Design and method: Heterozygote TGR(mREN2)27 rats were made diabetic with streptozotocin for 5 or 12 weeks. Rats were treated in the final 3 weeks with vehicle, irbesartan (15 mg/kg.day; IRB) or IRB plus the NEPi thiorphan (0.1 mg/kg.day; ARNI). Haemodynamics were measured by telemetry in the 5 week diabetic animals. In the 12 week diabetic animals vascular reactivity was determined in isolated mesenteric arteries, renal Na+ transporters were analysed by immunoblotting, and plasma and urine were collected for biochemical analysis.

Results: Baseline mean arterial blood pressure (MAP) was 156.8 ± 5.4 mmHg. IRB and ARNI lowered MAP identically over the 3-week period, a maximum reduction of ~50 mmHg being reached around day 7. Heart weight/tibia length ratio was reduced after treatment with ARNI only. Proteinuria and albuminuria were observed from 8 weeks of diabetes onwards and proteinuria was significantly reduced by ARNI treatment only. Urinary volume and plasma and urinary creatinine did not change. No ET-1 rises were observed, vascular reactivity was not influenced, and the pattern of kidney sodium transporters was not affected by ARNI or IRB treatment.

Conclusions: ARNI reduces cardiac hypertrophy and proteinuria in diabetic TGR(mREN2)27 rats independently of blood pressure.

PP.14.08 SUBTLE CHANGES IN THE GENE EXPRESSION PATTERN OF ADIPOCYTES AND VSMCS TREATED WITH MANIDIPINE

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Objective: Manidipine is a dihydropyridine calcium channel blocker (CCB) used in the treatment of hypertension and coronary heart disease. Its beneficial effects extend beyond those expected to accompany reductions in blood pressure. Of particular interest are studies with manidipine which suggest that this agent may be associated with greater improvements in insulin sensitivity. We evaluated the expression pattern of adipocyte differentiation-related genes in murine fibroblasts differentiated to adipocyte and treated with manidipine. Analyses were also conducted in vascular smooth muscle cells (VSMCs) exposed to angiotensin-II and treated with manidipine.
In mature and hypertrophied adipocytes manidipine preserves PPARγ activity, promoting adipocytes differentiation. However, we observed subtle differences in Scarb1 and Cd36 gene expression. Thus, while manidipine treatment preserves Scarb1 expression, Cd36 gene expression was downregulated respect to mature untreated adipocytes.

Design and method: These facts take us to think that manidipine associated with preserving PPARγ activity in mature adipocytes.

Results: Regarding VSMCs gene expression, we observed that PPARγ is upregulated in manidipine treated cells, even when cells have been previously exposed to Ang II. A decreased expression of PPARγ by exposing the cells to Ang II compared to those treated only with manidipine was noted. We observed that Cd36 is upregulated in treated cells with manidipine even being previously exposed to Ang II. Interestingly, Scarb1 considered antiatherogenic because of its role as HDL-C receptor, were also upregulated in manidipine treated cells after exposure to Ang II.

Conclusions: In VSMCs, PPARγ and its response genes, Scarb1 and Cd36 increases with manidipine treatment in cells that are also treated with Ang II, though does not become as relevant as in cells treated only with manidipine.

PP.14.09 EFFECTS OF ANTI-BETA1- AND β3- ADRENERGIC RECEPTOR ANTIBODIES ON LEWIS RAT THORACIC AORTA

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Objective: To evaluate 1) whether β3–adrenergic receptor (AR) antibodies (ABs) possess an agonist-like activity on β3–ARs and 2) whether active immunization producing both β1– and β3–ABs has deleterious effects on vascular reactivity in Lewis rats.

Design and method: Lewis rats were immunized for 6 months with peptidic sequences corresponding to the second extracellular loop of β1– and β3–ARs. During the immunization, systolic blood pressure (SBP) was monitored using the tail plethysmography. The β3–ABs were purified and characterized by Enzyme-linked immunosorbent assay and their agonistic effect was evaluated on electrically field-stimulated isolated cardiomyocytes from adult rabbit by measuring the cell shortening. The vascular reactivity of immunized rats was assessed by ex vivo studies on isolated thoracic aorta using various β–AR agonists (isoproterenol, dobutamine, salbutamol, nebivolol) and phenylephrine.

Results: SR58611A (10 nM), a preferential β3–AR agonist and purified β3–ABs (25 μg/mL) induced a decrease of cell shortening (-39.56 ± 4.4% (n = 11) and -16.47 ± 3.5% (n = 12) respectively). This decrease was significantly inhibited when the cardiomyocytes were preincubated with the L–748337 (1 μM), a selective β3–AR antagonist ($p < 0.05$), and with pertussis toxin (0.3 μg/ml), a Gi protein inhibitor ($p < 0.05$). The immunizations producing functional β1– and β3–ABs had not affected the SBP. However, in β1–AR-immunized rats, the relaxations mediated by isoproterenol, dobutamine and salbutamol were significantly impaired, but nebivolol-induced relaxation was not modified. Moreover, phenylephrine-mediated contraction was improved in these rats. In contrast, immunization with β3–AR peptide led to the improvement of relaxations induced by isoproterenol and dobutamine but did not affect those induced by salbutamol, nebivolol and phenylephrine-induced contraction.

Conclusions: The results show that β3–ABs induced a β3–AR agonist-like activity. In addition, our study shows for the first time that β1– and β3–ABs, whose role is usually studied in the heart, can affect thoracic aorta reactivity. β1–ABs would have a pathogenic action by altering the β–AR vasorelaxation whereas β3–ABs would have a beneficial effect on aorta reactivity.

PP.14.10 EFFECT OF DIFFERENT LEVELS OF HYPOXIA (13% AND 10% O2), SEX OF THE ANIMAL AND ESTRADIOL ON THE DEVELOPMENT OF HYPOXIC PULMONARY HYPERTENSION IN GONADECTOMIZED RATS

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Objective: Pulmonary hypertension (PAH) is characterized by increased of right ventricular (RV) systolic pressure and hypertrophy of RV. Women of various ages are more subjected to PAH, compared with male, what contradicts the protective effect of estradiol on the cardiovascular system. Gender differences in PAH are recognized but not well understood. Previously we demonstrated that estradiol potentiates hypoxic PAH (hPAH with O2 = 6%) in female gonadectomized rats. The aim of current research was to test the hypothesis that the gender differences in effect of estradiol on the development of experimental hPAH depend on the level of oxygen in the inspired air.

Design and method: Female and male gonadectomized Wistar rats were divided into 8 groups, four from which (2 M and 2F) were injected subcutaneously during 4 weeks with 1,2-propandion (vehicle, 200mkl/rat/day, C.); and four groups with estradiol (15mkg/kg/day – 2ME and 2FE). The procedures followed the FELASA/ICLAS guide for use of laboratory animals. hPH was induced by exposure to hypobaric hypoxia in all 8 groups. 4 groups (MC, FC, FE, ME) had hypoxia with 13%O2, and 4 groups – 10%O2. Rats were housed in a hypobaric chamber 10 h/day, 2wk. Right ventricular systolic pressure (RVSP) was measured as indices of hPH.

Results: Two weeks after hypoxia exposure all gonadectomized rats developed hPH and RVSP was greater in MC, FC, FE, ME groups with 10% O2 than 13% O2. Chronic estradiol administration in groups with hypoxia 13% caused a decrease of RVSP in group FE on 14.6% compared with FC ($p < 0.05$) without any change in the group of ME. In groups with hypoxia 10% estradiol caused the increase of RVSP in group ME on 24% compared with MC ($p < 0,05$) without any change in the group of FE

Conclusions: Our data suggest that estradiol (15 mkg/kg/day) leads to differently changes of pulmonary system in female and male gonadectomized rats with hPH. This effect depends on levels of hypoxia (13% and 10% O2).

PP.14.11 MODE OF ACTION OF THE ANTIHYPERTENSIVE EFFECT OF THE CREATINE ANALOGUE BETAGUANIDINOPROPIONIC ACID: THE ROLE OF CK GENE EXPRESSION

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Objective: Increasing evidence indicates that the ATP regenerating enzyme creatine kinase (CK) is involved in hypertension. The enzyme catalyzes the reversible transfer of a phosphoryl group of phosphocreatine to ADP, thus rapidly regenerating ATP near ATPases involved in sodium retention and vascular contractility, thereby promoting high blood pressure. Accordingly, plasma CK after rest was reported to be the main predictor of blood pressure and failure of antihypertensive therapy in the general population. Furthermore, human resistance artery contractility was shown to be highly CK-dependent. The CK system is competitively inhibited by the creatine analogue beta-guanidinopropionic acid (GPA). This reduces blood pressure in the spontaneously hypertensive rat (SHR). However, it is unknown whether the mode of action of GPA involves reducing CK. Therefore, we assessed the expression of the CKM and B isoenzyme in tissues of GPA-treated SHR.