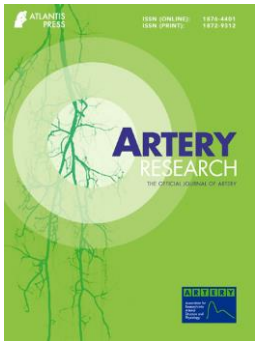

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P3.11: HEME OXYGENASE 1 EXPRESSION BY HUMAN UMBILICAL ARTERY ENDOTHELIAL CELLS (HUAECS) IN UREMIC VERSUS HEALTHY SERUM CONDITIONS

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VSMCs and activation of integrin mechanotransduction pathways represent potential mechanisms. Here, we examine whether mechanical stretch increases thrombin generation on cultured rat aortic VSMCs.
We used a model of cultured rat aortic VSMCs submitted to cyclic stretch (Flexcell, 10% at 1 Hz). Cyclic stretch during 60 and 360 minutes induced a differentiated contractile VSMC phenotype without apoptosis and up-regulated integrin $\alpha_v\beta_3$ expression 1.3 fold. Cyclic stretch stimulated binding of prothrombin to VSMCs and increased the subsequent thrombin generation by 67% and 30% respectively. It also produced time-dependent phosphorylation of Src, FAK and Akt as well as increased ILK phosphorylation at 15 minutes. Talin cleavage was increased between 5 and 60 minutes. The $\alpha_v\beta_3$ antagonist cRGDPV and α_v-siRNA blocked these responses. A talin-siRNA decreased stretch-induced α_v expression and the phosphorylation of Src, FAK, Akt and ILK. ILK-siRNA had no effect on α_v expression but inhibited phosphorylation of Akt and talin at 360 minutes.
These results demonstrate that cyclic stretch stimulates the generation of thrombin on rat VSMCs via activation of integrin $\alpha_v\beta_3$ pathways. They further suggest that intravascular generation of thrombin may be regulated by integrin $\alpha_v\beta_3$ antagonists in patients with high pulse pressure.

P3.07
QUANTITATIVE METHOD TO DETERMINE SMOOTH MUSCLE CELL ORIENTATION IN VITAL ARTERIES

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Arterial wall tension is actively regulated by smooth muscle cells (SMCs). To study the biomechanical behaviour of the arterial wall, SMC orientation should be accurately quantified. We evaluated the feasibility of a method to quantify SMC orientation in vital, excised arteries imaged by two-photon laser scanning microscopy. Arteries were mounted on micropipettes and pressurised to approximate in-vivo geometry. To enable unambiguous determination of orientation of individual cells, and assuming nuclear orientation to be representative of cell orientation, specimens were stained with a nuclear dye (SYTO13). Images were acquired with increasing imaging depth (Figure). Nuclei were automatically delineated in each image by applying vesselness filtering, a technique that enhances elongated structures. After thresholding, a momentum matrix was calculated for each nucleus, consisting of the sum of pixel to centre-of-gravity distances for each combination of coordinate axes. For each nucleus, the angle θ between its longest axis, as obtained through eigenvalue analysis, and the vessel's circumferential axis was calculated (Figure). Mean (θ_m) and SD (θ_{sd}) of SMC orientation were calculated by averaging SMC angles over a depth-ROI (Figure) using circular statistics. We analysed image stacks of murine carotid (n=3) and uterine (n=3) arteries. Orientation averaged over all stacks was approximately circumferential (2±5°, mean±SD). These results demonstrate the potential of our approach to quantify SMC orientation in vital arteries, circumventing artefacts associated with histological fixation and sectioning.

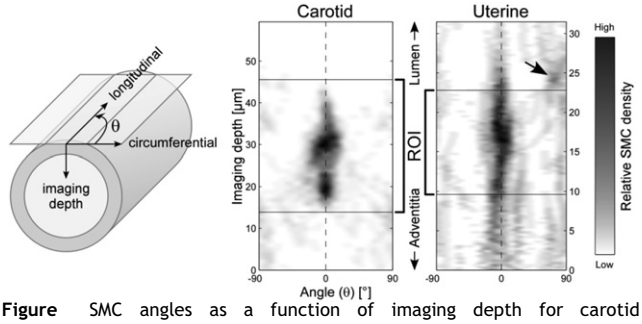

Figure SMC angles as a function of imaging depth for carotid ($\theta_m \pm \theta_{sd} = -4 \pm 25°$) and uterine artery ($\theta_m \pm \theta_{sd} = 5 \pm 32°$). Arrow indicates endothelial cells with elongated nuclei.

P3.08
SILENCING OF PKG1 GENE SENSITIZES VASCULAR SMOOTH MUSCLE CELLS TO THE PRO-FIBROTIC EFFECT OF THE ENDOGENOUS NA/K-ATPASE INHIBITOR, MARINOBUFAGENIN

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Marinobufagenin (MBG), an endogenous Na/K-ATPase inhibitor and a vasoconstrictor, induces vascular fibrosis via PKC-dependent inhibition of Fli-1, a nuclear transcription factor and a negative regulator of collagen synthesis. In vascular smooth muscle cells (VSMC), atrial natriuretic peptide (ANP), via cGMP/PKG1 dependent mechanism, reduces sensitivity of Na/K-ATPase to MBG. We hypothesized that VSMC from aged rats have a heightened sensitivity to the pro-fibrotic effect of MBG due to an age-associated down-regulation of cGMP/PKG-dependent signaling.
In response to acute NaCl loading (0.4g/kg), aged (24-month old) Sprague-Dawley rats exhibited exaggerated responses of urinary MBG (5.4±0.4 vs. 1.9±0.2 pmol/hr; $P<0.01$) and of systolic blood pressure (29 vs. 15 mmHg; $P<0.01$), and greater inhibition of Na/K-ATPase in aorta vs. 3-month old rats. In VSMC from young rats on a normal salt diet, 1 nmol/L MBG induced down-regulation of Fli-1 and a 50% increase in the levels of collagen-1, and these effects were blocked by 1 nmol/L ANP. In VSMC from aged rats levels of PKG1 and Fli-1 were markedly reduced. MBG (1 nmol/L) decreased Fli-1 by 60%, increased level of collagen-1 two-fold ($P<0.01$). In contrast to young rats, ANP failed to oppose these effects. Silencing of the PKG1 gene in VSMC from young rats sensitises these cells to the pro-fibrotic effect of MBG: 1 nmol/L MBG increased levels of collagen-1 2.5-fold ($P<0.01$).
Thus, the age-associated reduction in vascular PKG1 levels and resultant decline in cGMP signaling sensitize VSMC to the pro-fibrotic effect of MBG. Silencing of PKG1 in young VSMC mimics these effects of aging.

P3.10
VASORELAXATORY EFFECTS OF AMP-ACTIVATED PROTEIN KINASE ACTIVATION ARE NON-UNIFORMLY MANIFESTED IN DIFFERENT VASCULAR BEDS

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Aim: AMP-activated protein kinase (AMPK) is recently proposed to participate in functional hyperemia. It is expressed in both endothelial and smooth muscle cells and may be activated by metabolic influences or by an increase of shear stress on the endothelium. Functional hyperemia is not manifested uniformly in different vascular beds, being most pronounced in skeletal muscles, moderate in intestine and negligible in skin. This study tested the hypothesis that the effects of AMPK activation will show similar pattern of tissue specificity.
Methods: The segments of small arteries from diaphragm (DA), mesentery (MA) and skin (SA) were isolated from male Wistar rats and mounted in wire myograph (DMT A/S). We studied the effects of AICAR (10^{-4} M) on vessel contraction to α_1-adrenoceptor agonist methoxamine. Similar experiments were done in the presence of NO-synthase inhibitor, L-NNA (10^{-4} M).
Results: 60-min incubation with AICAR greatly reduced the contractile responses of DA and MA, but did not change SA contraction. L-NNA treatment abolished the effect of AICAR in DA, while in MA the effect of AICAR was smaller but still evident.
Conclusions: AMPK activation results in diminished vasocontractile responses and this correlates with magnitude of functional hyperemia in different organs; the effect is absent in skin, where blood flow is mainly regulated by sympathetic influences. Endothelial NOS represents a major target of AMPK in DA. In MA AMPK may also reduce contraction of smooth muscle cells by changing their calcium homeostasis or reducing calcium sensitivity of contraction. Supported by RFBR (grant 12-04-01665-a).

P3.11
HEME OXYGENASE 1 EXPRESSION BY HUMAN UMBILICAL ARTERY ENDOTHELIAL CELLS (HUAECS) IN UREMIC VERSUS HEALTHY SERUM CONDITIONS

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Background: In CKD, atherogenesis is accelerated, compared to the general population. Heme-oxygenase 1 (HO-1), an inducible heme-degrading

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