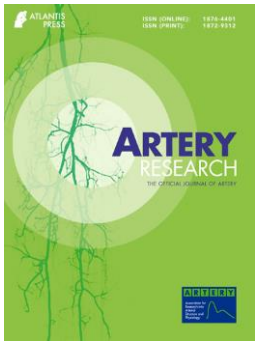

Artery Research

ISSN (Online): 1876-4401 **ISSN (Print): 1872-9312**
Journal Home Page: https://www.atlantis-press.com/journals/artres

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

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To cite this article: B. Spronck, J.J. Merken, W. Kroon, R.T.A. Megens, K.D. Reesink, T. Delhaas (2012) P3.07: QUANTITATIVE METHOD TO DETERMINE SMOOTH MUSCLE CELL ORIENTATION IN VITAL ARTERIES, Artery Research6:4, 179–179, DOI: https://doi.org/10.1016/j.artres.2012.09.134

To link to this article: https://doi.org/10.1016/j.artres.2012.09.134

Published online: 21 December 2019

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.

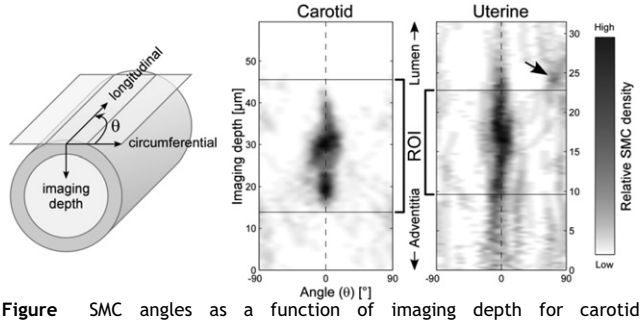

Figure SMC angles as a function of imaging depth for carotid ($\theta_m \pm \theta_{sd} = -4 \pm 25°$) and uterine artery ($\theta_m \pm \theta_{sd} = 5 \pm 32°$). Arrow indicates endothelial cells with elongated nuclei.

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