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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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 α[5]β[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 α[5]β[3] antagonist crGDVP and α[5]siRNA blocked these responses. A talin-siRNA decreased stretch-induced α[5] expression and the phosphorylation of Src, FAK, Akt and ILK. ILK-siRNA had no effect on α[5] expression but inhibited phosphorylation of Akt and talin at 360 minutes.


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Marinobufagenin (MBG), an endogenous Na/K-ATPase inhibitor and a vasocostritor, induces vascular fibrosis via PKC-dependent inhibition of Flt-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 VSMCs 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 Flt-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 Flt-1 were markedly reduced. MBG (1 nmol/L) decreased Flt-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 sensitizes VSMC to the pro-fibrotic effect of MBG. Silencing of PKG1 in young VSMC mimics these effects of aging.