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recovery of contractile function (57 ± 8% in IPostC, 41 ± 12% in CsA vs. in Control 18 ± 5% of initial rate pressure product, $P < 0.05$).

Conclusions: Our results show, for the first time, that IPostC and CsA induced-cardioprotection are linked to an overshoot of PCr in early reperfusion. Nevertheless, cardioprotective effect induced by inhibition of mPTP opening was less important than IPostC effect, suggesting that supplementary signalling pathway would be recruited in response to IPostC.

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Mitochondrial localization unveils a novel role for GRK2 in the regulation of oxidative metabolism

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Recent studies demonstrate the role of GRK2 in the control of insulin signaling and cellular metabolism. Here, we investigate the role of this kinase in cellular energy production.

In HEK-293 cells, GRK2 targets within mitochondrial compartments and the RH domain within the amino-terminus of GRK2 is required and sufficient for mitochondria localization. Within the mitochondria, GRK2 binds and phosphorylates some proteins that are still to be identified. In HEK-293s, stable overexpression of GRK2, increases the number of mitochondria, which are giant, with regular crest organization, suggestive of increased biogenesis. Accordingly, two mitochondrial genes, NADHd and cytochrome B, are increased in copy numbers and expression after GRK2 overexpression. This effect is lost when a catalytically inactive mutant is overexpressed in HEK-293. The reciprocal evidence was collected in mouse aorta cells lacking the GRK2 gene. In this setup, we found a reduction in the number of copies and the level of expression of the NADHd and cytocrome B as compared to control cells. Since increased mitochondrial DNA content often associates with elevated oxidative respiratory chain activity, we assessed total and mitochondrial ATP, and found that GRK2 overexpression increases while inactive GRK2 decreases total and mitochondrial ATP levels. Acute hypoxia promotes a transient increase of total and mitochondria-associated GRK2 levels, the successive re-oxygenation for 15 minutes and 1 hour restores the levels of the kinase. Also, ATP decreases during hypoxia and slowly returns to basal levels after oxygen replenishment. The overexpression of GRK2 attenuates the loss of ATP production during hypoxia and after oxygen restoration, compared to controls. Finally, in mice, GRK2 gene deletion in the skeletal muscle causes a significant reduction of ATP levels under basal conditions and attenuates the recovery of ATP levels following ischemia/reperfusion.

Our data show an unexpected role of GRK2 in the mitochondria, supporting a positive regulation of the bioenergetic pathway.

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Activation of soluable guanylate cyclase by cinaciguat reduces ischemia/reperfusion injury after heart transplantation

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Ischemia/reperfusion injury is one of the main limiting factors of cardiac surgery. We previously showed that upregulation of the cGMP-signaling has protective effects on cardiac tissue. In the present study, we investigated the effects of cinaciguat, an activator of the soluble guanylate cyclase (sGC), on ischemia/reperfusion injury in a rat model of heterotopic heart transplantation.

In our study young Lewis rats were used for allogenic intraabdominal heart transplantation. Three experimental groups were assigned: A cinaciguat preconditioning group, an ischemic control and a nonischemic control. The treatment group received 10mg/d/kg body weight cinaciguat orally for 4 days prior explantation. Heterotopic heart transplantation was performed by anastomosing aorta and pulmonary artery of the donor heart with the abdominal aorta or the caval vein of the recipient, respectively. Nonischemic transplantation was achieved by a slight modification of the model of heterotopic heart transplantation using cuff technique. The ischemic time period in the treatment group and the ischemic control group was standardized to 1h. The reperfusion time was 1h in all groups. Left ventricular pressure (LVP), its first derivative (dP/dt) and end-diastolic pressure (LVEDP) were evaluated. ATP-content of the myocardial tissue was measured and the energy charge potential, as marker for the energy state of the myocardial tissue, was calculated.

Hemodynamic parameters were significantly increased in the cinaciguat group (Pmax: 82 ± 4 vs. 123 ± 13* vs. 127 ± 13* mmHg; dP/dtmax: 1740 ± 116 vs. 3350 ± 444* vs. 4397 ± 602* mmHg/s; ischemic control vs. cinaciguat vs. nonischemic control; *$p < 0.05$ vs. ischemic control). Furthermore we recorded increased ATP-levels and an improved energy charge potential (ATP: 1.86 ± 0.41 vs. 6.56 ± 0.84* vs. 6.58 ± 1.12* μmol/g; energy charge potential: 0.49 ± 0.04 vs. 0.76 ± 0.08* vs. 0.69 ± 0.07*; ischemic control vs. cinaciguat vs. nonischemic control; *$p <$ 0.05 vs. ischemic control).

Our current results support the concept that the NO-cGMP-PKG-pathway plays an important role in ischemia/reperfusion injury. We conclude that upregulating this pathway using cinaciguat has a preconditioning-like effect and can effectively reduce ischemia/reperfusion injury.

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Myocardial ischemic tolerance and expression/distribution of protein kinase C in various forms of chronic hypoxia

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The adaptation to chronic hypoxia (CH) confers long-lasting cardiac protection against acute ischemia/reperfusion injury, and protein kinase C (PKC) appears to be involved in its protective mechanism. Studies dealing with the mechanism of cardioprotection induced by CH provide controversial results, which may be caused by using different CH models. The aim of our study was to compare the expression and distribution of PKCδ, PKCε and their phosphorylated forms in the left ventricular myocardium of rats exposed to various forms of CH. Adult male Wistar rats were either kept under normoxic conditions or exposed to intermittent hypobaric hypoxia (IHH); (8 hours/day, 5 weeks, 7000 m), continuous normobaric hypoxia (CNH); (24 hours/day, 3 weeks, 10.5 % O_2) and intermittent normobaric hypoxia (INH); (23 hours/day, 3 weeks, 10.5 % O2). The susceptibility of the hearts to myocardial infarction was evaluated in rats subjected to 20-min coronary artery occlusion and 3-h reperfusion (tetrazolium staining). The expression of PKC isoforms δ, ε and their phosphorylated forms was determined by Western blotting. The adaptation to IHH decreased the infarct size from 60.6 ± 2.2% of the area at risk in normoxic controls to 48.0 ± 2.2%. IHH increased the expression of PKCδ in homogenate, cytosolic and particular fractions (to 160 %, 193 % and 126 % of normoxic controls, respectively) and decreased the expression of PKCε in homogenate and particular fraction (to 58 % and 60 % of normoxic controls, respectively) without affecting the level of phosphorylated PKCε (Ser 729). CNH decreased the infarct size to 41.5 ± 3.0%, but did not influence the relative amount of PKCδ, PKCε and phosphorylated PKCε. Surprisingly, INH did not affect the infarct size and the expression of the total and phosphorylated PKCε, but led to the translocation of PKCδ from the membranes to the cytosolic fraction. Our study suggests that degree of protection and involvement of PKC isoforms in protective signaling pathway strongly depends on the models of CH and adaptation protocol.

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Heme oxygenase over-expression reduces tissue damage and improves microvascular function in the rat model of chronic myocardial infarction

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Occlusion of a coronary artery causes metabolic derangement and immediate loss of contractile function in downstream ischaemic myocardium as well as irreversible myocardial damage with tissue necrosis followed by scar formation. Both the infarcted segment and the residual viable myocardium undergo profound remodeling leading to progressive loss in pump efficiency up to heart failure. Although size of area at risk and occlusion duration influence cell death type and distribution, other factors as microvascular vasoconstriction, oxidative stress and inflammation affect both infarct size and remodeling via apoptosis and hypertrophy. Heme-oxygenases are a complex system modulating vascular tone, oxidative stress and cellular apoptosis. The two isoforms HO-1 and HO-2 break down heme, thereby generating carbon monoxide (a vasodilator gas with anti-inflammatory properties), bilirubin (antioxidant) and iron. HO-2 is constitutive, while HO-1 is induced by free heme, as well as by oxidative stress.

Purpose: To assess the effect of HO-1 over-expression during acute event, on ischemic damage, ventricular remodeling and microvascular reactivity in a rat model of chronic infarct.

Methods: Male rats underwent permanent ligation of the descendant anterior coronary artery (LAD). In one group of animals HO-1 expression was pharmacologically induced by cobalt protoporphyrin (CoPP) administration 10 min following LAD ligation. Thereafter, CoPP was administered i.p. once a week over four weeks. In parallel, a second group was injected with saline (placebo). The effect of CoPP treatment was evaluated in comparison with placebo at one month from surgery on the basis of in vivo ventricular function (2D-echography), plasma levels of natriuretic peptide B (BNP), amount of fibrosis as assayed by morphometric analysis and response of coronary resistance to changes of perfusion pressure in isolated heart experiments.

Results: In a rat model of myocardial infarct HO-1 over-expression in the early phase of ischemia and during the following weeks, as compared to placebo, significantly: a) preserved ejection fraction (75% ± 2 vs 52% ± 4); b) reduced BNP levels (50 ± 23 vs 92 ± 17 pg/ml) and infarct size (23% ± 1 vs 36% ± 1 of left ventricle); c) prevented coronary microvascular constriction in response to lowering perfusion pressure (paradoxical vasoconstriction).

Conclusion: The current findings support the concept that HO-1 over-expression might be a useful strategy for myocardial protection against ischemic damage and progression toward heart failure.

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Cardioprotection at reperfusion by magnesium orotate in Langendorff perfused rat hearts and isolated mitochondria

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Orotic acid, and its salt, magnesium orotate, have been previously shown to markedly improve the energy status and heart function in several pathological settings associated with ischaemia/reperfusion injury. This study was purported to characterize the effects of magnesium orotate (Mg-orotate) administrated at reperfusion on contractile function in Langendorff perfused rat hearts and oxidative phosphorylation in rat heart mitochondria. To this aim isolated rat hearts were subjected to 30 min of global ischemia and 60 min of reperfusion in the presence or absence of 1 mM Mg-orotate given for the whole duration of the postischaemic reperfusion. Recovery of post-ischaemic ventricular function was assessed at 30 min of reperfusion by the left ventricular developed pressure (LVDP) and the maximal and minimal first derivatives of left ventricular pressure and (dLVP/dtmax and dLVP/dtmin) as indices of contractility and relaxation, respectively. All contractile parameters were expressed as percentage of their pre-ischaemic values. In an additional set of experiments, mitochondria were isolated at 30 min of reperfusion from both treated and non-treated hearts and oxygen consumption was measured at 37°C by polarographic oxymetry in the presence of NAD and FAD-linked substrates, respectively. Basal (state 2) and ADP-stimulated (state 3) respiratory rates were recorded and expressed as nanoatoms oxygen/min/mg mitochondrial protein. Respiratory control index (RCI) was calculated as the ratio between state 3 and 2 respiratory rates. At 30 min of reperfusion, Mg-orotate induced a substantial recovery of LVDP (62 ± 3% in treated vs. 45 ± 4% in non-treated animals, $p < 0.01$) and a similar significant improvement of dLVP/dtmax values. With respect to mitochondrial function, no differences were found in respiratory rates between the two groups (13% increase in state 2 and 21% increase in state 3, respectively, p =