Topic 30 – Cardiac and vascular signalling – B

April 03rd, Friday 2015

0270

CaMKII inhibition prevents cardiac arrhythmias elicited by phosphodiesterases 3 and 4 inhibitors

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β-adrenoceptors (β-AR) stimulation increases cardiac function by rising cAMP that activates protein kinase A (PKA) and enhances Ca^{2+}-induced Ca^{2+}-release by phosphorylating proteins such as ryanodine receptors and phospholamban, which are also targets of the Ca^{2+}/Calmodulin Kinase II (CaMKII). Any dysregulation of the β-AR pathway promotes arrhythmias. Local cAMP concentration in heart is mainly regulated by phosphodiesterases (PDE) type 3 and 4. Here, we investigated the proarrhythmic effects of PDE3 and PDE4 inhibition and evaluated the relative contribution of PKA and CaMKII to these mechanisms. An IonOptix system was used to record intracellular Ca^{2+} transients in isolated adult rat and pig ventricular myocytes (ARVMs and APVMs) loaded with 1μM Fura-2AM and paced at 1Hz. In ARVMs, PDE4 inhibition with Ro20-1724 (Ro, 10μM) potentiated the inotropic effect of the β-AR agonist isoproterenol (Iso, 1nM) but induced spontaneous Ca^{2+} waves (SCWs) recorded during 10s pacing pauses (2.3±0.2 SCWs/10s, n=15; p<0.001). Sarcoplasmic reticulum (SR) Ca^{2+} load increased by 30±5% (n=13; p<0.001) in Iso, but doubled (p<0.001) in Iso+Ro leading to SR Ca^{2+} leak (measured in a $0Na^{+},0Ca^{2+}$ solution±1mM tetracaine, n=7; p<0.001). PKA inhibition by H89 (10μM) suppressed SCWs, SR Ca^{2+} leak and the inotropic effect of Iso±Ro. The CaMKII inhibitor KN93 (10μM), unlike its inactive analogue KN92, reduced SR Ca^{2+} leak by 85% (n=16; p<0.001) and SCWs incidence by 72% (n=8; p<0.001) without affecting the inotropic effect of Iso+Ro. KN93 blunted the SR Ca^{2+} leak induced by PDE3 inhibition with cilostamide (Cil, 1μM, n=10; p<0.001) by 81% but preserved Ca^{2+} transients amplitude. Similarly, CaMKII inhibition prevented SCWs evoked by Cil and Ro in APVMs.

These results show that PDE inhibitors exert inotropic effects *via* PKA but lead to SCWs *via* both PKA and CaMKII activation. They also suggest the potential use of CaMKII inhibitors as adjuncts to PDE inhibitors to limit their proarrhythmic effects.

0392

AMPKα1 regulates cell adhesion and migration of human cardiac fibroblasts via cytoskeletal remodelling pathway

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Background: A key detrimental characteristic of left ventricular (LV) remodelling is fibrosis. The pathogenesis of cardiac fibrosis includes migration of cardiac fibroblasts. Remodelling of actin cytoskeleton is a hallmark of the migrating cells. AMP-activated protein kinase (AMPK) regulates actin cytoskeleton organization and has been shown to influence LV remodelling. In this study, we investigated whether A-769662, a pharmacological AMPK activator, modulates human cardiac fibroblast (HCF) migration and adhesion by affecting actin organization.

Methods: Single-cell migration was assessed by time-lapse imaging. Focal adhesions (FA) were visualized by immunofluorescence together with fluorescent phalloidin to stain actin filaments. Phosphorylation state of myosin regulatory light chains (MLC) was determined by western blot analysis and immunofluorescent staining. RhoA activity was assessed using a pull down method.

Results: A-769662 significantly decreased HCF motility, in an AMPKα1-dependent manner. In addition, A-769662-treated cells shifted from an elongated shape, characterized by leading and trailing edges, to a more polygonal one with numerous and thicker stress cables, mainly dorsal fibres and transverse arcs. This was accompanied by markedly increased cell adhesion and focal adhesion size. Concomitantly, AMPKα1 activation also resulted in a sustained activation of RhoA that was associated with a significant increase in MLC phosphorylation and its colocalization with transverse arcs.

Conclusion: We postulate that A-769662 counteracts HCF migration through an AMPK-dependent sustained activation of the RhoA/MLC pathway, promoting actin polymerization and contraction. Myosin II-generated tension into FA is expected to promote their maturation and subsequently alter migration.

0148

Effects of atrial natriuretic peptide on rat ventricular fibroblasts during differentiation into myofibroblasts

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Atrial natriuretic peptide antifibrotic properties are mainly described in cardiac myocytes or in induced cardiac myofibroblasts (Angiotensin II or TGF-β induced differentiation). In the present work, we investigate the effects of ANP/NPRA/cGMP system in modulating rat cardiac fibroblasts function. Cardiac fibroblasts were isolated from adult Wistar male rats and cultured in the presence of serum in order to induce fibroblasts differentiation. Cultures were then treated with ANP (1μM), 8-Br-cGMP (100μM) or IBMX (100μM), a non-specific phosphodiesterases inhibitor. ANP significantly decreased proliferation rate and collagen secretion. Its effect was mimicked by the cGMP analog, while combining ANP with 8-BrcGMP did not lead to additional effects. Moreover intracellular cGMP levels were elevated when cells were incubated with ANP confirming that ANP intracellular pathway is mediated by cGMP. Additionally, immunoblotting and immunofluorescence were used to confirm the presence of guanylyl cyclase specific natriuretic peptide receptors A and B. Finally we scanned specific cGMP dependent PDEs *via* RT-qPCR, and noticed that inhibiting all PDEs led to an important decrease in proliferation rate. Effect of ANP became more prominent after 10 culture days, confirming the importance of ANP in fibroblasts to myofibroblasts differentiation. Uncovering cellular aspects of ANP/NPRA/cGMP signaling system provided more elements to help understand cardiac fibrotic process.

0452

MTOR inactivation during early postnatal development of mice myocardium leads to severe dilated cardiomyopathy due to altered translational efficiency and hypoxia-induced apoptosis

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Mechanistic target of rapamycin (mTOR) is a central regulator of cell growth, proliferation, survival and metabolism. mTOR inhibition is increa-

singly used in antitumoral therapies and mTOR inhibition with rapamycin was shown to be cardioprotective during aging and cardiac stress. Studies in genetic mice models have shown that mTOR is essential for heart development and cardiac function in adult. However, mTOR functions during postnatal cardiac development are not fully elucidated. We have therefore generated a cardiac-specific mTOR knockout mouse using α-MHC-Cre mice leading to mTOR inactivation in early postnatal mouse myocardium. The mutant mice develop a severe lethal dilated cardiomyopathy due to defects in cardiomyocyte growth, survival and subsequent fibrosis. In contrast to adult myocardium, both mTORC1 and mTORC2 activities are impaired in juvenile heart, as shown by hypophosphorylation of the translation inhibitor 4E-BP1 and loss of the cardioprotective AKTS473 phosphorylation. We find that translation initiation defects and altered ribosome biogenesis both contribute to impaired cardiomyocyte growth. In addition, we show that increased apoptosis is associated with activation of JNK kinase and p53 accumulation. Moreover mTORcmKO hearts display a strong decreased expression of the primary oxygen carrier, myoglobin, and HIF1α accumulation suggesting hypoxia. However, mTORcmKO hearts do not display HIF1 hypoxic response consistently with mTOR being essential for HIF1-dependant trancriptionnal activity. These observations indicate that hypoxia-induced apoptosis likely contribute to DCM in mTORcmKO mice. Altogether, our results demonstrate that mTOR is a key regulator of cardiomyocyte growth, viability and oxygen supply in early postnatal myocardium. Our findings highlight potential cardiotoxicity of new mTOR inhibitors and the importance to set up optimal treatments in cardiology to both target mTOR hypertrophic functions and maintain adequate oxygen supply.

0406

Effects of FGF23 and Klotho on adult rat cardiomyocytes in culture

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The bone derived hormone fibroblast growth factor 23 (FGF23) and its co-receptor Klotho represent a novel endocrine axis regulating mineral metabolism in health and disease.

FGF23-Klotho signalling inhibits renal phosphate reabsorption and activation of vitamin D, and reduces secretion of parathyroid hormone. Serum levels of FGF23 rise in chronic kidney disease (CKD). In contrast, tissue expression of Klotho decreases in parallel with CKD progression and reaches low or undetectable levels in end stage renal disease.

Numerous studies identify elevated FGF23 as a predictor of adverse clinical outcome. In particular, elevated FGF23 has recently been associated with greater risks of major cardiovascular events and mortality.

However, there have been very few studies that have attempted to address the direct effects of FGF23 on myocardium. Moreover whether Klotho is involved in FGF23 – mediated actions on cardiomyocytes is still unclear.

In this context, we investigate the role of FGF23 and Klotho in adult rat ventricular myocytes (ARVMs). Using video-edge-detection, epifluorescent microscopy and an Ionoptix® system, performed in isolated cardiomyocytes subjected to FGF23 or Klotho alone, or in association, we showed that FGF23 increases cell size and cell shortening in ARVMs, and induces arrhythmia in the presence of Isoprenaline. In addition Klotho prevents FGF23 effects on adult cardiomyocytes. Indeed, ARVMs subjected to Klotho showed marked protection from FGF23-induced hypertrophic responses and from FGF23-induced arrhythmias in the presence of Isoprenaline.

Altogether these preliminary data provide a direct evidence of the role FGF23 in adult cardiomyocytes and suggest that Klotho may have a beneficial effect in preventing adverse cardiovascular outcomes in patients with or without CKD.

0379

Cytoarchitectural and metabolic alterations induced by ER stress in heart

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The rough endoplasmic reticulum (rER) is the site for synthesis, folding and quality control of secreted and membrane proteins. Impairment of ER function in response to stresses such as oxidative stress, disruption of calcium homeostasis or ischemia causes the accumulation of misfolded proteins in the rER lumen, resulting in ER stress. Over the past decade, ER stress has emerged as an important mechanism involved in the pathogenesis of cardiovascular diseases including heart failure. However, the molecular mechanisms underlying the contribution of ER stress to cardiac dysfunction remain poorly understood. In the present study, we evaluated the effect of the ER stressor tunicamycin (TN) on cardiac function in mice. TN injection (2mg/kg, 72h) induced a significant impairment of systolic function as indicated by the decrease in ejection fraction and fractional shortening. However, the heart rate, left ventricular internal diameters in diastole and systole and wall thickness were not affected. Transmission electron microscopy analysis revealed that TN induced an important ultrastructural remodeling of the cardiomyocytes with an increase in the occurrence of rER. Whereas rER was essentially located near the nucleus in cardiomyocytes of control mice, we observed an expansion of the rER network near sarcomeres and around T-tubules and mitochondrial clusters after TN treatment. In addition, mitochondrial structure and network were also disorganized. When measured in skinned fibers, the rate of mitochondrial oxidation was slower and an impairment of the function of the creatine kinase energy shuttle was observed in response to TN. In addition, ER stress triggered a metabolic remodeling characterized by a shift from fatty acid to glycogenic substrates consumption. Taken together our results show for the first time that the cytoarchitectural and metabolic alterations of cardiomyocytes contribute to the cardiac injury induced by ER stress.

0116

Phosphodiesterases regulate cAMP level in the mitochondria of adult cardiomyocytes

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The heart is highly energy-dependent with most of its energy provided by mitochondria. Mitochondria also play a role in many essential cellular processes including metabolite synthesis, redox balance, calcium homeostasis and cell death. Therefore, maintaining a functional population of mitochondria is critical for cardiac function and identification of novel regulatory mechanisms. Recently, a soluble adenylyl cyclase (sAC) has been revealed to serve as a local source of the second messenger cAMP in the mitochondrial matrix in response to bicarbonate and calcium. PDE2A, a cGMP-stimulated cAMP-hydrolyzing phosphodiesterase (PDE), was also shown to be expressed in the mitochondrial matrix of liver and brain. To gain new insights into the control of mitochondrial pool of cAMP, we investigated the role of various isoforms of PDEs in isolated cardiac mitochondria. Western blot analysis of PDE protein expression revealed positive bands for PDE2A, 3A, 4A and 4B subtypes in cardiac mitochondria. Basal cAMP-degrading enzymatic activities were determined by radioenzymatic assay in cardiac mitochondrial lysates with 1μm cAMP as substrate. PDE2 represented the largest mitochondrial cAMP PDE activity (35% of total) and its activity was enhanced ~2-fold by 5μM cGMP and was inhibited by the PDE2 inhibitor, Bay 60-7550 (100nm). PDE3 and PDE4 activities represented, respectively, 30% and 25% of total, and they were inhibited by their respective inhibitors, cilostamide (1μm) and Ro 20-1724 (10 μM). Accordingly, measurements by ELISA of mitochondrial cAMP levels confirmed that inhibition of PDE2, 3 and 4 families leads to an increase in cAMP level. In conclusion, at least three PDE families and four PDE subtypes are located in the cardiac mitochondrial matrix, participating to a local signaling pathway with sAC to control cAMP level. Our findings unravel a cAMP signaling cascade in cardiac mitochondria which may have implications for the metabolic control of cardiac function.