J013

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

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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 0 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 0 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 (ser479). 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. Wortmanin, 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/Frz 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 CAMP OR CALCIUM TRANSIENTS REVEAL DIFFERENTIAL REGULATION OF CARDIAC CONTRACTILITY BY SPECIFIC PHOSPHODIESTERASES

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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 0.5 Hz 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

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La fonction contractile du cardiomyocyte 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 ATPases. Il semblerait que l'architecture cellulaire joue un rôle primordial dans l'efficacité de la CD. Etant donné que cette architecture subit des remaniements au cours de la période postnatale, nous avons étudié la mise en place de la signalisation énergétique et de l'architecture du cardiomyocyte de souris au cours du développement (3, 7, 21, 42 et 63 jours).

L'efficacité de la CD a été étudiée sur fibres ventriculaires perméabilisées. 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 rigor 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 rigor 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 cardiomyocytes par microscopie électronique a