Myocardial reperfusion injury management: erythropoietin compared with postconditioning

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Submitted 20 May 2009; accepted in final form 15 July 2009

CORONARY HEART DISEASE remains the leading cause of morbidity and mortality in Western countries. Prompt reperfusion of the ischemic myocardium is critical for salvaging the heart (5, 7, 35). However, a restoration of blood flow can paradoxically have deleterious consequences and lead to lethal myocardial ischemia-reperfusion injury (35). During the last two decades, numerous strategies have been used in an attempt to reduce infarct size and improve ventricular function (46). However, none has shown a consistent clinical benefit. Therefore, new cardioprotective strategies, aimed at limiting myocardial infarct size, are required to attenuate reperfusion injury and to improve clinical outcomes. Ischemic postconditioning (IPost), defined as brief intermittent episodes of ischemia and reperfusion performed at the onset of reperfusion after a prolonged period of ischemia, offers a novel approach to myocardial protection (48). IPost has been shown to exert its cardioprotective effect by upregulating prosurvival kinases named the reperfusion injury salvage kinase (RISK), consisting of phosphatidylinositol 3-kinase/Akt (PI3K/Akt) and extracellular signal-regulated kinase (ERK1/2), at the time of reperfusion (14, 39, 44). Although IPost is clinically applicable and has been successful in attenuating infarct size (42), its use as a clinical cardioprotective strategy to decrease ischemia-reperfusion injury is limited to patients with ongoing acute myocardial infarction subjected to coronary angioplasty. Therefore, the pharmacological activation of the RISK pathway at the time of myocardial reperfusion by administering specific pharmacological agents may provide a more amenable approach to cardioprotection. The hematopoietic cytokine erythropoietin (EPO), known to be an effective agent to treat renal anemia, has recently been shown to have a cardioprotective action against ischemia-reperfusion injury beyond its hematopoietic action (20). The presence of EPO receptors has been reported in cardiomyocytes (45), and the administration of EPO at the time of myocardial reperfusion has been shown to reduce ventricular dysfunction and apoptosis in a number of experimental studies (2, 3, 34). PI3K-Akt-GSK-3β signaling plays a crucial role in protection by EPO against myocardial necrosis and apoptosis (11, 13, 23–26, 28, 30, 36, 43). However, despite a growing body of evidence indicating the cardioprotective effect of both IPost and EPO, no studies to date have ever compared both cardioprotective strategies in the same experimental model. The present study was designed to examine whether EPO is as effective as IPost to decrease postischemic myocardial injury in both Langendorff-isolated-heart and in vivo myocardial ischemia-reperfusion rat models.

MATERIALS AND METHODS

Female Wistar rats, 10 wk old and weighting ~200–250 g, were used in this study. All animals were treated in accordance with the Guide for the Care and Use of Laboratory Animals, published by the National Institutes of Health (NIH Publication No. 85-23, Revised...
1996). The protocol was approved by our regional Animal Care and Use Committee.

Study 1: Langendorff-Isolated Heart

Heart preparation. Rats were anesthetized with an intraperitoneal injection of pentobarbital sodium (100 mg/kg). Heparin (1,500 IU/kg) was administered intravenously to prevent intracoronary clot formation. The heart was rapidly excised and immediately immersed in ice-cold heparinized-modified Krebs-Henseleit buffer containing (in mmol/l) 118 NaCl, 5.6 KCl, 1.2 MgCl2, 1.2 Na2HPO4, 20 NaHCO3, 12 glucose, and 2.4 CaCl2 (pH 7.4) (37). The heart was mounted on a Langendorff-perfusion apparatus (EMKA Technologies, Paris, France) and retrogradely perfused through the aorta with nonrecirculating buffer saturated with 95% O2-5% CO2 at 37°C. The heart was maintained in a thermostatic chamber at 37°C. Perfusion was maintained at a constant pressure of 75 mmHg. A fluid-filled latex balloon was inserted in the left ventricle (LV) via the left atrium for pressure recording. The balloon was connected to a pressure transducer (EMKA Technologies) and inflated to an initial LV end-diastolic pressure between 8 and 10 mmHg.

Isolated heart experimental protocol. All hearts were allowed to stabilize for 20 min. Hearts failing to develop LV systolic pressure over 70 mmHg when the end-diastolic pressure was set at about 10 mmHg were excluded. After the stabilization period, hearts underwent 25 min global ischemia and 30 min reperfusion (37) for hemodynamic study. Hearts were randomly assigned to one of following groups: 1) control (no intervention, n = 12); 2) postconditioning, i.e., three cycles of 10 s of full reperfusion followed by 10 s of global ischemia initiated immediately at the onset of reperfusion (IPost, n = 16) (22); 3) EPO, a single dose of darbepoetin alfa (1,000 IU/kg donor body wt), directly administered to isolated hearts at the onset of reperfusion (EPO, n = 12) (17); 4) IPost + wortmannin (1 μmol/l, initiated at the onset of reperfusion and continued throughout the reperfusion period, n = 10); 5) EPO + wortmannin (initiated at the onset of reperfusion and continued throughout reperfusion, n = 10); 6) IPost + PD-98059 (10 μmol/l, initiated at the onset of reperfusion and continued throughout the reperfusion period, n = 11); and 7) EPO + PD-98059 (initiated at the onset of reperfusion and continued throughout reperfusion, n = 8). In a subset of experiments, reperfusion was maintained for 120 min to evaluate infarct size (38).

Hemodynamic parameters assessment. The following parameters of cardiac function were continuously monitored and simultaneously recorded by Iox 1.593 software (EMKA Technologies): coronary flow (CF) rate, heart rate, LV systolic pressure, LV end-diastolic pressure, and the maximal (dP/dtmax) and minimal (dP/dtmin) value of the first derivative of LV pressure. LV developed pressure (LVDP) was calculated as the difference between the systolic and diastolic pressure of the LV.

Infarct size measurement. Infarct size was determined as previously described in five consecutive randomly selected rats from each group: control, IPost, and EPO (33). After 2 h of reperfusion, the hearts were harvested and the LVs were sectioned from apex to base into five to six 1-mm sections by using a coronal heart slicer matrix (Braintree Scientific). Sections were incubated in 1% triphenyltetrazolium chloride (TTC, Sigma) in phosphate-buffered solution (pH 7.4) at 37°C for 20 min and then fixed in 10% formalin. For each section, the area of necrosis was quantified by planimetry using ImageJ software (NIH, Bethesda, MD) and expressed as a percentage of the total LV area.

Western blot analysis. For analysis of Akt, ERK1/2, and GSK-3β phosphorylation, hearts were collected at the end of the 30 min reperfusion period and the LV was freeze clamped in liquid nitrogen (between stainless steel tongues precooled with liquid nitrogen) before being stored at −80°C. Frozen myocardial tissue samples were powdered in a mortar and pestle precooled to the temperature of liquid nitrogen. Approximately 200 mg of powdered ventricular tissue were used for protein extraction. Frozen myocardial tissue samples were homogenized on ice in 1 ml ice-cold lysis buffer containing 30 mM HEPES, 20 mM KCl, 2.5 mM EGTA, 2.5 mM EDTA, 40 mM sodium fluoride, 4 mM sodium pyrophosphate, 1 mM sodium orthovanadate, 10% glycerol, and 1% Nonidet P-40, a phosphatase inhibitor cocktail (Sigma), and protease inhibitor cocktail (Complete mini, Roche Applied Science, Mannheim, Germany). The homogenate was centrifuged at 13 000 rpm at 4°C for 30 min, and the resulting supernatant was collected. Protein concentration was determined using Bio-Rad DC protein assay kit (Bio-Rad, Hercules, CA) according to the manufacturer’s instructions. Aliquots of the supernatant containing equal amounts of proteins (40 μg) were heated to 95°C for 5 min in sample loading buffer. Proteins were separated on a 10% SDS-PAGE gel and transferred to a nitrocellulose membrane (Amersham Bioscience). After the nonspecific binding sites with 5% nonfat milk for 1 h were blocked in Tris-buffered saline Tween (TBST) containing 20 mM Tris-HCl, 137 mM NaCl (pH 7.6), and 0.1% Tween-20, the membranes were incubated overnight at 4°C with rabbit antibodies against 47Ser-phospho-Akt, total Akt, phospho-ERK1/2, total ERK1/2, P-ser-phospho-GSK-3β, and total GSK-3β (1:1000; Cell Signaling). After being washed in TBST, the membranes were incubated for 1 h at room temperature with horseradish peroxidase-conjugated anti-rabbit IgG secondary antibody (1:2000, Santa Cruz Biotechnologies) and washed, and bound antibody was detected using an enhanced chemiluminescence Western bloting kit (Santa Cruz Biotechnologies). The densities of the bands with appropriated molecular mass (60 kDa for Akt, 42/44 for ERK1/2, and 46 for GSK-3β) were determined semiquantitatively using a lumino-image analyser, LAS-3000 mini (Fujifilm, Tokyo, Japan).

Study 2: In Vivo Ischemia-Reperfusion

Myocardial infarction model. Rats were anesthetized with 60 mg/kg pentobarbital sodium intraperitoneally and ventilated with an endotracheal tube (Series small animal ventilator, SAR-830/P, CWE). Body core temperature was monitored during the surgical procedure with a rectal thermometer and maintained at 36–38°C using a homeothermic blanket linked to a temperature control unit (HB101/2 RS, BIOSEB France). Animal transmitters (CTA-F40 implant, DSI, St. Paul, MN) were subcutaneously implanted for ECG monitoring. A left thoracotomy was performed in the fifth intercostal space. After the pericardium was removed to expose the heart, a 7-0 monofilament suture was placed around the proximal portion of the left anterior descending coronary artery (LAD). The ligature ends were passed through a small length of plastic tube to form a snare. For coronary artery occlusion, the snare was pressed onto the surface of the heart directly above the coronary artery. Ischemia was confirmed by blanching of the myocardium, dyskinesia of the ischemic region, and ST-segment elevation on the ECG. After 45 min of occlusion, reperfusion was achieved by loosening the snare and then confirmed by a marked hyperemic response at reperfusion.

In vivo experimental protocol. Rats were randomly assigned to four groups: 1) control (ischemia-reperfusion only); 2) sham-operated group (same procedure without tightening the snare); 3) postconditioning, i.e., 3 cycles of 10 s of reperfusion and 10 s of ischemia performed immediately after reperfusion, as described previously (IPost) (18, 19, 47); and 4) EPO, a single bolus of darbepoetin alfa (1,000 IU/kg), administered intravenously at the onset of reperfusion (EPO) (1, 15, 29). The rats were weaned from mechanical ventilation and returned to their cages to recover.

Infarct size measurement. Twenty-four hours after reperfusion, the rats were reanesthetized (100 mg/kg pentobarbital sodium ip). Heparin (1,500 IU/kg) was administered intravenously to prevent intracoronary clot formation. The heart was then removed, and the LAD was reoccluded using the 7-0 monofilament suture kept in place. The ascending aorta was retrogradely perfused ex vivo with Evans blue. The LV was sliced transversely from apex to base into five to six
1-mm slices. The slices were incubated in TTC as described above in Study 1: Langendorff-Isolated Heart. Computerized planimetry was used to determine the area at risk (AAR, nonblue) and the area of necrosis (white area). The percentage of myocardial infarction was calculated as the total infarcted area unstained by TTC divided by the total AAR for the heart.

Evaluation of apoptosis. The detection of apoptotic cells in five hearts from each group was carried out using the terminal deoxynucleotidyl transferase-mediated dUTP nick end labeling (TUNEL) method. After infarct size assessment, LV tissues from the AAR and from the remote myocardium were fixed in formalin for 24 h and embedded in paraffin, and 5-μm sections were obtained. The sections were then deparaffinized and rehydrated with xylene and graded alcohol series. The sections were stained using the in situ DeadEndTM Colorimetric Apoptosis Detection System (Promega, Madison, WI) according to the manufacturer’s instructions. Briefly, tissue sections were washed in PBS and then fixed in 4% paraformaldehyde solution before incubation in 20 μg/ml proteinase K for 10 min. Sections were washed in PBS and were incubated with terminal deoxynucleotidyl transferase enzyme in a humidified chamber at 37°C for 60 min for incorporation of biotinylated nucleotides at the 3’-OH DNA ends. The reaction was terminated by transferring the slides to 2× saline sodium citrate. Endogenous peroxidase activity was quenched by an incubation in 0.3% hydrogen peroxide. Finally, streptavidin horseradish peroxidase was bound to the biotinylated nucleotides, and peroxidase activity in each section was demonstrated by the application of a stable chromogen diaminobenzidine. With the use of this procedure, apoptotic nuclei are stained dark brown. The sections were counterstained with hematoxylin for total nuclei. Staining was viewed with an Olympus BX40 microscope and was analyzed. Three sections from each myocardial sample were randomly selected, and 10 microscopic fields per section were evaluated by two independent blind observers. In each field, the nuclei were counted and the percentage of TUNEL-positive nuclei was calculated.

Drugs
Darbepoetin alfa (Aranesp) was provided from Amgen. A darbepoetin alfa dose of 1,000 IU/kg donor body wt is approximately equivalent to 5 μg/kg [Amgen (33)]. Pentobarbital sodium was obtained from Ceva Sante Animal and heparin (Heparin Choay) from Sanofi-Aventis. Wortmannin and PD-98059 were purchased from Sigma. Wortmannin and PD-98059 were dissolved in DMSO and diluted into buffer such that the vehicle constituted <0.02% of the total volume.

Statistical Analysis
All values are expressed as means ± SE. Statistical analyses were performed by using Sigma Stat software version 3.5 (Systat software). Differences between groups were evaluated using one-way analysis of variance (ANOVA), followed by Student-Newman-Keuls post hoc test. For the Langendorff data, ANOVA for repeated measurements was used. A P value < 0.05 was considered to be statistically significant.

RESULTS

EPO is Better than IPost in Preventing Postischemic Cardiac Injury

Study 1: Langendorff-isolated heart. There were no significant differences in baseline hemodynamic parameters (heart rate, LVDP, dP/dt max, dP/dt min and CF levels) between groups before ischemia. LVDP, dP/dt max, and dP/dt min were all impaired after 25 min ischemia in the control hearts (Fig. 1). The mean LVDP of control hearts at 30 min reperfusion was 18.57 ± 4.67 compared with 73.58 ± 4.42 mmHg before ischemia, which represents a recovery of 25%. Hearts treated with either IPost or EPO at the onset of reperfusion showed, respectively, a 55% and 88% contractile function recovery of LVDP at 30 min reperfusion. LV function was significantly improved in both IPost and EPO groups throughout the reperfusion period, with a significantly better LVDP in the EPO group (Fig. 1A).
Table 1. Heart rate and coronary flow data

<table>
<thead>
<tr>
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<th>Baseline</th>
<th>5 min Reperfusion</th>
<th>30 min Reperfusion</th>
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<tbody>
<tr>
<td>Heart rate, beats/min</td>
<td></td>
<td></td>
<td></td>
</tr>
<tr>
<td>Control</td>
<td>243.7±17.5</td>
<td>220.6±28.3</td>
<td>214.7±29.1</td>
</tr>
<tr>
<td>IPost</td>
<td>211.1±17.2</td>
<td>218.3±22.4</td>
<td>203.7±24.5</td>
</tr>
<tr>
<td>EPO</td>
<td>184.9±19.6</td>
<td>214.7±25.3</td>
<td>167.5±19.5</td>
</tr>
<tr>
<td>Coronary flow, ml/min</td>
<td></td>
<td></td>
<td></td>
</tr>
<tr>
<td>Control</td>
<td>14.2±1.2</td>
<td>10.4±1.0</td>
<td>6.8±0.8</td>
</tr>
<tr>
<td>IPost</td>
<td>15.9±0.7</td>
<td>14.6±0.9*</td>
<td>12.3±0.7*</td>
</tr>
<tr>
<td>EPO</td>
<td>17.1±1.1</td>
<td>17.2±1.5*†</td>
<td>13.9±1.3*</td>
</tr>
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</table>

Values are means ± SE. IPost, ischemic postconditioning; EPO, erythropoietin. *P < 0.05 vs. control; †P < 0.05 vs. IPost.

Study 2: in vivo ischemia-reperfusion. Sixty-two rats were used for surgery. Four were excluded because of technical troubles with the ventilator, and 16 died during or immediately after surgery (5 during ischemia, 4 within the first minutes after reperfusion, and 7 within the following 2 h). The mortality rate was similar in all myocardial infarction groups. Finally, 42 rats were available for analysis 24 h after reperfusion (6 sham-operated, 12 control, 12 IPost, and 12 EPO).

The ischemic area induced by LAD ligation (AAR/LV) did not differ among the three myocardial infarction groups (control = 32.5 ± 3.2%, IPost = 29.6 ± 2.9%, and EPO = 32.0 ± 4.8%, not significant). Both IPost and EPO treatment induced infarct size reduction compared with control (40.5 ± 3.6% and 28.9 ± 3.1%, respectively, vs. 53.7 ± 4.3% of the AAR, P < 0.05). Similar to the data observed in study 1, EPO decreased infarct size significantly more than IPost (P < 0.05).

As shown in Fig. 3, TUNEL-positive cells expressed as a percentage of total nuclei were significantly increased in the ischemic zone in the control group (15.09 ± 1.71%) compared with the remote myocardium (1.92 ± 0.24%). IPost and EPO treatments significantly reduced the number of TUNEL-positive cells in the ischemic zone (8.80 ± 1.41% and 7.09 ± 1.51%, respectively) versus the control group (P < 0.05). Whereas this single shot of the apoptosis at 24 h may not reflect the global apoptotic cell death after reperfusion, it is interesting to note that the apoptosis level assessed 24 h after reperfusion in the ischemic zone was not significantly different between EPO- and IPost-treated rats.

P13K/Akt and ERK1/2 Pathways are Both Required in IPost- and EPO-Mediated Cardioprotection

To evaluate the respective role of the PI3K and ERK1/2 pathways in EPO- and IPost-mediated cardioprotection, we used pharmacological inhibitors in the Langendorff-isolated rat
heart study. As illustrated in Fig. 4, the addition of wortmannin, an inhibitor of PI3K/Akt signaling pathway, to IPost- and EPO-treated hearts fully prevented the postischemic recovery of LV function. Furthermore, the protection induced by EPO and IPoT was completely abolished by the treatment with PD-98059, an inhibitor of ERK1/2.

**EPO Induces Higher Phosphorylation of ERK1/2 and the Downstream Target GSK-3β**

To explain the better cardioprotection afforded by EPO compared with IPoT, we evaluated the phosphorylation levels of survival kinases Akt and ERK1/2 and the downstream target GSK-3β in the isolated perfused hearts at 30 min of reperfusion. As shown in Figs. 5 and 6, there was no significant difference in the levels of total Akt, ERK1/2, and GSK-3β in the study groups. Thus the levels of phospho-Akt, phospho-ERK1/2, and phospho-GSK-3β were expressed as their densitometric levels normalized by those levels of each total protein. As illustrated in Fig. 5A, at 30 min of reperfusion, the phosphorylation of myocardial Akt was significantly increased in both IPoT- and EPO-treated hearts compared with control hearts. The administration of wortmannin significantly reduced the level of phosphorylated Akt in the myocardium treated with IPoT and EPO by 31% and 38%, respectively. As shown in Fig. 5B, phospho-ERK1/2 levels were increased in both IPoT and EPO groups compared with controls, although the difference in the IPoT group did not reach statistical significance. This EPO-induced phosphorylation of ERK1/2 was significantly attenuated by PD-98059. Contrary to our findings on phospho-Akt, phospho-ERK1/2 levels were significantly higher in the EPO group compared with the IPoT group (P < 0.05). Furthermore, EPO induced a significantly stronger phosphorylation of GSK-3β than IPoT, which is in line with a higher increased phosphorylation of ERK1/2 (Fig. 6).

**DISCUSSION**

The novelty and significance of this study arise in the demonstration that using three cycles of 10 s ischemia/10 s reperfusion, EPO administered at the time of myocardial reperfusion was significantly more effective than IPoT to protect the rat heart against postischemic myocardial injury. To our knowledge, this is the first demonstration that EPO has a clear advantage over IPoT using head-to-head comparisons in isolated heart and in vivo. The mechanism underlying IPoT- and EPO-induced cardioprotection involves the activation of PI3K/Akt and the mitogen-activated protein kinase ERK1/2 and their downstream target GSK-3β. The increased resistance to myocardial ischemia induced by EPO seems to be mediated by an enhanced phosphorylation of GSK-3β as a consequence of a greater ERK1/2 phosphorylation.

**EPO as a Pharmacologic Postconditioning Agent**

The cardioprotection afforded by IPoT has been demonstrated in several animal species, both in vivo as well as ex vivo (10, 19, 44, 48) and recently in humans (42). Previous studies have demonstrated the antiapoptotic effect of IPoT (18, 31). EPO has also been shown to induce cardioprotection in experimental models (2, 20, 24, 26), mainly by decreasing cardiomyocytes apoptosis after the ischemic insult (8). Whereas IPoT is limited to patients with ongoing acute myocardial infarction subjected to coronary angioplasty, the present study suggests that EPO is a valuable pharmacological postconditioning agent, maybe more effective that IPoT in preventing reperfusion injury. In a pilot clinical study, Lipsic et al. (21) examined the effect of EPO in acute myocardial infarction and showed that a single IV bolus of the EPO analog darbepoetin alfa in patients with first acute ST-segment elevation myocardial infarction is safe and well tolerated.

![Fig. 3. Effect of IPoT and EPO on apoptosis after myocardial infarction. A: control. B: IPoT. C: EPO. Apoptotic cardiomyocyte nuclei appear brown stained, whereas terminal deoxynucleotidyl transferase-mediated dUTP nick end labeling (TUNEL)-negative nuclei appear blue with hematoxylin. Heavy staining of numerous TUNEL-positive cardiomyocytes was observed in the central ischemic region of the LV after 45 min of left anterior descending coronary artery occlusion and 24 h of reperfusion. In contrast, few TUNEL-positive cells were detected in the LV ischemic region of IPoT- and EPO-treated hearts. *P < 0.05 vs. control; n = 5 hearts in each group.](http://www.ajpheart.org/doi/figure/10.1152/ajpheart.00390.2009)
multicenter studies are underway to determine whether this new treatment strategy is able to improve the clinical outcome in patients presenting with acute myocardial infarction.

**Role of Akt-ERK1/2-GSK-3β in EPO- and IPost-Mediated Cardioprotection**

We showed here that inhibitors of the prosurvival kinases Akt and ERK1/2 significantly suppressed the protective effects of IPost and EPO in the isolated-heart model, underlying the pivotal role of PI3K/Akt and ERK1/2 signaling pathways in protecting the heart from ischemia-reperfusion injury. These observations are in accordance with other studies that have also implicated these prosurvival kinases in EPO- and IPost-mediated cardioprotection in other experimental models, in vitro and or in vivo (2, 15, 34). In isolated rat hearts, U-0126, an inhibitor of MEK1/2 administered at the time of reperfusion, blocked EPO-induced protection (2). In an infant rabbit-perfused heart model, the protection observed after the administration of EPO for 15 min before ischemia was abolished when...
EPO was coadministered with an inhibitor of ERK1/2 (PD-98059) (47). Consistent with their findings, our study also observed that PD-98059 abolished cardioprotection achieved by EPO. In contrast, Hanlon et al. (13) showed that U-O126, infused just before EPO treatment, blocked the EPO-induced phosphorylation of ERK1/2 but did not abolish the improvement of postischemic recovery of LVDP in isolated rat hearts. Our study provides further support for the importance of the ERK1/2 pathway in EPO-induced cardioprotection. Contrary to that shown in EPO-treated hearts, Western blot analysis showed no significant increases in the phosphorylation of ERK1/2 in the IPost group compared with controls. However, PD-98059, an inhibitor of ERK1/2, significantly inhibited IPost-induced cardioprotection. This may be due to the small time window of activation of ERK1/2 around the beginning of reperfusion. The activation of ERK1/2 occurs within the first minutes following IPost (2). By the time the samples were taken after 30 min of reperfusion, the signal may have become dissipated or regulated by phosphatases. However, ERK1/2 phosphorylation remained significantly stronger at 30 min in EPO-treated hearts compared with IPost, highlighting a critical role for ERK1/2 in EPO-induced cardioprotection. Taken together, our findings suggest that the phosphorylation of both Akt and ERK1/2 is necessary to trigger the mechanism of EPO- and IPost-induced protection at the time of reperfusion after a prolonged ischemia.

Opposite results exist regarding the role of GSK-3β phosphorylation in the postconditioning mechanisms (9, 27, 40). GSK-3β is a putative regulatory factor of the threshold for opening of the mitochondrial permeability transition pore, and several cytoprotective signals converge to this kinase (16), including ERK1/2 and Akt. Activated ERK can phosphorylate Thr43 of GSK-3β, which primes GSK-3β for subsequent Ser9 phosphorylation (6). The inactivation of mitochondrial GSK-3β by Ser9 phosphorylation has been shown to be primarily important for antiapoptosis resistance of cardiomyocytes afforded by EPO receptor activation (28). The infarct size-limiting effect of EPO has been shown to be linked to the phosphorylation of Ser9 in GSK-3β (26). The increased resistance to myocardial ischemia observed here with EPO may be mediated by the higher phosphorylation of GSK-3β as a consequence of the greater ERK1/2 phosphorylation.

**Experimental Rat Models and Cardioprotection**

Although investigators have not observed any benefit of IPost on myocardial infarct size using in vivo rat models (7, 22), we found the cardioprotective effects of three cycles of 10 s ischemia/10 s reperfusion in our both Langendorff and in vivo rat models. Our results are in accordance with other studies using the same postconditioning regimen in an in vivo rat model (18, 19, 47). The reasons for these discrepancies are unknown. It may involve a different duration of global ischemia, different postconditioning regimens, and a possible effect of sex difference (7).

Conflicting results exist regarding the efficacy of EPO and IPost in female rats (4, 7, 12, 32). Dow and Kloner (7) showed that IPost was ineffective in reducing cardiac infarct size induced by either 30 min or 45 min ischemia in female rats (7). More recently, Penna et al. (32) showed that IPost significantly reduced infarct size and postischemic systolic dysfunction in females after 30 min ischemia, although the effect on infarct size was smaller in female group compared with males. Chan et al. (4) showed a protective effect of EPO in isolated female hearts.
rat hearts, whereas Hale et al. (12) showed that the administration of EPO failed to improve long-term healing or cardiac function after myocardial infarction in the female rat (12). In our models of ischemia-reperfusion, both IPost and EPO exhibited positive effects regarding postischemic cardiac function recovery and RISK signaling pathways activation in female.

Limitations

Only one postconditioning protocol with three cycles of 10 s reperfusion/10 s ischemia at the time of reperfusion has been compared with EPO. However, we chose this protocol as it has been well validated by previous experimental studies, in both isolated-heart (22) and in vivo ischemia-reperfusion rat models (18, 19, 47). We used only female rats to avoid variability in response to treatments. However, it would be of interest to directly compare the efficacy of both IPost and EPO in male and female rat models. Moreover, we did not explore the temporal changes in myocardial salvage kinases, Akt, ERK1/2, and GSK-3β phosphorylation during reperfusion since our results at 30 min of reperfusion show significant differences between the groups. Finally, we did not evaluate Akt, ERK1/2, and GSK-3β activity. An increase in phosphorylation is not necessarily accompanied by increased kinase activity (41).

Conclusion

EPO and IPost share similar intracellular cardioprotective pathways. However, in our models, EPO administered at the time of reperfusion exhibited better protective effects than IPost against reperfusion injury through a higher increase of GSK-3β. These data reinforce the great hope in EPO as a pharmacological agent of postconditioning.

ACKNOWLEDGMENTS

We thank Pierre Legras and Jerome Roux from the animal facilities for taking care of animals, Robert Filmon (Service Commun d’Imageries et d’Analyses Microscopiques) for technical assistance, and Danielle Feuvar for helpful comments and discussions.

GRANTS

N. Ghaboura and S. Tamareille were supported by a fellowship from the Conseil Général du Maine et Loire.

DISCLOSURES

No conflicts of interest are declared by the author(s).

REFERENCES

ERYTHROPOIETIN VERSUS POSTCONDITIONING


