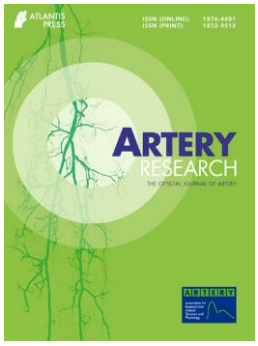

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P3.12: A MONOCLONAL ANTIBODY TO THE ENDOGENOUS NA/K-ATPASE LIGAND, MARINOBUFAGENIN, REDUCES PROFIBROTIC GENE EXPRESSION AND REVERSES CARDIOVASCULAR FIBROSIS IN SALT-SENSITIVE HYPERTENSION

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enzyme, with anti-oxidative and anti-inflammatory properties, results in protection against atherogenesis. We investigated whether oxidative stress affects HO-1 induction differentially uremic or control conditions.
Methods: HUAECs were conditioned in 30% human serum conditions (pooled from 40 hemodialysis patients or 10 healthy volunteers) for 72h, followed by exposure to increasing concentrations of peroxynitrite (0.1-1mM) for 10 minutes in 100mM phosphatebuffer, pH 7.5. Additionally, HUAECs were incubated with 100μM Hemin during 6h. Cell viability was measured by MTT assay, 30 minutes after peroxynitrite exposure with or without hemin (50μM for 6h).
HO-1 expression was evaluated by RT-PCR, Western blot and ELISA.
Results: Equal cell viability was found when conditioned in uremic or control serum. Hemin treatment did not affect cell viability, but peroxynitrite treatment reduced cell viability by 23% and 43 % in control and uremic serum ($P<0.05$ vs. control). Hemin induced a 250-fold increase in HO-1 expression in both conditions. Consistent with mRNA induction, WB and ELISA confirm the induction of HO-1 by hemin in uremic and control conditions.
Conclusion: Although uremia is considered pro-oxidative, pro-inflammatory state, uremic serum per se does not affect cell viability. Oxidative stress however affects endothelial cell viability to a larger extent in uremic conditions compared to control. More work will be needed to determine whether the induction of HO-1 by Hemin is capable of abrogating oxidative-stress-induced processes, implicated in atherogenesis, in cells exposed to uremic toxins in the circulation.

P3.12
A MONOCLONAL ANTIBODY TO THE ENDOGENOUS NA/K-ATPASE LIGAND, MARINOBUFAGENIN, REDUCES PROFIBROTIC GENE EXPRESSION AND REVERSES CARDIOVASCULAR FIBROSIS IN SALT-SENSITIVE HYPERTENSION

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Salt-sensitive hypertension is accompanied by elevated levels of an endogenous Na/K-ATPase inhibitor, marinobufagenin (MBG). Because MBG is implicated in cardiac fibrosis in experimental uremic cardiomyopathy (Hypertension 2007;49:215-24), we hypothesized that immunoneutralization of heightened levels of MBG in hypertensive Dahl salt-sensitive rats (DS) with monoclonal antibody may impact the profibrotic gene expression and cardiac remodeling.
We studied the following groups ($n=6$ each): (a) DS on a low salt (0.3% NaCl) diet (LS); (b) DS on a high salt (8% NaCl) diet for 7 weeks (HS); (c) DS on a high salt diet for 7 weeks, followed by monoclonal anti-MBG antibody treatment for 5 days (HSAB). Levels of MBG and of proteins implicated in pro-fibrotic signalling, and mRNA expression (microarray analysis) in left ventricles (LV) and aortae were assessed.
In HS vs. LS, BP increased by 74 mmHg ($p<0.01$), plasma MBG doubled ($p<0.05$), renal MBG excretion increased 6-fold ($p<0.01$), tissue weights increased (LV: 2.37 ± 0.05 vs. 1.62 ± 0.04 g/kg BW, $p<0.01$; aorta: 4.44 ± 0.17 vs. 3.01 ± 0.06 mg/mm*kg BW, $p<0.01$), and LV collagen rose 3.5-fold. In HSAB, BP was reduced by 35 mmHg ($p<0.01$), collagen-1 and LV and aortic weights were reduced ($p<0.01$) vs. HS group. In hypertensive DS there was a tissue-specific pattern of up-regulation of expression of genes, implicated in TGFβ-signaling (LV: TGFβ1-β2, MAPK3, CTGF, SMADs, collagen-1; aorta: TGFβ1-β3, PDGF, fibronectin, SNAIL1, PCOLCE, collagens), that was down-regulated following immunoneutralization of MBG.
Thus, immunoneutralization of MBG produces an anti-remodeling effect associated with down-regulation of genes implicated in TGFβ-induced fibrosis initiated by MBG in salt-sensitive hypertension.

P3.13
ROLE OF THE SEMICARBAZIDE-SENSITIVE AMINE OXIDASE (SSAO) IN CELL DIFFERENTIATION: CONSEQUENCES IN ATHEROSCLEROSIS

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The « semicarbazide sensitive amine oxidase » (SSAO) transforms primary amines into aldehydes, ammonia and hydrogen peroxide and is widely expressed by vascular smooth muscle cells (SMC) from the arterial wall. SSAO expression is increased during VSMC differentiation like in others cell types (adipocytes, chondrocytes). Disregulation of VSMC phenotype participates to the development of atherosclerosis lesion. To evaluate if the absence of SSAO could participate to the development of atherosclerosis, the laboratory established a double ApoE/SSAO knock out mice model (ApoE-/-SSAO-/-).
25 week-old ApoE-/-SSAO-/- mice presented a significant 50 % increase in plaque surface associated with an 80% decrease in a-actin expression in the media of aortic sinus from ApoE-/-SSAO-/- mice compared to ApoE-/- mice. We noticed a small T-cell infiltration in the media from ApoE-/-SSAO-/- mice whereas no T-cell infiltration was observed in the media from ApoE-/- mice. No difference was detected in monocytes/ macrophages infiltration in the plaque in aortic sinus from APOE-/-SSAO-/- mice and ApoE-/-. ApoE-/-SSAO-/- and ApoE-/- mice were transferred with labelled CD45.1 peripheral bone marrow cells from wild type mice. 5 day after PBMC injection, the number of labeled CD45.1 cells was increased in aorta, decreased in blood and unchanged in spleen from ApoE-/-SSAO-/- mice compared to ApoE-/- mice.
In conclusion, the absence of the SSAO increases the atherosclerosis in ApoE-/- mice. This result could be explained by a switch of VSMC to an inflammatory phenotype.

P3.14
THE CONTRIBUTION OF RHO KINASE AND PROTEIN KINASE C IN CONTRACTION OF RAT RENAL SMALL ARTERIES: SMOOTH MUSCLE AND ENDOTHELIAL EFFECTS

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Noradrenaline released from sympathetic nerves by activating $alpha_1$-adrenergic receptors causes renal artery contraction, in particular, through an inhibition of the myosin light chain phosphatase by Rho kinase (RhoK) and/or protein kinase C (PKC). In addition, both these kinases are expressed in endothelium and may influence the contraction through inhibition of endothelial NO-synthase (eNOS). The role of these kinases in contraction of renal arteries is poorly understood. Therefore, the goal of our study was to estimate the participation of RhoK and PKC in $alpha_1$-adrenergic contraction of rat renal arteries and in regulation of eNOS activity.
Interlobar arteries (2-3-order branches of the renal artery) were dissected from the kidney of male Wistar rats and mounted in a wire myograph (DMT A/S, Denmark). The arteries were contracted for 10 min with methoxamine ($alpha_1$-adrenoceptor agonist, 0.8-1.6 microM) up to 70-80% of the maximal response level. Preincubation with the RhoK inhibitor (Y27632, 3 microM) or PKC inhibitor (GF109203X, 1 microM) decreased the contractile response by 60-70%. However, in the presence of eNOS inhibitor L-NNA (100 microM) Y27632 or GF109203X reduced the contractile response to methoxamine by only 40-50%. Therefore, NOS inhibition attenuated the effects of RhoK and PKC inhibitors.
In conclusion, RhoK and PKC with almost equal potency strongly potentiate smooth muscle contraction in rat renal small arteries. Along with that, vasocontractile effects of these kinases in rat kidney are due to inhibition of endothelial NOS. Supported by RFBR (grant N10-04-01723-a).

P3.15
LARGE EDDY SIMULATION OF AORTIC COARCTATION BEFORE AND AFTER SURGERY

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The blood flow through an aortic coarctation before and after corrective surgery was simulated using computational fluid dynamics (CFD) with a state-of-the-art scale-resolving turbulence model (Large eddy simulation, LES). In this manner, the transitional and turbulent effects in the pulsating blood flow could be accounted for. Aortic geometry and in-plane velocity profiles in the ascending and descending aorta were measured using MRI. The velocity profiles provided patient-specific flow boundary conditions for the fluid model. The simulation computed the turbulent kinetic energy (TKE) of the flow, which is a measure of the turbulent velocity fluctuations. High