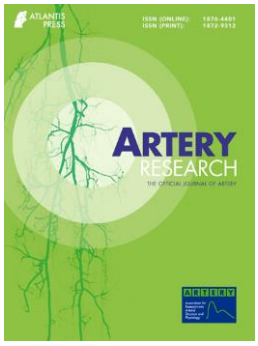

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P3.06: CYCLIC STRETCH-INDUCED THROMBIN GENERATION BY RAT VASCULAR SMOOTH MUSCLE CELLS IS MEDIATED BY THE INTEGRIN (V(3) SIGNALING PATHWAY)

H. Louis, Z. Li, M. Bourhim, V. Regnault, P. Lacolley

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P3.03

NEW METHOD TO ASSESS ARTERIAL STIFFNESS IN CONSCIOUS UNRESTRAINED RATS BY TELEMETRY

M. Isabelle, S. Chimenti, D. Gransagne, S. Chariglione, C. Vayssettes-Courchay, N. Villeneuve, J. P. Vilaine
Institut de recherches SERVIER, Suresnes, France

Pulse wave velocity (PWV) is considered as "the gold standard" to assess arterial stiffness. However, PWV is very dependent of blood pressure (BP) and is affected by anaesthesia, largely used in animal experimentation. Thus, the goals of the present study were: 1) To validate the PWV measurement in awake unrestrained rats using a new telemetry implant from Data Science International equipped with two pressures probes. 2) To measure PWV at different BP levels by using the circadian change of BP during the day or after acute BP reduction.
One catheter was placed in aortic arch and the other in abdominal aorta at the level of iliac bifurcation in Wistar Kyoto rats (18weeks-old, n=5). Hemodynamic parameters were recorded for 24h during baseline period and during an acute decrease in BP induced by diltiazem (100mg/kg/po). PWV was calculated by using the foot-to-foot method.
This new implant allows to measure heart rate, BP, BP amplification and PWV (Table1). The changes in PWV due to circadian or pharmacological changes in BP are shown in Fig.1. Both conditions exhibit similar linear regressions, allowing the assessment of PWV at different BP levels and thus independently of the BP.
In conclusion, we show for the first time that the evaluation of arterial stiffness dependent and independent of the BP in chronically instrumented awake unrestrained rats is now possible. It will be a good tool to assess the effects of drugs on arterial wall stiffness.

Table 1

	Central BP	Distal BP
SBP mmHg	118 ± 2	122 ± 2
DBP mmHg	101 ± 2	99 ± 2
MBP mmHg	110 ± 2	110 ± 2
PP mmHg	18 ± 1	22 ± 1
Heart rate bpm	355 ± 8	
Amplification	1,25 ± 0,11	
PWV m/s	4,7 ± 0,2	

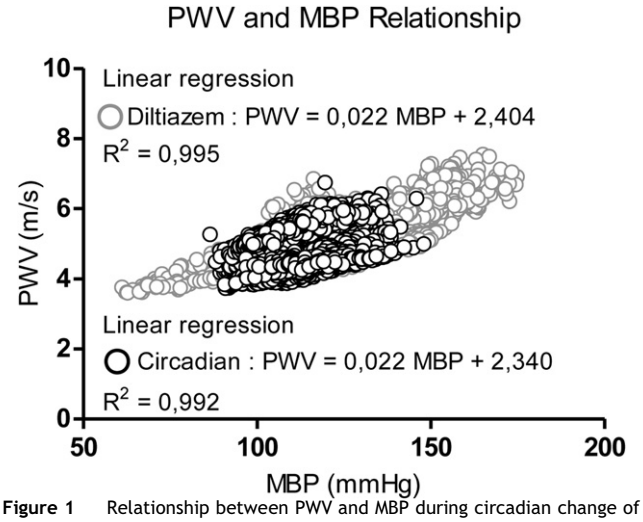

Figure 1 Relationship between PWV and MBP during circadian change of BP or acute administration of diltizem on 24h period. PWV : Pulse Wave Velocity, MBP : Mean Blood Pressure

P3.04

INCREASED THROMBIN GENERATION AND VASCULAR REMODELING IN OBESE ZUCKER RATS

J. Lagrange [1,2], A. Benetos [1,2], L. Walton [4], K. Cruickshank [3], P. Lacolley [1,2], V. Regnault [1,2]
[1]*Inserm U961, Nancy, France*
[2]*Université de Lorraine, Nancy, France*
[3]*King's College of London, Londres, United Kingdom*
[4]*School of Medicine, Manchester, United Kingdom*

The metabolic syndrome associates obesity, inflammation and arterial stiffness. We characterized the coagulation phenotype in 25 and 80 week old Zucker rats, that mimics human metabolic syndrome. We adapted a calibrated automated thrombography technique which follows thrombin activity after in vitro stimulation by tissue factor. The endogenous thrombin potential (ETP) which represents the area under the curve of thrombin generation was higher in 25 week old obese rats than in control lean rats of the same age (428 ± 29 nM.min versus 328 ± 27 nM.min) and still higher at 80 weeks (422 ± 30 versus 306 ± 11 nM.min). The most striking finding was an increase in thrombin generation characterized by a widening of the area under the curve associated with an increase in plasma fibrinogen. This hypercoagulability was corroborated by F1+2 test *in vivo* at 25 weeks and did not depend on platelets because it was observed in platelet free plasma. Endothelial dysfunction was shown by a high plasma concentration of von Willebrand factor and inflammation by an increase in several cytokines in a cytokine array and in metalloproteinase activity by zymography. In contrast, there was no increase in thrombin generation *in vitro* with ageing whatever the strain. To conclude, we have shown that thrombin generation increased *in vitro* with obesity, independently of platelet activation as early as 25 weeks of age. We suggest an implication of fibrinogen whereby thrombin interacting with fibrinogen is protected from its inhibition by antithrombin.

P3.05

THE ROLE OF HYALURONAN IN AORTIC STIFFENING IN PATIENTS WITH RHEUMATOID ARTHRITIS

K. M. Maki-Petaja, R. Patel, S. E. Cleary, M. Yasmin, I. B. Wilkinson
University of Cambridge, Cambridge, United Kingdom

Introduction: Patients with rheumatoid arthritis (RA) have increased aortic stiffness, which can be improved with anti-inflammatory therapies. However, how inflammation leads to aortic stiffening remains unclear. One potential mechanism is by overproduction of hyaluronan (HA) in the extracellular matrix, which results in stiffening of the arterial wall by thinning of elastic lamellae in animal models. However, the effect in man is unknown. The aim was to evaluate 1) whether serum HA concentration is a valid surrogate of aortic tissue level and 2) to compare serum HA in patients with RA and control subjects and to relate this to aortic pulse wave velocity (aPWV).
Methods: 18 aortic tissue samples were homogenised and HA concentration in the homogenate and corresponding serum sample was assessed using commercially available ELISA kit (DY3614, R&D Systems, U.K). Also, aPWV using SphygmoCor system, and Serum HA was assessed in 40 patients with RA and in 40 matched control subjects.
Results: There was a correlation between tissue and serum HA (R=0.68; P=0.01). Patients with RA had higher serum HA compared to controls (66.6±65.2 v. 11.0±7.9 ng/ml; P<0.0001). In patients with RA, the serum HA levels correlated with aPWV (R=0.3; P=0.01).
Conclusion: This study demonstrates that serum HA is increased in patients with RA in comparison to control subjects, and this correlates with aortic stiffening. The data suggests that increased HA synthesis may be the mechanism behind inflammation-induced aortic stiffness in patients with RA. However, further experiments are needed to study causality between tissue HA concentration and aortic stiffness.

P3.06

CYCLIC STRETCH-INDUCED THROMBIN GENERATION BY RAT VASCULAR SMOOTH MUSCLE CELLS IS MEDIATED BY THE INTEGRIN (V(3) SIGNALING PATHWAY)

H. Louis [1,2], Z. Li [2], M. Bourhim [1,2], V. Regnault [1,2], P. Lacolley [1,2]
[1]*Université de Lorraine, Nancy, France*
[2]*Inserm U961, Nancy, France*

Vascular smooth muscle cell (VSMC) phenotypic modulation plays a pivotal role in atherothrombotic diseases. Thrombin generation at the surface of

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

B. Spronck [1], J. J. Merken [1], W. Kroon [1], R. T. A. Megens [2], K. D. Reesink [1], T. Delhaas [1]
[1]*Maastricht University Medical Centre, Maastricht, Netherlands*
[2]*Ludwig-Maximilians-University Munich, Munich, Germany*

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.

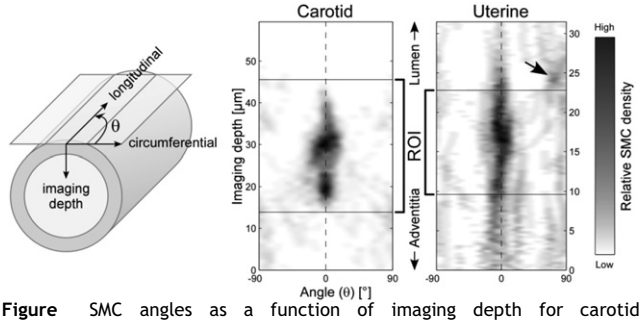

Figure SMC angles as a function of imaging depth for carotid ($\theta_m \pm \theta_{sd} = -4 \pm 25°$) and uterine artery ($\theta_m \pm \theta_{sd} = 5 \pm 32°$). Arrow indicates endothelial cells with elongated nuclei.

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

O. V. Fedorova, V. Shilova, E. G. Lakatta, A. Y. Bagrov
National Institute on Aging, NIH, Baltimore, United States

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

A. A. Borzykh [1], G. V. Morgunova [2], O. S. Tarasova [1,2], O. L. Vinogradova [1]
[1]*SRC RF - Institute for Biomedical Problems RAS, Khoroshevskoe shosse 76A, 123007, Moscow, Russian Federation*
[2]*Faculty of Biology, M.V. Lomonosov Moscow State University, Leninskie Gory 1/12, 119234, Moscow, Russian Federation*

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

K. E. L. Daenen, M. Hoylaerts, B. Bammens
KU Leuven, Leuven, Belgium

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