feeding. To induce obesity, 8 week old female C57 ETB overexpression mice were placed on a high fat diet (42% calories from fat) for 8 weeks. There was no significant difference in parameters of insulin sensitively, including fasting insulin and glucose, oral glucose tolerance and insulin tolerance, between normal diet (NMD) or HFD fed control mice; however, female adipocyte ETB overexpression mice (adETbOX) had a significant increase in fasting insulin and glucose and impaired glucose and insulin tolerance compared to both NMD and HFD fed control. Further, plasma cholesterol was significantly elevated in adETbOX compared to NMD and HFD controls, indicative of dyslipidemia. Insulin resistance and dyslipidemia in adETbOX were associated with reduction in Akt2, a key mediator in the insulin signally pathway. Metabolic impairments of HFDOX were also associated with increased mRNA expression TNFα and IL-10 compared to NMD, suggesting increased inflammation in adETbOX females. These data indicate that protection of female mice against obesity and insulin resistance in response to HFD may be mediated by lower expression of ET-1 and ETB receptor in adipocytes.

APSET17.91

Clinical Rationale for a Potent and Selective ETA Blocker for the Treatment of IgAN and other Glomerular Diseases: the ALIGN and AFFINITY Trials

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Glomerular diseases are the leading cause of ESKD worldwide. Immunoglobulin A nephropathy (IgAN), focal segmental glomerulosclerosis (FSGS), Alport syndrome,

and diabetic kidney disease (DKD) are all characterized by proteinuria, which is a strong predictor of disease progression and ESKD. Currently, there are no approved therapies for IgAN, FSGS, or Alport syndrome and despite the recent approval of sodium glucose co-transporter 2 inhibitors (SGLT2i), residual risk in DKD remains high, leaving an important unmet need for new therapies to lower proteinuria and preserve kidney function in high-risk patients. Endothelin A (ETA) receptor activation drives proteinuria, along with kidney inflammation and fibrosis. Atrasentan, a potent and selective ETA antagonist, targets a key pathogenic pathway common to the progression of proteinuric glomerular disease of different underlying etiologies. Atrasentan has been studied in over 5,300 patients with DKD, showing clinically significant and sustained reductions in proteinuria when administered on top of a maximum tolerated dose of RAS inhibitor (RASi). It was overall well tolerated, and the most common adverse event was fluid retention. Selective ETA blockade represents a potential approach to reduce proteinuria and preserve kidney function in proteinuric glomerular diseases. Selective ETA blockade represents a promising approach to reduce proteinuria and preserve kidney function in patients at high risk of progression. ALIGN, a global, phase 3, double-blind, placebo-controlled study is in progress to determine the effect of atrasentan in IgAN patients at high risk of kidney function loss. Approximately 320 patients across North America, South America, Europe, and Asia-Pacific with biopsy-proven IgAN will be randomized to receive 0.75 mg atrasentan or placebo daily for 132 weeks. Patients will continue receiving a maximally tolerated and stable dose of a RASi as standard of care. The study will also include patients that are unable to tolerate RASi therapy. Additional eligibility criteria include urine protein creatinine ratio (UPCR) ≥1 g/g and eGFR ≥30 mL/min/1.73 m2. The primary objective is to evaluate change in proteinuria at Week 24. Secondary objectives include change from baseline in eGFR, safety, and tolerability, and quality of life. AFFINITY, a global, phase 2, open-label basket study is on-going to study the efficacy and safety of atrasentan in IgAN, FSGS, Alport syndrome and DKD patients at risk of progressive loss of kidney function. Approximately 80 patients in the United States, Australia, South Korea, Spain, Italy, and the United Kingdom with proteinuric glomerular diseases will be enrolled in a basket study to receive 0.75 mg atrasentan orally for 52 weeks. The study has 4 cohorts, each consisting of 20 patients with the following diseases: IgAN, Alport syndrome, FSGS, and DKD. Patients must be receiving a maximally tolerated and stable dose of RASi and patients with DKD must also be on a stable dose of a SGLT2i. Proteinuria must be present in all patients: IgAN (urine protein creatinine ratio (UPCR) between 0.5 and < 1.0 g/g), FSGS (UPCR > 1.5 g/g), Alport syndrome (UPCR > 0.5 g/g), and DKD (urine albumin creatinine ratio (UACR) ≥ 0.5 g/g)). Patients must also have an eGFR ≥ 30 mL/min/1.73 m2 (≥ 45 mL/min/1.73 m2 for the DKD patients). The primary objective is to evaluate the effect of atrasentan on change in proteinuria (IgAN, FSGS, AS) or albuminuria (DKD) from baseline at Week 12. Key exploratory objectives include changes in eGFR from Baseline to Week 52 and changes in audiology assessments in patients with Alport syndrome.

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Impaired diurnal mitochondrial bioenergetics in Bmal1 knockout rats is associated with loss of diurnal ET-1 production in the kidney

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Endothelin is an essential regulator of Na+ homeostasis in the kidney. In the kidney, 95% of O2 consumed is used for Na+ transport through mitochondria respiration. Mitochondrial dysfunction has been shown to drive the progression of acute kidney injury to chronic kidney disease. The circadian clock regulates both endothelin and Na+ transport with a necessary diurnal variation for optimal health. Furthermore, the clock gene, BMAL1, is known to regulate mitochondrial O2 consumption, but whether this occurs in the kidney has not been investigated. Therefore, we tested the hypothesis that mitochondrial respiration is impaired in the kidney of a novel BMAL1 clock gene knock-out rat and whether this aligns with loss of diurnal control of ET-1 and Na+ excretion. Male and female global BMAL1+/+ and BMAL1-/- rats at 12-14 weeks of age were used to measure renal mitochondrial O2 consumption. Outer renal medullary tissue was dissected and prepared for respiration measurements at either inactive (ZT2-4) or active (ZT14-16) periods to correspond with the minimum and maximum whole-body energy consumption and peak and trough Bmal1 protein expression, respectively. Respiration was assessed using permeabilized tissue in the Oroboros Oxygraph and Data Lab 2 software (O2K, Oroboros Instruments GmbH). Mitochondrial gene expression analysis was conducted using RT-PCR. Data were analyzed using two-way ANOVA between combined male and female BMAL1+/+ (n=6) and BMAL1-/- (n=9). O2 consumption during state three respiration was significantly higher (main effect p=0.0310) in BMAL-1-/- (118.5±156 pmol/s*mg) compared to BMAL1+/+ rats (74.7±15.0 pmol/s*mg Tukey posthoc p=0.0303). An unpaired t-test with Welch's correction showed a decreased rate of uncoupling complex IV activity in BMAL1+/+ rats during active vs. inactive (163.8±40.1 vs. 305.5±40.2 pmol/s*mg) times of day (p=0.0031). Mitochondrial genes, optic atrophy 1 (OPA1), and Mitofusin 1 (Mfn1) show a dampened rhythmicity of mitochondrial fusion in BMAL1-/- rats. Our lab previously reported that male BMAL-1-/- rats do not have the typical night-day difference in Na+ excretion consistent with a lack of rhythmicity in mitochondrial function. In a separate cohort, male BMAL1+/+ and BMAL-1-/- rats (n=3 each) were maintained on a high salt diet (0.4%NaCl) for two weeks to stimulate renal ET-1 production. During the last 48hr, rats were placed in metabolic cages for 48hr to facilitate 12hr urine collections. BMAL1-/- rats had significantly lower ET-1 excretion rates (p=0.0148 for genotype) and lacked the normal diurnal difference (p=0.042 genotype X time of day). Therefore, deletion of BMAL1 resulted in changes in ET-1 excretion that corresponded to the reduced complex IV activity. These results are consistent with the hypothesis that BMAL1 plays a key role in maintaining renal mitochondrial respiration as

reflected in impaired diurnal Na+ handling and ET-1 production.

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Endothelin, the kidney & cardiovascular disease

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Chronic kidney disease (CKD) is an increasingly common public health concern with a global prevalence of ~10%. It results from a heterogeneous group of conditions that lead to a progressive and irreversible impairment in kidney function. CKD now ranks as the 12th leading cause of death worldwide with much of the mortality associated with CKD due its commonest complication, cardiovascular disease. Endothelin-1 (ET-1) plays important roles in renal physiology and pathophysiology. The kidney is an important source of ET-1 and has the body's highest concentration of endothelin-B receptors. This talk will cover the major advances made in uncovering the roles of ET-1 in kidney physiology and disease, and possible future directions. It will include: 1. selective and mixed ET receptor blocking approaches to reducing blood pressure (including resistant hypertension) and proteinuria, the two key biomarkers for CKD progression2. the role of endothelin blockade in improving circulating lipids and arterial stiffness 3. the synergy of ET-blocking approaches with blockade of the renin-angiotensin system & sodium glucose cotransporter 2 (SGLT2) inhibition4. ET blockers in specific kidney diseases: Alport's syndrome, FSGS, IgA nephropathy 5. potential for ET blockers in acute kidney injury and in patients with kidney failure