J013 INHIBITION OF MTOR SURVIVAL PATHWAY BY AN EMBRYONIC DEVELOPMENTAL WNT PATHWAY SUPPRESSES PRECONDITIONING INDUCED CARDIOPROTECTION

F. VIGNERON 1, B. VINASSA 2, L. TARIOSS 1, M. CAMPAGNAC 1, T. COUFINHAL 1, S. BONORON-ADELE 2, P. DOS SANTOS 1, C. DUPLAA 1
1 Inserm U.828, Pessac, France
2 IFR 4, Pessac, France

Ischemic preconditioning (IPC) protects against heart prolonged lethal ischemia by activating a cardioprotective signalling cascade involving Akt and GSK3β; however molecular pathways are still incompletely known. In an in vivo model of IPC, we previously demonstrated that the Wnt pathway can inhibit cardioprotection via a direct target, GSK3β.

Here we go further in the preconditioning pathway comprehension. On a retrograde isolated heart perfusion model, two models of preconditioning were set up: IPC via four sequences of 5 min of flow ischemia followed by 5 min reperfusion; a Pharmacological Preconditioning (PCP), by 25 min perfusion of diazoxide, a direct activator of mitoKATP. Preconditioning signalling was studied by Western blot analysis, in hearts harvested just after preconditioning, and cardioprotection was analyzed after 40 min flow ischemia followed by 120 min reperfusion, by TTC staining.

We verified that PCP, like IPC, induce an inhibition/phosphorylation of GSK3β (ser9) by activation of Akt (ser473). These results were correlated with a significant reduction of infarct size after ischemia-reperfusion (10.8% and 18% (n=6, p<0.05) of risk area, after PCP and IPC respectively, vs 37.5% (n=7, p<0.05) in non preconditioned control hearts). We showed then that both IPC and PCP were able to induce the mTOR survival pathway by increasing P-mTOR (ser2448) and its subcellular targets P70S6K (thr389) and 4E-BP1 (ser65) via Akt activation. Wortmannin, an AKT inhibitor, blocked preconditioning induced mTOR activation and infarct size reduction (39.9% of risk area, n=6, p<0.05 vs control, NS). These effects of preconditioning were also inhibited either by 5-hydroxydecanoate, an antagonist of mitoKATP (44.4% of risk area, n=6, p<0.05 vs control, NS) or by rapamycin, a specific inhibitor of mTOR (40% of risk area, n=7, p<0.05 vs control, NS).

Hearts of transgenic mouse overexpressing sFRP1 (α-MHC/sFRP1), a Wnt/β-catenin antagonist, cardioprotections induced by IPC and PCP were inhibited: sFRP1 impaired GSK3β inhibition and mTOR activation (P70S6K and 4E-BP1 phosphorylation), independently of Akt.

We evidenced for the first time that cardioprotection involves a crosstalk between an embryonic developmental Wnt pathway and a survival pathway, mTOR/P70S6K.

J014 SIMULTANEOUS RECORDINGS OF CELL SHORTENING AND CALCIUM TRANSIENTS REVEAL DIFFERENTIAL REGULATION OF CARDIAC CONTRACTILITY BY SPECIFIC PHOSPHODIESTERASES

D. MIKA 1, J. LEROY 1, P. LECHENE 1, R. FISCHMEISTER 1, G. VANDECASTEELE 1
1 Inserm UMR-S 769, Univ Paris-Sud 11, Faculty of Pharmacy, Châtenay-Malabry, France

Multiple cyclic nucleotide phosphodiesterases (PDEs) belonging to four families (PDE1 to PDE4) hydrolyze cAMP in cardiac cells, but the functional significance of this diversity is not well understood. The goal of this study was to characterize the involvement of different PDEs in excitation-contraction coupling in cardiomyocytes. For this, sarcomere shortening and Ca2+ transients were recorded simultaneously in rat ventricular myocytes field stimulated at 0.5Hz with an IonOptix system. Selective inhibition of PDE2 with Bay 60-7550 (Bay, 100 nM) or PDE4 with Ro-201724 (Ro, 10µM) had no effect on basal cell contraction, whereas selective inhibition of PDE3 with cilostamide (Cil, 1µM) or β-adrenergic stimulation with isoprenaline (Iso, 1nM) increased myocyte shortening. Inhibition of PDE4 potentiated the response to Cil and Iso, showing that PDE4 becomes important when cAMP is prestimulated. Similar results were obtained on Ca2+ transients. cAMP measurements by FRET in beating cardiomyocytes indicate that Iso strongly increases cAMP levels. Effects of selective PDE inhibitors are under investigation. These results show that PDE2, PDE3 and PDE4 differentially regulate excitation-contraction coupling in cardiomyocytes.

J015 RÔLE DE L’ARCHITECTURE CELLULAIRE DANS LA SIGNALISATION ÉNERGÉTIQUE DU CŒUR DE SOURIS AU COURS DU DÉVELOPPEMENT POSTNATAL

J. PIQUEREAU 1, F. JOUBERT 1, F. FORTIN 1, M. NOVOTOVA 2, R. VENTURA-CLAPIER 1, V. VEKSLER 1
1 Inserm U-769 Signalisation et Physiopathologie Cardiaque, Chatenay Malabry, France
2 Institut de Physiologie Moléculaire et Génétique, Bratislava, Slovak Republic

La fonction contractile du cardioméocyte requiert un contrôle local du rapport ATP/ADP au niveau des ATPases du réticulum sarcoplasmique (SERCA) et des ATPases des myofilaments. Les principaux systèmes, contrôlant l’approvisionnement des ATPases en énergie, sont les créatines kinases et la canalisation directe (CD) des nucléotides adényliques entre les mitochondries et les myofilaments. During the development (3, 7, 21, 42 et 63 days).

L’efficacité de la CD a été étudiée sur fibres ventriculaires perfusées ou isométriques. L’évaluation de l’apport d’énergie à SERCA par la CD repose sur la mesure de la charge calcique du réticulum sarcoplasmique (RS) qui est révélatrice de l’efficacité du système énergétique. Le rendement du système au niveau des myofilaments est estimé, pour sa part, en suivant la tension de rigueur des fibres à mesure que la concentration d’ATP est diminuée dans le milieu. A sept jours, la CD, entre les mitochondries et SERCA, est aussi efficace que chez l’adulte (l’estimation à trois jours n’a pu être réalisée en raison de l’immaturité du RS) ; elle est en revanche significativement moins efficace à trois jours qu’à sept jours au niveau des myofilaments. En effet, la concentration de MgATP pour laquelle apparaît la tension de rigueur diminue de 8,1 ± 1,4 fois à sept jours et de seulement 4 ± 0,6 fois à trois jours. Cependant, l’étude de la fonction mitochondriale, par mesure de consommation d’oxygène ou de l’activité enzymatique, a montré qu’il n’existe pas de différence notable entre les mitochondries de trois et sept jours. L’observation des cardiomycocytes par microscopie électronique a
Ad-shMRP4 demonstrated a significant increase in the calcium intracellular cAMP level. Adult rat cardiomyocytes infected with using a FRET technique MRP4 inhibition was shown to increase and over-expressed in case of increased cardiac intracellular cAMP.

Results in a cage with free access to a running monitored wheel. Mice. As a model of physiological hypertrophy, animals were housed implanted subcutaneously into 3-months old MRP4 KO and wild type containing isoproterenol delivering 20 μg/g/day for 2 weeks were treated MRP4 KO mice displayed a significant increase in cardiac hypertrophy compared to stimulated WT mice (HW/BW: 6±0.38 vs 5.29±0.34; p=0.001). In contrast to the regulation of pathological cardiac hypertrophy, MRP4 inhibition did not affect the physiological cardiac growth response associated with physical training (HW/BW: 4.83±0.01 vs 4.84±0.13, p=NS). In contrast to the regulation of pathological cardiac hypertrophy, MRP4 inhibition did not affect the physiological cardiac growth response associated with physical training (HW/BW: 4.83±0.01 vs 4.84±0.13, p=NS), indicating the absence of a regulatory role of MRP4 in physiological cardiac hypertrophy.

Conclusion — These results reveal a unique and important function for MRP4 in stress-dependent cardiac growth by controlling cyclic nucleotides signalling pathways in cardiomyocytes.

J017
THE RHO/RAC EXCHANGE FACTOR VAV2 CONTROLS NITRIC OXIDE–DEPENDENT RESPONSES IN VASCULAR SMOOTH MUSCLE CELLS
V. SAUZEAU 1, X.-R. BUSTELO 2
1 Inserm U915-Institut du thorax, Nantes, France
2 Centro de Investigacion del Cancer, Salamanca, Spain

The regulation of arterial contractility is essential for blood pressure control. The GTase RhoA promotes vasoconstriction by modulating the cytoskeleton of vascular smooth muscle cells. Whether other Rho/Rac pathways contribute to blood pressure regulation remains unknown. We have previously demonstrated that vav2 null mice suffered from serious defects in the cardiovascular system of, including tachycardia, systemic arterial hypertension, extensive cardiovascular remodelling, heart fibrosis, and loss of kidney homeostasis. By studying this hypertensive knockout mouse lacking the Rho/Rac activator Vav2, we have discovered a new pathway composed of Vav2, the GTase Rac1, and the serine/threonine kinase Pak that is critical for nitric oxide–triggered blood vessel relaxation and normotensia. This pathway mediates the Pak–dependent inhibition of phosphodiesterase type 5, a process that favors the inactivation of the RhoA pathway and the depolymerization of the F–actin cytoskeleton in vascular smooth muscle cells. The inhibition of phosphodiesterase type 5 requires its physical interaction with autophosphorylated Pak1 but, unexpectedly, occurs without detectable transphosphorylation events between those two proteins. The administration of phosphodiesterase type 5 inhibitors prevents the development of the hypertension and the cardiovascular disease in Vav2–deficient animals, demonstrating the key role of this signaling route in blood pressure regulation. Taken together, these results unveil the cause of the cardiovascular phenotype of Vav2 knockout animals, identify a new Rac1/Pak1 signaling element, and provide a mechanistic framework to better understand blood pressure control in physiological and pathological states.

J018
THE DEUBIQUITINASES USP33 AND USP20 COLLABORATORILY REGULATE BETA2 ADRENERGIC RECEPTOR RECYCLING AND RESENSITIZATION
M. BERTHOUZE 1,2, R.-J. LEFKOWITZ 1, S.-K. SHENOY 1
1 Department of Medicine and Cell Biology, Division of Cardiovascular Medicine, Duke University Medical Center, Durham, USA
2 Inserm U769, Signalisation et Physiologie Cardiaque, Chatenay-Malabry, France

Chronic agonist stimulation of the β2 Adrenergic Receptors (β2ARs) leads to their lysosomal trafficking and degradation. Previous studies demonstrated that agonist-induced β2AR ubiquitination is necessary for lysosomal targeting and degradation of the receptor. We have now found that the de-ubiquitinating enzymes USP33 and USP20 are recruited to the β2AR complexes, by using cellular co-immunoprecipitation assays. This led to our hypothesis that USP33