SESSION V

BODY FLUID HOMEOSTASIS
RENAL FUNCTION

Friday (September 17, 2021; 10:00 – 10:45)
Friday (September 17, 2021; 11:45 – 13:35)

Chair:

Prof. Elzbieta Kompanowska-Jezierska
Department of Renal and Body Fluid Physiology, M. Mossakowski Medical Research Institute, Polish Academy of Science, Warsaw, Poland

Prof. Maciej Jankowski
Department of Clinical Chemistry, Medical University of Gdansk, Gdansk, Poland

DETAILED SESSION V SCHEDULE

Opening lecture (September 17, 2021; 11:45 – 12:15; *virtual stream B*)

S5.L1 BLOCKADE OF ENDOTHELIN RECEPTOR AS AN ADJUVANT THERAPY IN THE TREATMENT OF CHRONIC KIDNEY DISEASE – STUDIES IN REN-2 TRANSGENIC RATS. **I. Vaneckova** (Institute of Physiology, Czech Academy of Sciences, Prague, Czech Republic).

Oral presentations (September 17, 2021; 12:15 – 13:35; *virtual stream B*)

S5.L2 RENAL EFFECTS OF β,γ-METHYLENE ATP, P2X RECEPTOR AGONIST, IN NORMO- AND HYPERGLYCEMIC RATS. G. Chyla, E. Kreft, M. Jankowski (Department of Clinical Chemistry, Medical University of Gdansk, Poland).

S5.L3 ANGIOTENSIN II IN THE MEDIAL PREOPTIC AREA MEDIATES CARDIOVASCULAR REGULATION, BUT NOT MICTURITION CONTROL. **S. Daiuto[1], P. Aronsson[2], M. Sato[1]** ([1]Centro Universitario FMABC, Santo Andre, Brazil, [2]Department of Pharmacology, Institute of Neuroscience and Physiology, Sahlgrenska Academy, University of Gothenburg, Gothenburg, Sweden).

S5.L4 HYPOTENSIVE EFFICIENCY OF EPOXYEICOSATRIENOIC ACID ANALOG (EET-A) AND 20-HETE AGONIST (AAA) IN SPONTANEOUSLY HYPERTENSIVE RATS (SHR). **I. Baranowska[1], O. Gawrys[1,2], A. Walkowska[1], Z. Huskova[2], Z. Honetschlagerova[2], J.R. Falck[3], J.D. Imig[4], L. Cervenka[2,5], E. Kompanowska-Jezierska[1]** ([1]Department of Renal and Body Fluid Physiology, M. Mossakowski Medical Research Institute, Polish Academy of Science, Warsaw, Poland; [2]Center for Experimental Medicine, Institute for Clinical and Experimental Medicine, Prague, Czech Republic; [3]Department of Biochemistry, University of Texas Southwestern Medical Center, Dallas, TX, U.S.A; [4]Department of Pharmacology and Toxicology, Medical College of Wisconsin, WI, U.S.A; [5]Department of Pathophysiology, 2nd Faculty of Medicine, Charles University, Prague, Czech Republic).

S5.L5 THE ROLE OF ADENOSINE (ADO) IN CONTROL OF RENAL HAEMODYNAMICS AND EXCRETION IN NORMO-(NG) AND HYPERGLYCAEMIC (HG) SPRAGUE DAWLEY RATS. **J.D. Sitek, A. Walkowska, M. Kuczeriszka, L. Dobrowolski** (Department of Renal and Body Fluid Physiology, Mossakowski Medical Research Institute, Polish Academy of Sciences, Warsaw, Poland).

S5.L6 DIFFERENTIAL EFFECTS OF HIGH-FAT DIETS VARYING IN FATTY ACID COMPOSITION ON THE KIDNEY HISTOLOGY AND EXPRESSION OF GENES RELATED WITH CELLULAR STRESS AND WATER-ELECTROLYTE HOMEOSTASIS. **A. Grzesiak[1], A. Dunislawska[2], M. Grabowska[3], K. Michalek[1], M. Ozgo[1], K. Liput[4], M. Pierzchala[4], A. Herosimczyk[1] A. Lepczynski[1]** ([1]West Pomeranian University of Technology, Szczecin, Poland, [2]University of Science and Technology in Bydgoszcz, Poland, [3]Pomeranian Medical University, Szczecin, Poland, [4]Institute of Genetics and Animal Biotechnology, Jastrzebiec, Poland).

Session summary

Poster session (September 17, 2021; 10:00 – 10:45; *virtual stream C*)

S5.P1 URINARY EXCRETION OF EXTRACELLULAR VESICLES IN PATIENTS IN THE EARLY PERIOD AFTER KIDNEY TRANSPLANTATION. **K. Salaga-Zaleska[1], A. Kuchta[1], B. Bzoma[2], A. Ploska[3], L. Kalinowski[3], A. Debska-Slizien[2], M. Jankowski[1]** ([1]Department of Clinical Chemistry; Medical University of Gdansk, Poland, [2]Department of Nephrology, Transplantology and Internal Diseases, Medical University of Gdansk, Poland, [3]Department of Medical Laboratory Diagnostics - Biobank Fahrenheit BBMRI.pl; Medical University of Gdansk, Poland).

S5.P2 AGING AFFECTS URINARY BLADDER REACTIVITY IN FEMALE WISTAR RATS: AN IN VIVO PREPARATION. **V. Correia[1], C. Magaldi[2], M. Moreno[2], F. Magaldi[2], B. Do Vale[1], L. Maifrino[2], M. Sato[1], E. Cafarchio[1]** ([1]Department of Morphology and Physiology, Centro Universitario FMABC, Santo Andre, SP, Brazil, [2]Universidade Sao Judas Tadeu, Sao Paulo, SP, Brazil).

S5.P3 SODIUM APPETITE IS INDUCED BY SWIMMING EXERCISE IN WISTAR RATS. **D. Vantini[1], G. Petri[2], J. Dos Santos[2], F. Fonseca[3], R. de Almeida[1], M. Sato[1]** ([1]Department of Morphology and Physiology, Centro Universitario FMABC, Santo Andre, SP, Brazil, [2]Department of Animals Care, Technical and Experimental Surgery, Centro Universitario FMABC, Santo Andre, SP, Brazil, [3]Laboratory of Clinical Analysis, Centro Universitario FMABC, Santo Andre, SP, Brazil).

S5.P4 THE ROLE OF PURINE P1 RECEPTORS IN CONTROL OF THE RENAL EXCRETION DEPENDS ON RAT'S AGE AND DURATION OF STREPTOZOTOCIN INDUCED HYPERGLYCAEMIA. **L. Dobrowolski, M. Kuczeriszka, A. Walkowska, J.D. Sitek** ([1]Department of Renal and Body Fluid Physiology, Mossakowski Medical Research Institute, Polish Academy of Sciences, Warsaw, Poland).

S5.P5 THE ROLE OF ANGIOTENSIN 1-7 IN CONTROL OF BLOOD PRESSURE, RENAL HAEMODYNAMICS, AND EXCRETION IN RATS MODEL OF STREPTOZOCIN-INDUCED DIABETES. **L. Dobrowolski, A. Walkowska, E. Kompanowska-Jezierska, J.D. Sitek, M. Kuczeriszka** ([1]Department of Renal and Body Fluid Physiology, Mossakowski Medical Research Institute, Polish Academy of Sciences, Warsaw, Poland).

S5.P6 HYPERGLICAEMIA MODULATES P1 RECEPTORS IMPACT ON THE RENAL CIRCULATION AND URINE EXCRETION BUT NOT TISSUE NO. **A. Walkowska, M Kuczeriszka, J.D Sitek, L. Dobrowolski** ([1]Department of Renal and Body Fluid Physiology, Mossakowski Medical Research Institute, Polish Academy of Sciences, Warsaw, Poland).

S5.L1

BLOCKADE OF ENDOTHELIN RECEPTOR AS AN ADJUVANT THERAPY IN THE TREATMENT OF CHRONIC KIDNEY DISEASE - STUDIES IN REN-2 TRANSGENIC RATS

I. VANECKOVA

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Chronic kidney disease (CKD) is a growing problem both in the developed and developing countries. Apart from significant morbidity and mortality and substantial decrease of quality of life, it is a growing financial burden for both the patients and societies. CKD arises as a frequent complication of diabetes, obesity and hypertension. Since it is usually unrecognized for a long time, it often progresses to the end-stage renal disease. Due to the ageing of world population, the prevalence of CKD is sharply increasing in recent decades, affecting more than 10% of the adult population. CKD is characterized by the development of progressive glomerulosclerosis, interstitial fibrosis and tubular atrophy along with a decreased glomerular filtration rate. This is associated with podocyte injury and a progressive rise in proteinuria. Although blockers of renin-angiotensin system (angiotensin converting enzyme inhibitors and angiotensin type 1 receptor blockers) are used as the "gold standard" in the treatment of CKD due to their antihypertensive and renoprotective effects, they only partially slow down the progression of CKD to end stage renal disease. Therefore, new therapeutics are urgently needed. Endothelin receptor blockers belongs to the promising drugs in this field. There are two types of G-protein coupled receptors, ET_A and ET_B – ET_A receptors located on vascular smooth muscle cells mediate vasoconstriction and cell proliferation, while ET_B receptors are located mainly on endothelial cells and mediate vasodilation, ET-1 clearance and inhibition of sodium reabsorption in the renal collecting duct, thus contributing to the regulation of sodium and water homeostasis. As endothelin-1 (ET-1) leads through the activation of ET_A receptors to renal cell injury, inflammation, fibrosis and finally to proteinuria, it is not surprising that ET_A receptor blockers were proven to have beneficial renoprotective effects in both experimental and clinical studies. Hypertensive Ren-2 transgenic rats (TGR) in combination subjected to partial nephrectomy are a good model of chronic kidney disease. Our results in heterozygous TGR rats with ablation nephropathy indicated the positive antiproteinuric effects of ET_A blockade using atrasentan as additional therapy to RAS blockade (especially in combination with diuretics).

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S5.L2

RENAL EFFECTS OF B,Γ-METHYLENE ADENOSINE TRIPHOSPHATE (ATP), P2X RECEPTOR AGONIST, IN NORMO- AND HYPERGLYCEMIC RATS

G. CHYLA, E. KREFT, M. JANKOWSKI

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Extracellular nucleotides affecting target cells through activation of purinergic receptors P2 (P2X and P2Y) play important role in control of renal hemodynamic and excretion function. This action may be modified in pathophysiological conditions, including hyperglycemia. Thus, we evaluated renal hemodynamic and tubular system sensitivity to action of β,γ-methylene ATP (β,γ-meATP), P2X receptor agonist, in diabetic rats. Clearance studies with β,γ-meATP (intravenous infusion rate 2 μmol/kg + 20 nmol/kg/min) were performed on streptozotocin-induced diabetic Wistar rats. Using laser Doppler flowmetry renal cortical and medullary blood perfusions (CBP, MBP) were measured. Results: β,γ-meATP decreased glomerular filtration rate (GFR) about 14% (1.30±0.05 vs.1.12±0.06 ml/min, $p < 0.01$) and increased CBP about 11% (580 ± 10 vs. 644 ± 16 PU, $p < 0.01$) in normoglycemic rats. However β,γ-meATP did not statistically significant effect affect GFR (0.73 ± 0.07 vs. 0.71 ± 0.08 ml/min) or CBP (570 ± 22 vs. 617 ± 22 PU) in hyperglycemic rats. MBF was not affected by β,γ-meATP in both experimental groups. In normoglycemic rats, β,γ-meATP increased diuresis about 113% (13 ± 2 vs. 29 ± 4 μl/min, $p < 0.01$) and sodium excretion in urine about 66% (1.20 ± 0.11 vs. 1.99 ± 0.21 μmol/min, $p < 0.01$) but in hyperglycemic rats the influence of β,γ-meATP was not observed. Conclusions: P2X receptors are involved in regulation of glomerular filtration, cortical blood flow and natriuresis. The lack of β,γ-meATP effects in diabetic rats suggests that hyperglycemia affecting vascular and tubular responses to agonist of P2X receptors may lead to disturbances in renal function and maintenance of homeostasis.

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S5.L3

ANGIOTENSIN II IN THE MEDIAL PREOPTIC AREA MEDIATES CARDIOVASCULAR REGULATION, BUT NOT MICTURITION CONTROL

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The medial preoptic area (mPOA) is a hypothalamic area known to participate in thermoregulatory control and blood pressure modulation, demonstrated by electrical stimulation or cobalt chloride administration, a non-selective synapse inhibitor. Retrograde labeling of mPOA by pseudorabies virus administrated in the urinary bladder has been previously shown. Immunohistochemical labeling for angiotensin II (Ang II), angiotensinogen mRNA, and AT-1 receptors have also been reported in mPOA neurons. Nevertheless, the role of Ang II in this area is still unknown. This study investigated whether Ang II acts or not in the mPOA to mediate the micturition and/or cardiovascular control. Male Wistar rats (~260 g) were submitted to implantation of a guide cannula in the mPOA 7 days prior to the experiments. On the day of the experiment, animals were anesthetized with 2% isoflurane in 100% O_2, and submitted to catheterization of the femoral artery and vein, and cannulation of the urinary bladder for mean arterial pressure (MAP), heart rate (HR) recordings, infusion of drugs, and intravesical pressure (IP) measurements, respectively. After the baseline MAP, HR and IP recordings for 15 min, Ang II (0.1 nM/µL, 1 µL) or saline (1 µL) was injected into the mPOA, and the variables were measured for additional 30 min. Data was expressed as mean ± SEM and analyzed using the Student's t-test ($P < 0.05$). Results: The injection of Ang II into the mPOA evoked a significant reduction in MAP (-50±11 mmHg, n=6, $P<0.05$) and HR (-42±26 bpm, $P<0.05$) compared to the saline injection (0 ± 0.7 mmHg and 1 ± 2 bpm, n=6). In contrast, no significant changes were observed in IP (9.12±9.68% vs. 5.65±5.65%, saline) after the injection of Ang II into the mPOA. We conclude that The Ang II in the mPOA causes hypotension and bradycardia, but does not seem to be involved in the micturition control.

Acknowledgments: FAPESP.

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S5.L4

HYPOTENSIVE EFFICIENCY OF EPOXYEICOSATRIENOIC ACID ANALOG (EET-A) AND 20-HETE AGONIST (AAA) IN SPONTANEOUSLY HYPERTENSIVE RATS

I. BARANOWSKA[1], O. GAWRYS[1,2], A. WALKOWSKA[1], Z. HUSKOVA[2], Z. HONETSCHLAGEROVA[2], J.R. FALCK[3], J.D. IMIG[4], L. CERVENKA[2,5], E. KOMPANOWSKA-JEZIERSKA[1]

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The precise pathomechanisms underlying the development of hypertension remain largely unclear and more efficient therapeutic strategies are still in a great demand. Latest studies suggest the important role of cytochrome P-450 dependent metabolites of arachidonic acid (AA) in blood pressure regulation. The epoxyeicosatrienoic acids (EETs) possess vasodilatory activity, they exhibit renoprotective and anti-inflammatory properties. In the present study the efficiency of EET-A (a stable analogue of 14,15-EET), together with AAA, a novel receptor antagonist of 20-HETE was tested. As a model we employed male spontaneously hypertensive rats (SHR) in two stages of disease development (6 and 16 week old rats). Rats were treated daily for 5 weeks with EET-A only, the combination of EET-A and AAA (administered in drinking water in the dose of 10 mg/kg/day each) and compared to age-matched untreated SHR. Systolic blood pressure (SBP) was measured by telemetry. Once a week observations in metabolic cages were performed; urine, blood and tissue samples were collected for further analysis. EET-A given alone had no significant effect on blood pressure of SHR (both young and adult). However the combined treatment with AAA + EET-A was not only effective in young rats, in which we observed significant attenuation of the disease development, but also it was significantly antihypertensive in adult animals (161 ± 5 vs. 180 ± 3 mmHg, $p < 0.05$). Additionally, combined treatment attenuated cardiac hypertrophy, decreased kidney ANG II level and increased the excretion of nitric oxide metabolites in adult rats. Taking into account all the beneficial impact of the combined treatment with EET-A and AAA on cardiovascular and renal function of adult SHR we suggest that it constitute a very promising novel antihypertensive strategy.

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THE ROLE OF ADENOSINE (ADO) IN CONTROL OF RENAL HAEMODYNAMICS AND EXCRETION IN NORMO-(NG) AND HYPERGLYCAEMIC (HG) SPRAGUE DAWLEY RATS

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The aim was to examine if the role of endogenous Ado in the regulation of kidney function in Sprague Dawley (Tac: Cmd: SD) rats depends on the duration of hyperglycemia in a pharmacological model of diabetes induced by streptozotocin (STZ). After administration of STZ (60 mg/kg i.p.) or its solvent to rats aged 6–7 weeks, in NG and HG animals short-term (14 days) or long-term (60 days) observations were conducted including blood glucose levels and body weight (b.w.) monitoring. Then, in anaesthetized (thiopental, 100 mg/kg i.p.), surgically prepared rats the effect of Ado deaminase (ADA, Ado metabolizing enzyme) on total renal blood flow (RBF, renal artery probe) and perfusion of kidney zones (laser-Doppler fluxes): upper cortex (CBF), outer- (OMBF) and inner-medulla (IMBF), along with urine (V) and sodium (UNaV) excretion was recorded. ADA infusion into the renal artery (140 U/kg b.w.) induced significant changes in NG-14 animals only: it increased CBF (5%) and decreased OMBF (9%). However, in NG-60 rats there was an increase in RBF (17%) and CBF (8%), while in HG-60 rats RBF decreased by 23%, CBF by 12%, with a transient decrease in OMBF (–8%) and IMBF (–20%). In NG-14 rats ADA administration did not change UNaV but a slight reduction in V was seen. In contrast, after 60 days' observation, ADA lowered V both in NG and HG rats (–20% and –25%, respectively), but it lowered UNaV in HG only (–34%). Thus, both the age of the animals (younger: 14 days' vs. older: 60 days' observation) and the duration of hyperglycemia influenced the effects of endogenous Ado on the regulation of renal function. In younger rats, irrespective of the glycemia level, the effects of Ado on renal blood perfusion and renal excretory function is slight and transient. On the other hand, in older rats, Ado modifies the perfusion of the kidney cortex; however the direction of changes depends on the level of glycaemia. The effect of Ado on the regulation of medullary circulation and sodium excretion was demonstrated only in the animals with hyperglycemia.

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DIFFERENTIAL EFFECTS OF HIGH-FAT DIETS VARYING IN FATTY ACID COMPOSITION ON THE KIDNEY HISTOLOGY AND EXPRESSION OF GENES RELATED WITH CELLULAR STRESS AND WATER-ELECTROLYTE HOMEOSTASIS

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Development of obesity is closely associated with the consumption of high-calorie diets. One of the diets considered as obesogenic is a western-type diet. It is characterized by a high content of saturated fatty acids (SFA) and a low level of omega-3 PUFA, often accompanied by an imbalance in the n-6/n-3 PUFA ratio. An overconsumption of the n-6 PUFA and SFA is strongly related with pathogenesis of many modern diet-related chronic diseases including chronic kidney disease (CKD). The high-fat diet consumption may lead to renal metabolic disorders and concomitantly may enhance both oxidative stress and inflammation. The above is a hallmark of apoptosis and a leading cause of the structural changes in the kidney microarchitecture. On the other hand n-3 PUFA consumption reduces the prevalence of CKD. Considering the above, we hypothesized that feeding mice with three different high-fat diets with a saturated fatty acids and a significant proportion of different ratios of unsaturated n-6 and n-3 FA would differentially impact the kidney histological structure and expression pattern of the selected genes. The analysis were performed on 24, two-month-old, Swiss-Webster mice. Animals were divided into 4 dietary groups (n=6) and for 12 weeks were fed the following diets: the standard diet (STD group), the high-fat diet (HFD) rich in SFAs (SFA group), and HFDs dominated by PUFAs with linoleic acid to α-linolenic acid ratios 14:1 (14:1 group) and 5:1 (5:1 group). After the experimental period animals were euthanized using CO_2, than kidney were collected for further analysis. Histological analysis included (H&E, PAS, trichrome and TUNEL staining and IHC labeling of aquaporins – AQPs 2, 3).RT qPCR was used to assess the expression pattern of genes related with inflammation (*Kim-1, Ccl2, Il-6*), oxidative stress (*Sod1, Cat*), PUFA metabolism (*Cox2, Lox5, Cyp2c29, Lepr*) and water-electrolyte homeostasis (Ang, Ren, Aqp3). Morphological analysis of the kidney revealed the lipid vacuoles in the proximal tubules in the kidney of mice from SFA and 14:1 groups. These morphological changes were accompanied by an enhanced expression of *Kim-1* gene which is known as a marker of the proximal tubule epithelial cells injury. The same tendency was observed for apoptotic cell count measured by TUNEL assay which was significantly higher in the kidney of animals from SFA and 14:1 groups. The oxidative stress-related genes such as Sod1 and Cat were significantly up-regulated in the 14:1 group. However, in the light of the fact that lipotoxicity may decrease the activity of an antioxidative enzymes we were suspecting significant ROS generation in the group of mice fed the SFA diet. Expression of *Cyp2c29* gene that encodes the protein involved in the synthesis of active renoprotective metabolites of both n-6 and n-3 PUFA, respectively epoxyeicosatrienoic acids and epoxydocosapentaenoic acids was significantly upregulated in the kidney of animals fed all HFDs. Conversion of aforementioned eicosanoids to their less active forms is catalyzed by a soluble epoxide hydrolase expression. The *Ephx2* gene encoding this protein was significantly up-regulated in the kidney of animals fed both PUFA rich HFDs. Cox2 gene was significantly down-regulated in the kidney of 4:1 group in comparison to the STD group. Both PUFA rich HFDs influenced local renin angiotensin system-related genes. The animals of 4:1 group showed significantly decreased renin gene expression. The angiotensinogen gene was significantly down-regulated in the kidney of animals of both 14:1 and 5:1 groups. Expression of *Aqp3* gene was significantly increased in the both SFA and 14:1 group, however the ICH analysis showed decrease of both AQP2 and AQP3 protein expression in the respectively apical and basolateral membrane of collecting duct epithelial cells in animals fed the SFA rich HFD. The high fat diets varying in fatty acids composition differentially influenced kidney morphology. The severity of observed pathophysiological lesions seems to show trend to decrease with increased content of alpha linoleic acid in the HFD. Observed morphological changes are probably related with the generation of the oxidative stress. It seems that the SFA-based HFD may affect the facultative water reabsorption in the collecting duct as an effect of AQP2 and 3 down-regulation. On the other hand n-3 PUFA may be involved in the regulation of local renal RAA system. Above may suggest that the westernized diet with a high SFA and/or n-6 PUFA content may exert a negative impact on renal morphology and function.

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S5.P1

URINARY EXCRETION OF EXTRACELLULAR VESICLES IN PATIENTS IN THE EARLY PERIOD AFTER KIDNEY TRANSPLANTATION

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Extracellular vesicles have a size about 30–300 nm and can be found in various body fluids, including urine. Increased excretion of extracellular vesicles may have a diagnostic value in the initial early stages of renal dysfunction. The aim of the study was to investigate the urinary excretion of extracellular vesicles in patients in the early period after kidney transplantation. The study was approved by the Medical Ethics Committee of Medical University of Gdansk and the director of University Clinical Center in Gdansk. The preliminary study involved adult Patients from the Department of Nephrology, Transplantology and Internal Diseases at the University Clinical Centre in Gdansk, Poland, in the early period after kidney transplantation (1–4 weeks, n=3) and healthy volunteers (n=3). Urinary extracellular vesicles were isolated by ultracentrifugation-based method from the same volume of each first morning urine sample. The expressions of the specific marker CD63, nephrin and podocin were verificated by Western blot. The total number of extracellular vesicles per milligram of creatinine was determined by nanoparticle tracking analysis (NTA). All values are expressed as mean ± SEM. Statistical significance between the two group was determined by unpaired t test. Human urinary extracellular vesicles were identified by showing the expression of the exosomal marker CD63 in all samples. In early period after kidney transplantation the urinary excretion of extracellular vesicles was 1.5-fold higher compared to healthy volunteers ($2.6*10^{10} \pm 4.4*10^{9}$ vs. $3.9*10^{10} \pm 5.0*10^{9}$ particles/mg creatinine, p=0.1218), however, this difference was not statistically significant. The presence of nephrin and podocin were confirmed on isolated extracellular vesicles. The research is preliminary and prompted us to further analyze of urinary excretion of extracellular vesicles proteins in a larger group of patients, looking for early markers of renal graft function.

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S5.P2

AGING AFFECTS URINARY BLADDER REACTIVITY IN FEMALE WISTAR RATS: AN IN VITRO PREPARATION

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Urinary bladder diseases affect mostly women worldwide and the number of patients with bladder disorders increases with aging. This study investigated the urinary bladder reactivity to the neurotransmitters of the autonomic nervous system in elderly female rats. Female nulliparous Wistar rats with 8, 15 and 18 months-old were anesthetized with 2% isoflurane in 100% O_2 and submitted to the catheterization of the femoral artery and vein and cannulation of the urinary bladder for mean arterial pressure (MAP), heart rate (HR), and intravesical pressure (IP) recordings, respectively, in a data acquisition system (PowerLab 16 SP, ADInstruments). After a baseline recording of MAP, HR and IP, acetylcholine (Ach, 2.0 µg/mL, 0.1 mL), or noradrenaline (Nor, 2.0 µg/mL, 0.1 mL), or saline (vehicle, 0.1 mL) were topically(*in situ*) administrated onto the urinary bladder, and all the parameters were recorded for additional 15 min. Data are expressed as mean ± SEM and were submitted to paired Student's t-test or One-way ANOVA ($P < 0.05$). The rats with 8, 15 and 18 months-old (n=6/group) showed significant increases in IP after Ach (500.00 ± 22.81%, 174.55 ± 20.88%, and 83.01 ± 0.80%, respectively) and significant decreases in IP (–67 ± 12%, –27.83 ± 12.69%, and –31.41 ± 0.28%, respectively) compared to saline (–0.97 ± 2.54%, 3.71 ± 0.68%, and –1.69 ± 1.97%, respectively). The IP responses to Ach were significantly attenuated in 15 and 18 months-old compared to 8 months-old female rats. No changes were observed in MAP and HR after Ach or Nor or saline *in situ* onto the urinary bladder in all groups. The findings suggest that aging reduces the urinary bladder reactivity to Ach and Nor, the neurotransmitters of the autonomic nervous system, and the reactivity to Ach was the most affected by elderly.

Acknowledgments: FAPESP and NEPAS.

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S5.P3

SODIUM APPETITE IS INDUCED BY SWIMMING EXERCISE IN WISTAR RATS

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The loss of water and sodium evoked by physical exercise has been described in several studies. This study investigated if chronic swimming exercise induces sodium appetite in rats. Adult male Wistar rats were submitted to swimming exercise (SE) or maintained sedentary (SED) after a gradual adaptation period to the individual tanks with warmed water. Afterwards, the animals underwent daily bouts of exercise, with 1 h of duration, 5 consecutive days/week, for 6 weeks with 5% of body weight (b.w.) load. At the end of the 6 weeks of swimming, the animals were left in a recovery period for 3 weeks. Daily water and 0.3 M NaCl intakes were measured using drinking bottles, in SE and SED rats (n=6/group). Another group of animals underwent the same SE and SED (n=6/group) procedures, but they only had access to water in the drinking bottle. Urine sodium and potassium were analyzed at the adaptation period, after 3 and 6 weeks of exercise, and after 3 weeks of recovery from the exercise bouts in all groups. Plasma sodium and potassium were evaluated in blood samples of all animals at the end of the experiment protocol. Data area as mean ± SEM and submitted to two-way ANOVA followed by Tukey post-test ($P < 0.05$). We observed a significant higher sodium intake in SE rats compared to SED rats was observed after 2 weeks (4.6 ± 0.7 vs. 1.1 ± 0.4 mL/100 g of b.w.), 4 weeks (5.7 ± 0.7 vs. 1.8 ± 0.3 mL/100 g of b.w.), 5 weeks (6.3 ± 1.0 vs. 2.0 ± 0.3 mL/100 g of b.w.) and during the 2 weeks of recovery from SE bouts, without corresponding increase in water intake. No difference was observed in water intake in rats without 0.3 M NaCl comparing SE and SED groups. Urine and plasma sodium showed no difference in all groups. Urine potassium only reduced after 6 weeks of SE in rats with access to 0.3 NaCl (189.4 ± 22.9 mEq/L) compared to SED animals (260.5 ± 9.6 mEq/L). Our data demonstrated that SE with 5% b.w. load for 6 wks increases sodium appetite in rats, which remained higher even after the cessation of the exercise bouts, suggesting that long-term mechanisms activated by this approach of exercise can mediate the change in this ingestive behavior.

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S5.P4

THE ROLE OF PURINE P1 RECEPTORS IN CONTROL OF THE RENAL EXCRETION DEPENDS ON RAT'S AGE AND DURATION OF STREPTOZOTOCIN-INDUCED HYPERGLYCAEMIA

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The impact of purine P1 receptors (P1R) on renal function is altered under pathological conditions, e.g. in diabetes mellitus (DM). Recently, we found that the P1R role depends on the animals' age, however, this was studied only in normoglycaemic (NG) animals subjected to the treatment with theophylline (Theo), a nonselective P1R antagonist. Here, we examined the effects of Theo and CSC (8-(3-chlorostyryl)caffeine, a selective antagonist of A2a type of P1R) on renal excretion during short- (2-wks, DM-2) or long-term (8-wks, DM-8) streptozotocin (STZ, 60 mg/kg i.p.) induced hyperglycaemia and compared the effects with those in NG age-matched male Sprague Dawley rats. In NG and DM anaesthetized rats (thiopental, 100 mg/kg i.p.), we measured urine flow (V), urine osmolality (Uosm), total solute (UosmV), sodium (UNaV) and potassium excretion (UKV). Theo infusion (14 mg/kg/h i.v.) induced a transient elevation of V, UosmV, UNaV; the slight decrease of Uosm did not differ between the NG two age groups and DM-14 or DM-60 rats. Remarkably, a long-lasting increase of Uosm after cessation of Theo infusion was shown in the NG older rats only. CSC given into the renal artery (1.7 µmol/kg/h) did not affect renal excretion in either NG age group. The exception was a twofold UNaV increase noted after cessation of CSC infusion in older rats. However, in diabetic rats, a distinct decrease of V, UosmV, UNaV, UKV was shown in DM-14 group only; in DM-60 rats an increase in UKV was seen. Neither antagonist affected the arterial blood pressure, which suggests that the effects on RE depended mainly on the drug's direct action on the tubular transport. Our data show that short-term hyperglycaemia does not modify the joint effect of all P1R on renal excretion but can alter the contribution of individual subtypes, such as P1A2aR. On the other hand, long-term diabetes blunts the P1R impact on sodium excretion and urine concentration and abolishes P1RA2a contribution to the control of renal excretory function.

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S5.P5

THE ROLE OF ANGIOTENSIN 1-7 IN CONTROL OF BLOOD PRESSURE, RENAL HAEMODYNAMICS, AND EXCRETION IN RATS MODEL OF STREPTOZOTOCIN-INDUCED DIABETES

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We examined if angiotensin 1-7 (Ang1-7) could modulate blood pressure and renal function in animal model of diabetes (DM). In Sprague Dawley rats 3wks after STZ (60 mg/kg i.p.) or solvent injections (normoglycaemic control, NG), osmotic minipumps with Ang1-7 (400 ng/kg/min, s.c.) or 0.9% NaCl were implanted for 3 weeks. Along with the 5 wks water intake, diuresis and systolic blood pressure (SBP, tail-cuff method) were observed; thereafter in thiopental anaesthetized rats we recorded: mean arterial blood pressure (MABP), whole kidney blood flow (RBF) and perfusion of renal zones (laser-Doppler fluxes): cortex (CBF), outer - (OMBF) and inner- (IMBF) medulla also with urine flow (V), total solutes (UosmV) and sodium (UNaV) excretion. Regardless of the Ang1-7 treatment, a significant increase in water intake was shown in DM rats. However, this was associated with elevation of diuresis only in DM+Ang1-7 rats which was more pronounced than in NG+Ang1-7 rats. There was a significantly higher SBP in NG+Ang1-7 than in NG rats, while no difference in SBP was observed in DM rats after the addition of Ang1-7. MABP, RBF, CBF and OMBF did not differ among groups. Regardless of Ang1-7 treatment, IMBF was significantly higher in NG than in DM rats. V was significantly decreased by Ang1-7 in NG but increased in DM rats. Also, UNaV was lowered by Ang1-7 in NG rats. Only UosmV differs between NG and DM rats, it was higher in the latter and in addition increased in Ang1-7. In summary, data from chronic studies indicate that Ang1-7 contributes to blood pressure control in NG rats only while in DM rats could modulate water intake and urine excretion. Data from acute studies suggest that angiotensin 1-7 rather not contribute to systemic (no MABP changes) and renal haemodynamics both in NG and DM. However, independent of/from glycaemia Ang1-7 can modify tubular water and solutes reabsorption but in the opposite way, to improve in normo- and to impair in hyperglycaemia. This could depend on the selective affinity of Ang1-7 to the AT-1 receptors, which density is simultaneously increased in diabetes.

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S5.P6

HYPERGLICAEMIA MODULATES P1 RECEPTORS IMPACT ON THE RENAL CIRCULATION AND URINE EXCRETION BUT NOT TISSUE NITRIC OXIDE

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Chronic hyperglycaemia could affect in the kidney paracrine factors like adenosine (Ado), their P1 receptor (P1R) density and nitric oxide (NO) bioavailability. The impact of these modifications on renal function is unclear. Ado can induce either vasoconstriction or vasodilatation, depending on the prevailing stimulation of A1 or A2 receptors (A1R, A2R). Ado-induced renal excretion alterations may be secondary to haemodynamic changes, or reflect a direct influence on tubular transport by P1R located along the nephron; both could be mediated by fluctuated intrarenal NO activity. We compared the effects of an antagonist of A1R and A2R (theophylline, Theo) in anaesthetized normoglycaemic rats (NG) or after two weeks hyperglycaemia induced by streptozotocin, (DM). Whole kidney blood flow (RBF) and perfusion of the outer and inner medulla (OMBF, IMBF, respectively) were measured, together with renal excretion of water (V), total solute ($U_{osm}V$), and sodium ($U_{Na}V$), and measurement of cortical and medullary tissue NO changes using selective microprobes. *I.V*. Theo did not affect systemic blood pressure. However, renal haemodynamics and excretion were modified differently in NG and DM rats. RBF transiently increased in NG (10%), significantly in contrast to a gradual decrease in DM rats (18%). Simultaneously, a slight OMBF decrease without IMBF alteration were shown in both groups. Interestingly, the renal tissue NO signal increased similarly (13–15%) in the cortex and medulla, independent of glycaemia. Theo-induced renal excretion changes were uniform, which indicates they were independent of haemodynamics alterations. Notably, increases of V, $U_{osm}V$ were greater in NG than DM rats (236% and 178% vs. 76% and 51%, respectively), while $U_{Na}V$ increase did not differ between both groups (223 vs.193%, respectively). Thus, in DM, in opposite to NG, within the cortex A2R prevails over A1R effects, reflected by RP1 antagonist vasoconstriction, whereas within medulla a balance between receptors persisted. A1R/A2R impact on tubular transport of water and solutes (but not sodium) were also modified by hyperglycaemia. However, these alterations of P1R action on renal haemodynamics and excretion seem to not be dependent on *NO* bioavailability.

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