Topic 29 – Cardiac and vascular signalling – A

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0169

Segregated calcium stores are important for stretch-induced calcium signaling in smooth muscle cells: implication in pulmonary hypertension

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Pulmonary arterial smooth muscle cells (PASMC) are submitted to stretch forces exerted by the blood pressure. They can transduce a mechanical stimulus of stretch into a biological response of contraction, a mechanism called myogenic tone which involves Ca^{2+} influx through stretch-activated channels (SAC). We investigate how the subcellular organization of Ca^{2+} stores in PASMC (sarcoplasmic reticulum (SR), lysosomes and mitochondria) is important for the Ca^{2+} response to stretch during PH.

Studies were performed in freshly isolated PASMC from control rats and rats with a pulmonary hypertension induced by an injection of monocrotaline (MCT). Inward currents from SAC were recorded after a negative pressure applied by a patch-clamp pipette. Cytosolic Ca^{2+} was also measured with the fluo4 probe and mitochondrial Ca^{2+} with the rhod2 probe after an osmotic shock. A pharmacological approach coupled with different coimmunolabelings of ryanodine receptors (RyR) and SERCA2 pumps was used to investigate which RyR subtype is involved in Ca^{2+} response to stretch.

PASMC exhibit segregated Ca^{2+} stores: a subplasmalemnal SR store with RyR1 and SERCA2b, associated to mitochondria and a perinuclear SR store with RyR3 and SERCA2a. We showed that a stretch of PASMC induces an inward Ca^{2+} influx through SAC that is amplified by a Ca^{2+} release by RyR1, which is buffered by mitochondria. In MCT rats, the subcellular organization of RyR subtypes is modified: RyR3 and SERCA2a are not only expressed in a perinuclear area but also in a subplasmalemnal level. This is correlated with a different organization of mitochondria. In MCT rats, this new organisation leads to a greater amplification by all RyR subtypes and to a higher Ca^{2+} increase induced by stretch. Furthermore, the Ca^{2+} response to stretch is enhanced by a mechanism independent on extracellular Ca^{2+}, involving caveolae.

The spatial organization of Ca^{2+} stores in PASMC is important for cell signaling and plays a causal role in PH.

0316

Vascular smooth muscle mineralocorticoid receptor contributes to coronary and left ventricular dysfunction after myocardial infarction

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Aims: Because mineralocorticoid receptor (MR) antagonists have shown efficacy in slowing down the progression of heart failure after myocardial infarction (MI), there is interest to elucidate the cell-specific involvement of MR. Indeed, the role of MR in vascular smooth muscle cells (VSMC) in heart failure, especially its impact on coronary circulation, has never been investigated.

Methods and Results: Two months after MI, mice lacking the MR specifically in VSMC (MI-MRSMKO) and mice treated with the MR antagonist finerenone (MI-fine) had better coronary function than control (MI-CTL), as assessed by acetylcholine-induced relaxation of isolated arteries (relaxation %: MI-CTL: 36±5, MI-MRSMKO: 54±3, MI-fine: 76±4; P<0.05). Furthermore, MRI showed that the coronary reserve was increased (ml/mg/min: MI-CTL: 1.1±0.5, MI-MRSMKO: 4.6±1.6, P<0.05; MI-fine: 3.6±0.7, P<0.01). Incubation with the NAPDH-oxidase inhibitor apocynin of coronary arteries improved acetylcholine-induced relaxation in MI-CTL to a higher extent than in MI-MRSMKO and MI-fine mice, suggesting that MR antagonism reduces oxidative stress-mediated endothelial dysfunction. Indeed, incubation of coronary arteries from non-infarcted animals with 10-9M angiotensin II induced oxidative stress and impaired acetylcholine-induced relaxation in CTL mice, but not in MRSMKO or in 4 weeks finerenone-treated mice. These improvements in coronary function were accompanied in MI-MRSMKO mice by reduced LV fibrosis and improved LV function.

Conclusion: After MI, VSMC-specific MR invalidation benefits LV dysfunction, likely through improvement of coronary reserve and of coronary endothelial function, demonstrating for the first time the deleterious role of smooth muscle MR activation in heart failure. Furthermore, systemic MR blockade by finerenone confers additional functional improvements.

0335

Contribution of large conductance Ca(2+)-activated K(+) channels to vasorelaxation induced by cAMP-phosphodiesterases inhibitors is blunted in coronary arteries from rats with heart failure

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3'-5'-cyclic adenosine monophosphate (cAMP) is an important mediator of vasorelaxation. In vascular smooth muscle, cellular cAMP concentrations are mainly regulated by type 3 and 4 phosphodiesterases (PDE3 & PDE4). Large conductance Ca(2+)-activated K(+) (BK) channels are reported to mediate cAMP-induced relaxation. Here, we sought to clarify whether BK channels are key players in the regulation of tone by PDE activities in rat coronary arteries (CA), and if this contribution is altered in congestive heart failure (CHF). CA were isolated from rats with CHF sacrificed 22 weeks after surgical stenosis of the ascending aorta. Age-matched « sham » animals were used as controls. CA were isolated and mounted on a myograph. Maximal contraction was obtained using high K(+) solution. Relaxations induced by inhibitors of PDE4 (Ro-20 1724, Ro, 10µM) or the PDE3 (Cilolstamide, Cil, 1µM) were studied in CA contracted with the thromboxane analogue U46619 (1-2µM) and incubated or not with iberiotoxin (IBTX, 100nM), a selective BK channel blocker. CA isolated from rats displaying robust signs of CHF showed a 78% reduction of maximal contraction (P<0.001, N=5-7) and reduced relaxing response to acetylcholine (–55%, P< 0.01), compared to CA from sham rats. Ro and Cil relaxed sham CA to respectively 31±13% and 14±5% of U46619 tone (N=6). In the presence of IBTX, these responses decreased to 3±1% (N=5, P<0.05) and 4±3% (N=4). In CHF rats, Ro relaxed CA to 7±4% and Cil to 27±4% (N=4), whereas IBTX had no effect either on responses to Ro (17±8 %, N=5) or Cil (26±6%, N=4). Simultaneous addition of Cil and Ro relaxed sham CA to 79±10% (N=7), an effect reduced by IBTX (20±9%, N=6, P<0.01). Interestingly, Cil and Ro together relaxed CHF CA to 69±9% but this was not altered by IBTX (69±14%, N=5-6). These results show that, in rat CA, BK channels are important effectors of relaxing responses to PDE3 and PDE4 inhibition. In CHF, specific contribution of BK channels to these responses is lost.

0296

Regulating O-GlcNAcylation by AMP-activated protein kinase, a new way to prevent hypertrophy development

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Background: We have previously shown that the AMPK specific activator, A769662, is able to block phenylephrine (PE)-induced hypertrophy without affecting the previously identified AMPK-related key regulators of

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