HYPERTENSION - EXPERIMENTAL MODELS

MP084 GONADECTOMY PREVENTS THE INCREASE IN BLOOD PRESSURE AND SERUM ACE ACTIVITY IN ACE2 KNOCKOUT DIABETIC MALE MICE

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Introduction and Aims: Whereas ACE2 deletion worsens kidney injury; its amplification ameliorates diabetic nephropathy. We previously showed that circulating ACE2 activity is increased in male diabetic NOD mice. The effect of gonadectomy in diabetic ACE2 knockout (ACE2KO) male mice has not been previously studied.

Methods: We study the effect of ACE2 deletion on systolic blood pressure (SBP), urinary albumin excretion (UAE), kidney to body weight ratio (KW/BW) and serum (s) and kidney (k) ACE enzymatic activity in C57Bl/6 streptozotocin (STZ)-induced male mice and their respective controls. We also evaluated the effect of gonadectomy in diabetic ACE2KO mice. Mice were followed-up for 19 weeks after induction of diabetes with STZ injection. Citrate was administered as a vehicle (cont). Study groups: ACE2KO-cont, ACE2KO-STZ, gonadectomy before diabetes induction GDX-ACE2KO-STZ.

Results: Hyperglycemia was observed in all groups given STZ. KW/BW and UAE were increased in both diabetic wildtype (WT) (UAE 12-fold) and ACE2KO mice (UAE 27-fold), ACE2KO diabetic mice had increased SBP compared to diabetic WT. In addition, gonadotomized diabetic ACE2KO showed significantly lower values of blood glucose, SBP, UAE, KW/BW compared to non-gonadotomized diabetic ACE2KO. Circulating ACE activity positively correlated with SBP (r=0.28; p=0.04) and was significantly increased in WT diabetic mice compared with WT-cont. Circulating ACE activity was increased in ACE2KO control mice as compared to WT control mice. Gonadectomy significantly decreased circulating ACE activity in diabetic ACE2KO mice. In contrast, renal ACE activity was significantly reduced in diabetic ACE2KO and WT animals.

Conclusions: In ACE2KO mice circulating ACE activity was increased as compared to WT mice. In addition, in diabetic ACE2KO mice SBP was increased compared to diabetic WT mice. Gonadectomy reduced blood glucose, UAE, renal hypertrophy, blood pressure and circulating ACE activity.

MYOCARDIAL AND RENAL REMODELING IN MALE WISTAR RATS RECEIVING HIGH SALT DIET

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Introduction and Aims: Myocardium, kidney and vasculature, in particular, reacts to changes in dietary NaCl intake through a complex series of events that are independent of blood pressure. The aim of this study was to compare the effect of normal and high NaCl content in the diet on the remodeling of the heart and kidney, and the NFκB expression in the myocardium in rats.

Methods: The study was performed in male Wistar rats. Control group (C) of animals (n=8) received normal NaCl intake (0.34%), experimental (E; n=8) – high (8%). Experimental period was 8 weeks. Mean BP was measured in awaked rats by tail cuff method. Serum urea (Ur), creatinine (Cr), total calcium and sodium levels were determined. Daily volume of urine and concentration of sodium in the urine was also determined. The degree of left ventricular hypertrophy was estimated as a ratio: left ventricular mass/body mass (LVH; mg/g). The degree of left (LKH) and right (RKH) kidneys hypertrophy was estimated as a ratio: kidney mass/body mass, mg/g).

Results: Consumption during the 2 months of a diet high in NaCl, without causing a rise in BP in Wistar rats leads to an increase in mass of the kidneys and the activation of NFκB in the myocardium, which may be one of the ways of myocardial remodeling and fibrosis.

MP086 EFFECTS OF CHYMOSTATIN, A CHYMASE INHIBITOR, ON BLOOD PRESSURE AND KIDNEY HAEMODYNAMICS IN DIFFERENT MODELS OF HYPERTENSION IN THE RAT

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Introduction and Aims: Chymase is known to form angiotensin II in cardiovascular and renal tissues independent of angiotensin-converting enzyme (ACE), and its expression is increased in pathological conditions. It was proposed that chymase blockade with a significant decrease in blood pressure (154±7 mmHg) compared between control and experimental myocardium with the use of 2'- method.

Results: High salt intake does not lead significant rise (mean±SE) of BP (135.5±5 mmHg) compared between control and experimental myocardium with the use of 2'- method.

Conclusions: Consumption during the 2 months of a diet high in NaCl, without causing a rise in BP in Wistar rats leads to an increase in mass of the kidneys and the activation of NFκB in the myocardium, which may be one of the ways of myocardial remodeling and fibrosis.

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in control period) and in RBF (4.8±0.9 vs 6.1±0.9 ml/min in control period). Chymase inhibition caused a significant decrease in OMBF in all groups with one exception. In unilaterally nephrectomised rats on high sodium diet OMBF remained unchanged but there was a significant decrease in IMBF (139±32 vs 79±36 PU in control period). Effects of chymostatin infusion persisted or were even enhanced after discontinuation of the infusion.

Conclusions: The degree of MAP reduction after chymostatin was found to depend on the model of hypertension. The greatest decrease was observed in SHR rats aged 16 weeks, which suggests an important functional role of the alternative, ACE-independent pathway of the tissue RAS system in the stage of established genetically determined hypertension. The decrease in MAP is probably responsible for the observed decrease in renal perfusion. It appears that in the early stage of development of hypertension (7-weeks SHR) the role of the systemic RAS remains crucial.

**MP087**

**EFFECTS OF SPECIFIC INHIBITION OF CHYMASE ON RENAL EXCRETION IN DIFFERENT RAT MODELS OF HYPERTENSION**

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**Introduction and Aims:** Chymase is known as a tissue angiotensin II-generating enzyme engaged in the control of the cardiovascular system. Since the ACE-independent pathway of angiotensin II synthesis is known to be active in pathological conditions, chymase inhibitors could be useful in prevention of cardiovascular diseases. To explore this possibility, we examined effects of chymostatin, a commercially available chymase blocker, in different forms of experimental hypertension: dependent on renal artery stenosis, genetically determined and sodium-dependent.

**Methods:** Male spontaneously hypertensive rats (SHR) in the early (age: 7-weeks) and established stage of hypertension (16 weeks), male Sprague-Dawley rats with hypertension induced by renal artery stenosis (two kidney, one-clip model, 2K,1C), and Sprague-Dawley rats with hypertension induced by unilateral nephrectomy followed by two-weeks' high sodium diet (4% Na w/w) were used. In acute experiments all rats were anaesthetised with sodium thiopental, 100 mg/kg i.p. Chymostatin (dissolved in 0.05% DMSO) or its solvent was infused intravenously at 2 μg/kg/h, for one hour. Timed urine collections were made and blood was sampled to determine renal excretion, glomerular filtration rate (GFR,ulin clearance), and plasma osmolality (Posm), sodium (PNa) and potassium (PK) concentration. Urine volume was determined gravimetrically. To correct for major inter-group differences in kidney size, the values of diuresis and sodium excretion were expressed per g kidney weight (V/g, PNa/g).

**Results:** After administration of chymostatin, the diuresis and sodium excretion slightly decreased in all groups. Only in 16-week SHR there was a significant (17%) decrease in V/g (1.82±0.3 vs 1.42±0.56 μl/min/g in control period; P<0.05) and a 76% drop in UNa/Vg (0.10±0.03 vs 0.02±0.04 μmol/min/g in control period; P<0.05). In 16-week-old SHR there was also significant decrease in GFR (0.41±0.06 vs 0.61±0.09 ml/min in control period; P<0.05). In the other groups chymostatin caused only a slight decrease in MAP (144±3 vs 139±2 μmol/l in control period; P>0.05).

**Conclusions:** Remarkably, chymase blocking effects were increasing progressively over time, becoming statistically significant only in the 16-week-old SHR. After the 2nd and 3rd dose (3.7±0.5 with 3rd dose vs 8.4±1.0 μmol/l in CP; P<0.001), this was followed by a return to control value after discontinuation of infusion. In conclusion, P2R-Y play an important role in modulation of blood perfusion of the outer-zone despite changes in arterial blood pressure. In the tubules they might stimulate water and solute transport independent of renal haemodynamics.

**MP088**

**EFFECTS OF ADENOSINE DIPHOSPHATE, A PURINORECEPTOR AGONIST, ON BLOOD PRESSURE AND RENAL FUNCTION IN THE RAT**

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**Introduction and Aims:** P2 purinergic receptors (P2R) are expressed in kidney vessels and tubules, however, the functional role of P2R-Y, one of two P2R families, in physiological and pathological states, remains unclear. The available information on the impact of P2R-Y on renal functions based mainly on studies of isolated preparations. The present whole-kidney study explored renal haemodynamics and excretion as affected by adenosine diphosphate (ADP), a non-selective agonist of P2R-Y.

**Methods:** In adult male Sprague-Dawley rats anaesthetized with sodium thiopental, 100 mg/kg, effects of ADP or its subcutaneous injection on mean arterial pressure (MAP), heart rate (HR), renal haemodynamics and excretion were measured simultaneously. After control period (CP), three subsequent doses of ADP (2, 4, 8 mg/kg) were infused iv., followed by recovery period. The whole-kidney blood flow (RBF) was determined using left renal Transonic probe. Intrarenal regional blood perfusion was determined using laser-Doppler probes placed on the kidney surface (superficial cortex, CBF) or inserted into the outer (OMBF) and inner-medulla (IMBF). Urine flow (V), sodium and potassium excretion (UNa/Vg, UP/Vg), and total solute excretion (Uni/Vg) were measured and expressed per g kidney.

**Results:** In time control group no significant changes were shown. ADP induced a dose-dependent decrease of MAP, to 95±3 with the 3rd dose vs 111±1 mmHg in CP (P<0.001) and a concurrent increase of HR (394±8 vs 345±9 beats/min in CP; P<0.05). RBF increased with the lowest dose and remained elevated throughout ADP infusion (9.3±0.8 with the 3rd dose vs 8.3±0.6 ml/min in CP; P<0.02). After cessation of drug infusion MAP and RBF returned rapidly to the control value. CBF increased 10% with the lowest dose of ADP (P<0.04) and declined slightly after the second dose, down to the value not different from control. OMBF was not affected by ADP but in recovery period it decreased 10% (P<0.01), whereas IMBF remained stable throughout the experiment, similarly as in the control group. A small but persistent drop in V was induced by the highest dose of ADP (5.4±0.6 vs 5.7±1.7 μl/min in CP; P<0.02) associated with a slightly lower decrease in GFR (0.11±0.1 vs 0.9±0.3 μmol/min in CP; P<0.02), however the latter change was transient. Interestingly, ADP caused a significant dose-dependent decrease in UNa/Vg after the 2nd and 3rd dose (7.0±0.5 with 3rd dose vs 8.4±1.0 μmol/l in CP; P<0.001), this was followed by a return to control value after discontinuation of infusion.

**Conclusions:** In conclusion, P2R-Y play an important role in modulation of blood perfusion of the deep cortex (measured as RBF). Within the medulla, their activity could help stabilize perfusion of the outer zone despite changes in arterial blood pressure. In the tubules they might stimulate water and solute transport independent of renal haemodynamics.

**MP089**

**BIPHALIN, A NON-ADDICTIVE SYNTHETIC OPIOID, IS HYPOTENSIVE AND IMPROVES RENAL PERFUSION IN ANAESTHETISED SPONTANEOUSLY HYPERTENSIVE RAT**

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**Introduction and Aims:** Neurogenic factors contribute to the development of arterial hypertension, and activation of opioid receptors can modulate the central nervous system signals to the cardiovascular system, and thereby alter arterial pressure. Biphallin is an enkephalin related dipeptide (synthesized by A.W. Lipkowski), showing affinity for opioid μ, δ, and κ receptors. Biphallin is induces only minimal physical dependence (addiction) when compared with morphine. The preliminary studies suggested biphallin's hypotensive activity. Therefore, in spontaneously hypertensive rats, we examined the impact of biphallin on blood pressure response, the transient renal blood perfusion was determined by the highest dose of ADP (5.4±0.6 vs 5.7±1.7 μl/min in CP; P<0.02) associated with a slightly lower decrease in GFR (0.11±0.1 vs 0.9±0.3 μmol/min in CP; P<0.02), however the latter change was transient. Interestingly, ADP caused a significant dose-dependent decrease in UNa/Vg after the 2nd and 3rd dose (7.0±0.5 with 3rd dose vs 8.4±1.0 μmol/l in CP; P<0.001), this was followed by a return to control value after discontinuation of infusion.

**Methods:** In conclusion, P2R-Y play an important role in modulation of blood perfusion of the deep cortex (measured as RBF). Within the medulla, their activity could help stabilize perfusion of the outer zone despite changes in arterial blood pressure. In the tubules they might stimulate water and solute transport independent of renal haemodynamics.

**Conclusions:** The renal vascular resistance (RVR) and hind limb vascular resistance (IVR) were calculated.

**Results:** 1. Baseline values in SHR vs S-D rats. Elevated MAP in SHR (169±3 mmHg vs 121±1 mmHg in S-D rats) was associated with distinctly decreased HR values (375±5 vs. 406±5 beats/min). Perfusion of the kidney (RBF) and hind limb (IBF) was lower in SHR vs S-D rats but the difference was much higher for S-D rats. Therefore, hypertension was associated with about 26-fold greater RVR and 36-fold greater IVR vascular resistance. 2. Biphallin effects on systemic haemodynamics. In SHR biphallin modestly but significantly decreased MAP (-4.5%) and HR (-5%). In S-D rats MAP actually increased significantly (5%) while HR did not change. 3. Effects on renal perfusion. Biphallin significantly increased RBF (+8.2%) but not IBF in SHR, no perfusion changes were observed in S-D rats. In SHR, RVR decreased slightly and in S-D it increased slightly (both changes significant). No major changes in IVR were seen in either rat group.

**Conclusions:** In anaesthetized SHR, a hypertension model with pronounced renal haemodynamics and excretion, induced by bilateral nephrectomy, had a higher blood pressure than the S-D rats. In both models, biphallin decreased blood pressure and renal vascular resistance. The renal resistance vessel (RVR) and hind limb vascular resistance (IVR) were calculated.
output. A concurrent substantial increase in RBF indicates that in spontaneously hypertensive rats this non-addictive opioid improves renal circulation.

**Methods:** We previously reported that ATP2B1 was one of the genes for hypertension receptivity in a large-scale Japanese population, which has been confirmed recently in Europeans, Koreans and Japanese. ATP2B1 encodes the plasma membrane Ca(2+)-ATPase isoform 1, which plays a critical role in intracellular calcium homeostasis. In addition, it is suggested that ATP2B1 plays a major role in vascular smooth muscle contraction. Furthermore, it is suggested that ATP2B1 is associated with salt sensitivity. Since the ATP2B1 knockout mouse is embryo-lethal, we generated mice with vascular smooth muscle cell specific knockout of ATP2B1 using the Cre-loxP system, in order to identify the relationship among ATP2B1 and hypertension and salt sensitivity.

**Results:** The knockout mice expressed significantly lower levels of ATP2B1 mRNA and protein in the aorta compared to control mice. Knockout mice showed significantly higher systolic blood pressure as measured by tail cuff method and radiotelemetry methods measuring for 24 hours. Moreover, femoral artery isolated from knockout mice showed significant elevated contractile response to phenylephrine. Similarly, primary cultured vascular smooth muscle cells isolated from the aorta of knockout mice showed significant higher phenylephrine mediated increase of intracellular calcium concentration. This finding was associated with the decreased expressions of ATP2B1 in knockout mice compared to control mice. On the other hand, the knockout mice showed up-regulation of ATP2B4, which is the paracrine form of ATP2B1, in the clipped kidney. In the vasculature, ATP2B1 expression is significantly downregulated. Moreover, knockout mice showed significant blood pressure elevation with hypercalcuria while high salt loading. In contrast, knockin mice showed significantly lower blood pressure levels, higher systolic blood pressure, and improved renal phenotype compared to control mice.

**Conclusions:** These results suggest that knockout mice are more susceptible to salt-induced hypertension, while knockin mice are protected from salt-sensitive hypertension. This study provides new insights into the role of ATP2B1 in the pathogenesis of hypertension and salt sensitivity.
and Tukey post test. **P<0.001.

Conclusions: paricalcitol lowers blood pressure increase driven by a high salt diet in this experimental model in association with reduced inflammation and oxidative stress.

MP094 ALTERATIONS OF RENAL SODIUM TRANSPORTERS IN THE ERYTHROPOIETIN-INDUCED CHRONIC RENAL FAILURE RAT

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Introduction and Aims: Erythropoietin (EPO) administration in urmic rats leads to an increase in blood pressure. The present study was designed to investigate the change of sodium balance and alteration of major renal sodium transporters in EPO-treated chronic renal failure rat.

Methods: Renal failure was induced by a two-stage 5/6 nephrectomy in 30 Sprague-Dawley rats. Uremic rats were divided into two groups and received vehicle and EPO (150 U/kg, intraperitoneal injection, x2/week) for 4 weeks. Half of the rats were sacrificed after 1 week of treatment, and the rest after 4 weeks.

Results: Serum creatinine and sodium level, hematocrit, body weight, and systolic blood pressure were similar in both groups before treatment. After 1 week of treatment, hematocrit increased in the EPO group (48.9 ± 1.0% vs. 38.4 ± 1.0%, P = 0.001). Systolic blood pressure (SBP) was 153.5 ± 6.8 mmHg in the EPO group and 147.3 ± 4.7 mmHg in control group (P = 0.041) after 1 week. After 4 weeks treatment, SBP increased significantly (159 ± 3.3 vs. 146.4 ± 2.3 mmHg, P = 0.007). Urinary sodium excretion and daily sodium balance did not show significant difference between groups throughout the experiment period. Expression of ENaC α decreased significantly (58.6 ± 5.1% of the control, P = 0.001) after 1 week of EPO treatment on immunoblot analysis. After 4 weeks of treatment, the renal abundances of ENaC α, γ, and NHE3 significantly decreased (56.1 ± 6.1, 49.4 ± 9.7%, and 38.6 ± 9.4% of the control, P = 0.011, 0.026, and 0.007, respectively) in the EPO group. Renal medullary endothelin levels of EPO-treated group increased significantly (10.2 ± 2.9 pg/mg vs. 5.2 ± 0.8 pg/mg, P = 0.028, at the 1st week; 3.6 ± 0.8 pg/mg vs. 1.5 ± 0.4 pg/mg, P = 0.035, at the 4th week) than that of control group. Plasma renin and serum aldosterone levels were not different between groups.

Conclusions: EPO increases renal medullary endothelin-1, and inhibits renal ENaC and NHE3 expression. Increased production of renal medullary endothelin-1 and decreased expression of renal sodium transporters might work as compensatory mechanisms in EPO-treated hypertensive chronic renal failure model.

MP095 EFFECT OF LISINOPRIL AND A COMBINATION OF LISINOPRIL WITH THE DIURETIC HYDROCHLOROTHIAZIDE ON KIDNEY FUNCTION AFTER UNILATERAL NEPHRECTOMY IN DAHL SALT RATS WITH ESTABLISHED HYPERTENSION

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Introduction and Aims: Dahl Salt Sensitive Rats develop hypertension and renal injury under high salt diet. Aim of the present study was to investigate the effect of unilateral nephrectomy (UNx) in Dahl SS rats pre-fed for 2 weeks with 8% NaCl diet on the progression of kidney disease with established hypertension and the effect of Lisinopril and the combination with hydrochlorothiazide (HCTZ).

Methods: Kidney function was assessed by GFR, creatinine (CREA) clearance and plasma Cystatin C. GFR was measured using plasma clearance kinetics of fluorescent isothiocyanate inulin following a single bolus iv injection. Cystatin C was analyzed by Luminex Millipore assay. Albumin was measured by ELISA and CREA was quantified by COBAS System. Blood pressure measurement was done by tail cuff method (Kent CODA System). Kidney morphology was evaluated from Hematoxylin and Eosin and Periodic Acid Schiff stained formalin-fixed and paraffin-embedded tissue sections.

Results: UNx rats had increased albuminuria compared to sham rats (148±35 to 375±53 mg/day). CREA clearance and GFR worsened in the UNx rats (1.35±0.24 and 0.66±0.05 ml/min/g kidney to 0.72±0.05 and 0.44±0.03 ml/min/g kidney). In parallel Cystatin C rose after UNx. There was no change in mean arterial blood pressure after UNx (143±9 and 148±11 mm Hg, respectively). Both glomerular (mesangial matrix expansion and glomerulosclerosis) and tubulo-interstitial (tubular degeneration/ regeneration, tubular dilatation, arterial thickening/necrosis, inflammatory infiltrates and interstitial fibrosis) histopathologic lesions were significantly increased in UNx rats compared to sham rats. 4 week treatment of UNx rats with Lisinopril 100mg/kg/d as oral gavage did not lower albuminuria. Lisinopril 100 mg/kg with HCTZ 15mg/kg/d as diuretic reduced significantly the albuminuria compared to UNx controls (43±28 mg/day). Lisinopril alone neither improved CREA clearance nor GFR, whereas the Lisinopril/HCTZ combination significantly preserved both (1.22±0.09 and 0.56±0.04 ml/min/g kidney). Only Lisinopril/HCTZ treatment decreased Cystatin C significantly compared to the UNx control. Lisinopril had no effect on mean blood pressure (150±5.7 to 117±4.4 mm Hg), whereas Lisinopril/HCTZ significantly reduced blood pressure (117±4 mm Hg). Lisinopril alone was not able to decrease kidney tissue damage. The combination of Lisinopril/HCTZ was able to prevent both glomerular and tubulo-interstitial histopathological lesions significantly.

Conclusions: This experiment demonstrates that UNx exacerbates renal disease progression in Dahl SS rats on a high salt diet. ACE inhibitor Lisinopril was not able to protect kidneys from further damage under this experimental setting. Combining Lisinopril with diuretic, in this case HCTZ was able to prevent worsening of kidney function. This animal model offers the possibility to study effects on top of ACE inhibitor treatment.

MP096 THE DISTURBED CIRCADIAN RHYTHM AND SALT SENSITIVITY OF BLOOD PRESSURE IN ADRIAMYCIN NEPHROPATHY RATS

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Introduction and Aims: The prevalence of the disturbed BP circadian rhythm is striking in CKD patients. The kidney is the key peripheral organ involved with the BP circadian rhythm and shows sodium sensitivity.

Methods: In the present study the circadian characteristics of MAP, SBP, DBP, heart rate (HR), pulse pressure (PP) and locomotor activity were measured in conscious and unrestrained 12-week-old ADRs and age-matched SD control rats by the radiotelemetry system. After baseline studies were obtained, the rats were provided a high salt diet (8.0%) for a 1 wk period prior to the 7 day telemetry studies. Results: 1. Adriamycin Nephropathy rats presented with the reversed circadian rhythms of MAP, SBP, DBP, and PP compared with SD control rats respectively. However, there was no significant difference in 24-h mean value of BP. 2. In the ADRs the circadian rhythm of the urine sodium excretion was disturbed, the RUNa in Dark period was significantly lower than that in Light period of the same group ([4.69 ±3.65] μmol/h v.s [27.66 ± 5.84] μmol/h, P=0.001) and also significantly lower than that in Dark period of the control group ([44.69±3.65] μmol/h v.s [39.49±2.24] μmol/h, P=0.023). In the ADRs, the FENa in Dark period was significantly lower than that in the Light period of the same group (0.15±0.06 vs 0.29±0.06, P=0.008) and also significantly lower than that in Dark period of the control group (0.15±0.06 vs 0.31±0.19, P=0.050). 4. Under high salt diet, SBP and MAP in Dark period increased significantly by 19.8 mmHg and 18.5 mmHg respectively than those before high salt diet (P=0.001) in ADRs. In the control group, the SBP and MAP in Dark period increased significantly by 8.4 mmHg and 6.2 mmHg respectively than those before high salt diet (P=0.001). In the ADRs, the SBP and MAP in light period increased significantly by 20.1 mmHg and 17.7 mmHg respectively than those before high salt diet (P=0.001). In the control group, the SBP and MAP in Light period increased by 17.2 mmHg and 3.0 mmHg respectively than those before high salt diet, but both differences were not significant. After the high salt diet, the control rats maintained the normal BP rhythms while the ADRs still presented disturbed BP rhythm.

Conclusions: We concluded that the circadian patterns of blood pressure were dramatically altered in ADR nephropathy rats with the disturbed circadian rhythm of the urine sodium excretion and FENa. And the ADR nephropathy rats showed a

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striking salt sensitive of blood pressure. The ADR nephropathy rat was a suitable CKD animal model with disturbed circadian BP rhythm and sodium sensitivity.

**Introduction and Aims:** Endothelin-1 is considered as a pathogenic factor in hypertension and kidney disease development. However, it also demonstrates natriuretic and diuretic properties in the kidney. Previously we showed that chronic ET-1 synthesis inhibition aggravates 1kidney-1clip hypertension in adult male rats. It is well-known that androgens are prohypertensive and may alter renal function, but the influence of androgens on renovascular hypertension and ET-1 renal excretion has not been studied before. The aim of this study was to determine the effect of chronic ET-1 synthesis inhibition on 1kidney-1clip hypertension development and renal function in gonadectomised male rats.

**Methods:** Male Wistar rats were divided into 8 groups. Among them 4groups were treated with an endothelin-converting enzyme inhibitor (PP36) for 6weeks after the operation. The groups iH, iH-PP with intact testicles, and castrated ch and ch-PP groups were subjected to right nephrectomy and a clip on left renal artery. Other four groups were sham-operated (Sham-PP, iSham, chSham, PP-chSham). Blood pressure (BP) was controlled with the tail-cuff method. To evaluate creatinine clearance and water balance rats underwent 24h urine collection. Creatinine and urea in serum and urine were analysed by spectrophotometry. Urine osmolality was analysed by cryoscopy.

**Results:** Hypertension was less pronounced in castrated males. BP rise in group iH-PP was significantly higher than in iH (167±6/86±25 vs. 145±5/55±28 mmHg, p<0.05), but BP rise in group ch-PP was similar to ch (165±5/67±5 vs. 171±7/76±6 mmHg). Hypertension development resulted in ET-1 excretion rise in group iH by 74% and in group ch by 135% compared to sham-groups. PP36 treatment resulted in plasma ET-1 reduction by 28% in group iSham-PP. Urinary ET-1 excretion was reduced by 43% in group iH-PP compared to iH, but PP36 did not alter ET-1 excretion in castrated rats. The iH-PP group demonstrated elevated urinary creatinine and urea (799±200 vs 447±61 mOsm/kg p<0.05) and reduced free water clearance (-22 ±4.5vs -16±4 ml/24h) compared to iH. However, urine osmolality and free water clearance (-15±5 vs -16±4 ml/24h) were not altered in cH-PP group. Serum creatinine and urea were significantly enhanced in hypertensive rats. Creatinine clearance (Cr-C) was reduced by 37% in iH-PP rats compared to iH (0.78±0.1 vs 1.24±0.2 ml/min, p<0.05). However, ch-PP demonstrated elevated Cr-C vs compared to ch (0.88±0.05 vs 0.79±0.06ml/ min), but it was 16% lower than in group cSham.

**Conclusions:** We suppose that ET-1 excretion reduction by PP36 contributed to free water clearance reduction in adult male hypertensive rats, which might have potentiated hypertension development. Our results indicate that androgens interact with ET-1 system and probably have a role in inhibiting renal ET-1 synthesis, which may influence renal function.

**MP099 ENHANCED AT1 RECEPTOR-ASSOCIATED PROTEIN IN RENAL TUBULE SUPPRESSES ANGIOTENSIN-MEDIATED HYPERTENSION**

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**Introduction and Aims:** We have previously shown that ATRAP (angiotensin II type 1 receptor-associated protein) interacts with the angiotensin II type 1 receptor and promotes constitutive internalization of the receptor so as to inhibit the pathological activation of its downstream signaling. The present study was designed to investigate the role of renal ATRAP in angiotensin II-mediated hypertension. We hypothesized that renal enhancement of ATRAP can suppress angiotensin-mediated hypertension by influencing the handling of renal sodium.

**Methods:** We generated transgenic mice dominantly expressing ATRAP in the renal distal tubules in comparison with wild-type (Wt) littermate mice. Initially, we examined the effect of Ang II infusion on blood pressure of Wt and Tg mice using a radiotelemetry method. We also performed metabolic cage analysis during the Ang II infusion period and compared the sodium balance between the Wt and Tg mice. In addition, we compared the expression of the major sodium transporters (NHE3, NCC, and ENaC subunits) in the kidneys of Wt and Tg mice.

**Results:** In the renal ATRAP transgenic mice compared with wild-type mice, 1) the development of high blood pressure in response to angiotensin II infusion was significantly suppressed based on radio telemetry (Figure 1), 2) the extent of daily positive sodium balance was significantly reduced during angiotensin II infusion in metabolic cage analysis, and 3) the renal Na⁺-Cl⁻ cotransporter activation and a-subunit of the epithelial sodium channel induction by angiotensin II infusion were inhibited. Furthermore, adenosinergic overexpression of ATRAP suppressed the angiotensin II-mediated increase in the expression of a-subunit of the epithelial sodium channel in mouse distal convoluted tubule cells.

**Conclusions:** These results demonstrate that renal distal tubule-dominant overexpression of ATRAP in vivo exerts an inhibitory effect on angiotensin II-dependent hypertension, thereby suggesting that ATRAP is a target of interest in hypertension.
A NEW INDICATOR CALCULATED FROM ABPM ELUCIDATES THE RELATION BETWEEN ANTIHYPERTENSIVE PRESCRIBING AND BLOOD PRESSURE LOAD; FROM THE CKD-JAC STUDY

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Introduction and Aims: The Chronic Kidney Disease Japan Cohort (CKD-JAC) study started in Sep 2007, and 2,977 subjects were enrolled from 17 facilities. CKD and hypertension (HTN) are closely related; HTN causes and exacerbates CKD, and vice versa. Exploring this relation, the Ambulatory Blood Pressure Monitoring (ABPM) sub-study was conducted. In this presentation, we suggest a new indicator calculated from ABPM which reflects blood pressure (BP) load, and evaluate an actual condition of antihypertensive prescribing using this indicator.

Methods: ABP was measured every 30 minutes for 24 hour with TM-2421 device. A simple questionnaire was completed by each patient, and it collected the information such as bedtime, awakening time, the frequency of using lavatory at night and how the monitoring affected sleep. Data on medical history, medications, office BP and renal function were used from the registration data. The following indicators were calculated; 24hr mean BP, daytime and nocturnal mean BP, and hyperbaric area index (HBI). HBI is an area enclosed by ABPM polygonal line, 135/85 mmHg line (daytime), and 120/70 mmHg line (nighttime). The criterion for HTN was 140/90 mmHg for the office BP and 130/80 mmHg for the 24-hr mean BP. Total HBI is the sum of systolic and diastolic HBI.

Results: The data of 1,075 subjects (393 female, age 58.5±12.3; 682 male, age 62.0±10.6) was analyzed. Mean office BPs were 129.8/76.3 mmHg (female) and 132.1/77.6 mmHg (male). Based on the 24-hr mean BP and office BP, 37.5% were classified as normal BP. Only 100 subjects were prescribed no antihypertensives, while 374 subjects were prescribed more than 3 antihypertensives. Total HBI [mmHg×hour] was greater with male (+74.3 vs. female), low eGFR (+23.5 per 10ml/min/1.73m²), diabetes (+90.8), proteinuria (+119.8), nocturia (+67.2) and in winter (+77.3 vs. summer). The number of antihypertensives remained significant after adjustment for these factors (+29.6 for each additional one medicine, P=0.005).

Conclusions: HBI was a very sensitive indicator reflected various factors relevant to progression of CKD. It also reflected factors which had effects on ABP measurements such as nocturia and season. The more a patient’s BP control worsens, the more a doctor increases antihypertensives hoping for good BP control. However, even if adjusted by various background factors, HBI increased significantly with increasing antihypertensives. This shows actual condition of antihypertensive prescribing, where BP control of the CKD patients is quite difficult. More detailed BP control can be carried out using HBI.