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TOPIC 06-1 – Ischemia, reperfusion

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0240

Ouabain preconditioning protects rat myocardial Na/K-ATPase from ischemia/reperfusion injury

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Cardiac Na/K-ATPase (NKA) function is a key target for protection against Ischemia/Reperfusion (I/R) injury. Ouabain is an inhibitor of NKA ion transport activity and a trigger of NKA signaling pathway. Activation of this pathway by transient exposure to ouabain 10 μM prior to ischemia protects the heart against I/R injury. This phenomenon, known as ouabain preconditioning (OPC), presents several characteristics of ischemic preconditioning (IPC), but whether OPC can protect NKA function itself is not known. In Langendorff-perfused rat hearts, 30 min of ischemia followed by 30 min of reperfusion (IR) significantly decreased NKA activity (1.2±0.3 *vs*. 5.3±0.1 μmol of Pi/hr/mg of protein, $P < 0.01$) without decreasing NKA α-protein content in crude homogenates. OPC and IPC prevented this alteration (5.1±0.2 and 4.6±0.2, respectively. $P<0.01$*vs*. IR). In rat neonatal cardiac myocytes, 30 min of substrate/coverslip-induced ischemia followed by 30 min of reperfusion compromised viability (0.060±0.003 *vs*. 0.003±0.002 U of lactate dehydrogenase released/ml of media, $p<0.01$) and NKA activity (1.2±0.1 *vs*. 1.8±0.1, $P<0.05$). OPC prevented these alterations in a PKCε-dependent manner. This suggests that OPC prevents I/R-induced NKA alteration in rat whole hearts and cardiac myocytes.

0264

NDAE1, the Drosophila Na+ -driven Cl- /HC03- Exchanger, preserves cardiac function during acidosis

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The Na^+-Driven Anion Exchanger (NDAE) constitutes the only Na^+- dependent Bicarbonate Transporter in *Drosophila*, instead of the seven members of the SLC4 family in mammals. NDAE promotes the exchange of Na^+ with protons together with the Bicarbonate-Chloride exchange, and can function in both directions.

In regard to the putative role of Na^+-dependent Bicarbonate Transporters in cardiac pathologies consecutive to intracellular acidosis, we investigated the function of NDAE in the *Drosophila* cardiac activity. Surprisingly, specific inactivation of *ndae* in the cardiac tube did not impair any of the measured cardiac function in basal conditions and did not impact either on viability. By contrast, a function of *ndae* can be revealed in several stress conditions which impaired homeostasis of H^+ and ions which are transported by NDAE (Na^+, Cl^-) or osmolarity. During acidosis, NDAE is required to prevent arrhythmia and heart failure and to allow the full recovery of cardiac activity when the physiological pH is restored. Activity of NDAE is also required in low external concentrations of Na^+ and Cl^- and for the cardiomyocyte response to hypo-osmolarity.

Moreover, we showed that *ndae* displays a strong genetic interaction with *NCX*, which encodes the unique Sodium-Calcium Exchanger in *Drosophila*, enlightening the tight functional coupling between Na^+-dependent H^+ extruders and NCX.

These results constitute the first *in vivo* demonstration that Na^+-dependent Bicarbonate Exchangers constitute essential pH regulators in the cardiac myocytes.

0448

The effect of myocardial posconditioning on apoptosis and caspases expression, including caspase-3 and caspase-9 in isolated perfused mouse heart

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Purpose: Myocardial Ischemic postconditioning (IPostC) and pharmacologic postconditioning by Cyclosporine A (CsA) are known as cardioprotective against apoptosis, there are few reports of the mechanism involved in their anti-apoptotic effects. It was previously demonstrated that caspase 3 mediated IPostC induced anti apoptotic effect; but it is unknown whether CsA prevents activation of caspase 9 and 3, which are implicated in the initiation and execution step of apoptosis. Our study was designed to address these issues.

Methods: The hearts isolated from C57BL/6J mice were subjected to no flow ischemia for 40 min followed by 2 or 4 hours of reperfusion (control group). IPostC was performed by 10 cycles of 5s reperfusion/5s ischemia at the onset of reperfusion, postconditioning by CsA was performed by administration of CsA 0.2 μM during the first 10 min of reperfusion (n=6 in each group). At the end of protocols, apoptosis was assessed by terminal deoxynucleotidyl transferase (TdT)-mediated dUTP nick end-labelling (TUNEL) and the expression of apoptosis-related proteins (cleaved-caspase 3 and cleaved-caspase 9) was measured by immunohistochemistry. Infarct size analysis was performed by TTC staining after 2 and 4 hours of reperfusion.

Results: The important TUNEL-positive cells stain confirm the occurrence of apoptosis in control group, in comparison, IPostC and CsA attenuated apoptosis, evidenced by fewer TUNEL-positive cells. As compared to Control group, the expression of cleaved-caspase3 and cleaved-caspase 9 was lower in IPostC and CsA groups. IPostC and CsA significantly decrease the infarct size as compared to control group.These effects are observed after 2 hours and 4 hours of reperfusion.

Conclusion: IPostC and CsA attenuate ischemia-reperfusion induced apoptosis and necrosis. Reduction of apoptosis with IPostC and CsA is associated with a decrease of activation of caspase 3 and 9 through antiapoptotic signaling after myocardial ischemia-reperfusion.

0172

Mononuclear cell surface adenosine deaminase activity and dipeptidyl-peptidase IV activity, sensitive and early markers of ischaemia-reperfusion in coronary heart disease

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Aims: During ischaemia, the extracellular level of adenosine increases, which has cytotoxic effects. In human endothelial cells, cell surface adenosine deaminase-complexing CD26 is coordinately induced during ischaemia as part of an adaptative response by transforming adenosine into the non toxic compound inosine. We examined whether a similar mechanism exists for mononuclear cells. Thus we studied mononuclear cell surface ADA activity (MCADA) as well as the authentic dipeptidyl-peptidase IV activity (DPPIV) of membrane CD26 during percutaneous transluminal coronary angioplasty (PTCA) as a model of ischaemia-reperfusion. We compared the enzymatic activities with levels of ischaemia-modified albumin (IMA), a well known marker of ischaemia-reperfusion.

Methods and results: 18 patients (15 men and 3 women, mean age 66±8 years) with non-ST segment elevation acute coronary syndrome related to a significant stenosis of proximal left anterior descending artery were prospectively included before revascularization of the culprit lesion. MCADA, DPPIV, and IMA were measured before percutaneous coronary intervention (T0) and 15 (T15) or 120 (T120) minutes post reperfusion. Fifteen healthy control subjects were enrolled.

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At T0, MCADA, DPPIV, and IMA levels were significantly higher in patients than in control subjects (patients vs controls; MCADA: 11.2±3.7 IU vs 7±1.4 IU, p<0.01; DPPIV: 1.13±0.5 vs 0.74±0.22, p<0.01; IMA: 100.8±3.9 vs 86.7±7.7, p<0.01). We observed a decrease in MCADA at T15 (8.6±2.4, p<0.01) and T120 (9.6±3, p=0.02), as compared with T0. DPPIV also decreased at T15 (0.95±0.5, p=0.01) and further decreased at T120 (0.88±0.4, p<0.01) as compared with T0. IMA level remained unchanged at T15 but increased significantly at T120 (111±13; p<0.01) as compared with T0.

Conclusion: Mononuclear cell surface ADA and DPPIV are sensitive and early markers of ischaemia-reperfusion process during PTCA.

0273

STAT3alpha interacts with nuclear GSK3ß and cytoplasmic RISK pathway and stabilizes rhythm in the anoxic-reoxygenated embryonic heart

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Activation of Janus Kinase 2/Signal Transducer and Activator of Transcription 3 (JAK2/STAT3) pathway is known to play a key role in cardiogenesis and in cardioprotection against ischemia-reperfusion in adult. We previously showed that in ventricle of the anoxic-reoxygenated developing heart, ROS-dependent STAT3α activation leads to nuclear accumulation of STAT3α without DNA-binding. Possible interaction between STAT3α and other signalling pathways [in particular Reperfusion Injury Salvage Kinase (RISK) pathway] and the role of activated STAT3α in functional recovery of the embryonic heart remains unexplored.

Hearts isolated from 4-day-old chick embryos were submitted to anoxia (30min) and reoxygenation (80min) with or without the JAK2/STAT3 inhibitor AG490 or the PhosphoInositide-3-Kinase (PI3K)/Akt inhibitor LY-294002. Time course of phosphorylation of STAT3α and RISK proteins [PI3K, Akt, Glycogen Synthase Kinase 3ß (GSK3ß), Glycogen Synthase (GS), Extracellular signal-Regulated Kinase 2 (ERK2)] was determined in homogenate and in enriched nuclear and cytoplasmic fractions of the ventricle. The chrono-, dromo- and inotropic disturbances were also investigated by ECG and mechanical recordings.

Phosphorylation of STAT3α was reduced by AG490 but not affected by LY-294002. STAT3α and GSK3ß were detected both in nuclear and cytoplasmic fractions while PI3K, Akt, GS and ERK2 were restricted to cytoplasm. AG490 decreased the reoxygenation-induced phosphorylation of Akt, GS and ERK2 and phosphorylation/inhibition of GSK3ß in the nucleus, exclusively. Inhibition of JAK2/STAT3 delayed recovery of atrial rate, worsened variability of cardiac cycle length and prolonged arrhythmias compared to control hearts.

Thus, besides its nuclear translocation without transcriptional activity, ROS-activated STAT3α can rapidly interact with RISK proteins present in nucleus and cytoplasm, without dual interaction, and reduce the anoxia-reoxygenation-induced arrhythmias in the embryonic heart.

0242

Modulation of Na/K-ATPase surface abundance during ischemia/reperfusion injury in rat cardiac myocytes

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Na/K-ATPase is crucial to cardiomyocyte survival during ischemia/reperfusion (IR) injury. In epithelial cells, we recently reported that IR induces Na/K-ATPase internalization, and that increasing Na/K-ATPase membrane abundance by expressing a mutated form of its catalytic α1-subunit (α1-L499V) resulted in a significant decrease in IR-induced cell death (Pierre et al., 2011, Am J Physiol Cell Physiol). The aim of this study was to determine whether IR-induced internalization of Na/K-ATPase occurs and whether α1-L499V expression protects neonatal cardiac myocytes (NCM) in face of an ischemic attack. 30 minutes of substrate/coverslip-induced ischemia in primary cultures of rat NCM resulted in a decreased ratio of cell surface/intracellular α1immunofluorescent signal, as observed by confocal microscopy during reperfusion. After 30 min of reperfusion, cell injury was indicated by AnnexinV/PI labeling and increased lactate dehydrogenase (LDH) release in the media. Transient expression of α1-L499V significantly reduced LDH release compared to α1-expressing cells. These results suggest that IR induces Na/K-ATPase internalization and that modulation of Na/K-ATPase cell surface abundance can protect against IR injury in NCM.

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