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Morphine preconditioning confers cardioprotection in doxorubicin-induced failing rat hearts via ERK/GSK-3β pathway independent of PI3K/Akt

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ABSTRACT

Preconditioning against myocardial ischemia–reperfusion (I/R) injury can be suppressed in some pathological conditions. This study was designed to investigate whether morphine preconditioning (MPC) exerts cardioprotection in doxorubicin (DOX)-induced heart failure in rats and the mechanisms involved. Phosphatidylinositol-3 kinase/protein kinase B (PI3K/Akt), extracellular signal-regulated kinase (ERK) and glycogen synthase kinase (GSK)-3β pathways were examined. Normal and DOX-induced failing rat hearts were subjected to I/R injury using a Langendorff perfusion system with or without MPC or ischemic preconditioning (IPC). The PI3K inhibitor (wortmannin) or ERK inhibitor (PD98059) was infused before MPC. In normal hearts, both MPC and IPC significantly reduced infarct size and the rise in lactate dehydrogenase (LDH) level caused by I/R injury. Pretreatment with wortmannin or PD98059 abrogated the protective effects of MPC and suppressed the phosphorylation of Akt, ERK and GSK-3β. In failing rat hearts, however, MPC retained its cardioprotection while IPC did not. This protective effect was abolished by PD98059 but not wortmannin. MPC increased the level of p-ERK rather than p-Akt. The phosphorylation of GSK-3β induced by MPC was reversed by PD98059 only. IPC did not elevate the expression of p-ERK, p-Akt and p-GSK-3β in failing rat hearts. We conclude that MPC is cardioprotective in rats with DOX-induced heart failure while IPC is not. The effect of MPC appears to be mediated via the ERK/GSK-3β pathway independent of PI3K/Akt.

Introduction

Heart failure is often the end-stage of a variety of cardiovascular diseases including hypertension, ischemic heart disease, valvular heart disease and cardiomyopathy. Many of these patients will undergo cardiac surgeries and cardiopulmonary bypass (CPB). Failing hearts may be more vulnerable to ischemia–reperfusion (I/R) injury and altered myocardial function postoperatively (Hausenloy et al., 2012). Of note, even in patients subject to non-cardiac surgeries, the incidence of postoperative myocardial injury due to ischemia is still high and associated with increased 30-day mortality (van Waes et al., 2013). Hence, it is important to find effective strategies to protect the failing heart against I/R injury during both cardiac and non-cardiac surgeries.

Numerous treatment strategies have been investigated to protect the myocardium against I/R injury. Ischemic preconditioning (IPC) is well known as a powerful innate protective mechanism, although it is generally clinically impractical due to its invasive and mechanical nature. In addition, the cardioprotective effect of IPC is weakened or abolished in some pathological conditions such as hypercholesterolemia, hyperglycemia, hypertension, cardiac hypertrophy, aging and obesity (Balakumar et al., 2009). Comparatively, pharmacological approaches, which bypass the initial steps within the signaling cascade, may be capable of reducing irreversible tissue injury in such diseased hearts (Balakumar et al., 2009; Ferdinandy et al., 2007). Pharmacologic techniques are also more attractive in terms of practical application, avoiding mechanical ischemia.

Opioids are very commonly used perioperative analgesics and can be administered systemically during general anesthesia or neuraxially with

Abbreviations: AAR, area at risk; CPB, cardiopulmonary bypass; CF, coronary flow; DOX, doxorubicin; DMSO, dimethylsulfoxide; EGFR, epidermal growth factor receptor; ERK, extracellular signal regulated kinase; GPCR, G-protein coupled receptor; GSK, glycogen synthase kinase; HR, heart rate; IPC, ischemic preconditioning; I/R, ischemia–reperfusion; IS, infarct size; LAD, left anterior descending coronary artery; LDH, lactate dehydrogenase; LVDP, left ventricular developed pressure; LVEDD, left ventricular end-diastolic diameter; LVESD, left ventricular end systolic diameter; LVEF, left ventricular ejection fraction; LVFS, left ventricular fractional shortening; mitoK$_{ATP}$, mitochondrial ATP-sensitive potassium channels; MPC, morphine preconditioning; mPTP, mitochondrial permeability transition pore; NS, normal saline; PI3K/Akt, phosphatidylinositol-3-kinase/protein kinase B; PKC, protein kinase C; TTC, triphenyltetrazolium chloride.

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regional techniques. Pre- and post-conditioning with opioids has been clearly demonstrated to exert protective effects on the heart (Li et al., 2009; Peart et al., 2005; Schultz and Gross, 2001). Several signaling pathways and many mediators/end effectors have been implicated in this, including extracellular signal regulated kinase (ERK) (Fryer et al., 2001a), phosphatidylinositol-3-kinase/protein kinase B (PI3K/Akt) (Xu et al., 2011), glycogen synthase kinase (GSK)-3β (Gross et al., 2007b), protein kinase C (PKC) (Fryer et al., 2001b), and mitochondrial ATP-sensitive potassium channels (mitoK$_{ATP}$) (Huh et al., 2001). However, most studies on opioid mediated cardioprotection have been undertaken in normal animal hearts in which I/R and conditioning are imposed in the absence of other disease processes and it is still not known whether opioids maintain their cardioprotective effects in the setting of heart failure.

The aim of this study was to investigate whether morphine preconditioning (MPC) exerts cardioprotection in rats with heart failure and the mechanisms involved. Doxorubicin (DOX), a widely used chemotherapeutic drug for cancer treatment, was used in this study to induce heart failure in rats due to its known cardiotoxicity (Houser et al., 2012; Lu et al., 2009). The different responses of normal and DOX-induced failing rat hearts to MPC or IPC were examined. The PI3K and ERK inhibitors were administrated before MPC to explore the role of PI3K/Akt and ERK signal pathways in morphine-mediated cardioprotection.

Materials and methods

Animal model

All animal experiments were performed in accordance with the Guide for the Care and Use of Laboratory Animals and approved by the Institutional Animal Care and Use Committee of Anhui Medical University. All animals were maintained on a 12 hour light/dark cycle at an ambient temperature of 22 ± 2 °C, with food and water available ad libitum. All studies involving animals are reported in accordance with the ARRIVE guidelines for reporting experiments involving animals (Kilkenny et al., 2010).

Adult male Sprague–Dawley rats (200 ± 30 g) received DOX (Pfizer Italy) weekly via the tail vein in 6 equal injections (each containing 2.0 mg/kg in 0.9% saline) over a period of 6 weeks for a total cumulative dose of 12 mg/kg, to induce chronic heart failure as described previously (Croteau et al., 2012). The rats receiving an equal volume of 0.9% saline (normal saline, NS) intravenously served as controls. Rats were maintained for an additional 2 weeks after the final injection. All animals were observed daily for general appearance, behaviors, abnormal signs and mortality during the course of the study.

Echocardiography

Echocardiography was performed at baseline and at the end (8 weeks) of the study by an individual blinded researcher who specialized in cardiac ultrasound. Rats were anesthetized with 1.5% isoflurane. Two-dimensional and M-mode echocardiography was performed by ACUSON Sequoia 512 with 15L8W-S transducer (Siemens, Germany) to measure left ventricular end-diastolic diameter (LVEDD) and left ventricular end systolic diameter (LVESD), by which the left ventricular fractional shortening (LVFS) and the left ventricular ejection fraction (LVEF) were automatically calculated by the echocardiography system.

Histological examination of the heart

At the end of the 8th week, the rats in the NS and DOX groups ($n = 4$ each) were sacrificed for histological examination. Heart tissues were fixed in 10% neutralized formalin. The fixed samples were embedded in paraffin and sectioned into serial sections (5 μm) and then routinely stained with hematoxylin–eosin according to conventional procedures. The sections were examined under a light microscope (×200) and photographed for morphological analysis. Cardiomyopathy scores are based on the percentage of myocytes showing cytoplasmic vacuolization and/or myofibrillar loss and are graded from 0 to 3 as follows: 0 = no alterations, 1 = <5%, 1.5 = 5% to 15%, 2.0 = 16% to 25%, 2.5 = 26% to 35%, and 3 = 35% affected cells (Herman et al., 1999).

Preparation of Langendorff isolated heart perfusion

The experimental procedure was performed as previously described (Zhang et al., 2005). In brief, all rats were anesthetized with pentobarbital sodium (60 mg/kg, IP, Sigma, USA). The hearts were quickly excised and immediately suspended in the Langendorff apparatus and perfused retrogradely at 100 cm H_2O with KH solution which was oxygenated with 95% O_2 and 5% CO_2 mixture and kept at pH 7.4. Meanwhile, the solution temperature was maintained at 37 °C by a temperature-regulating device. A 5-0 silk thread was passed around the left anterior descending coronary artery (LAD) between the pulmonary artery and the left atrial appendage. Regional ischemia was achieved by the ligation of the LAD and myocardial ischemia was confirmed by regional cyanosis and decreased coronary flow (CF). A latex balloon connected to a pressure transducer was inserted into the left ventricle to monitor hemodynamics including heart rate (HR) and left ventricular developed pressure (LVDP) with a PowerLab System (AD Instrument, Australia).

Experimental protocol

The experimental protocol for the isolated rat heart studies is illustrated in Fig. 1. All DOX-induced failing rat hearts were confirmed by echocardiography and randomly divided into 8 groups: sham ($n = 10$), I/R ($n = 10$), IPC ($n = 10$), MPC ($n = 10$), MPC + wortmannin (MWT, $n = 10$), MPC + PD98059 (MPD, $n = 10$) ($n = 6$ for infarct analysis, $n = 4$ for Western blot at 10 min reperfusion in each group) and wortmannin (WT, $n = 6$ for infarct analysis), PD98059 (PD, $n = 6$ for infarct analysis). The NS-injected normal rat hearts were randomly divided into 8 groups as above. All rat hearts were stabilized for 15 min and subjected to 30 min ischemia followed by 120 min reperfusion except that the hearts in the sham group were perfused with KH solution continuously until the end of the experiment. IPC was elicited with 3 cycles of 5 min of ischemia and 5 min of reperfusion before lethal ischemia. MPC was performed with three cycles of 5 min infusion of 1 μM morphine hydrochloride (Shenyang First Pharmaceutical Factory, China) and 5 min drug-free infusion. The dose of morphine was based on preliminary experiment results (Supplementary Fig. 1). PI3K inhibitor wortmannin (Cell Signaling Technology, USA) and ERK inhibitor PD98059 (Cell Signaling Technology, USA) were dissolved in dimethylsulfoxide (DMSO, the final concentration was less than 0.1%) before use. PD98059 (10 μM), wortmannin (0.1 μM) or vehicle was perfused for a period of 10 min before MPC until 5 min after the end of MPC followed by I/R, respectively. In the PD and WT groups, the inhibitors or vehicle were perfused alone for 40 min without MPC before I/R. The concentrations of the two inhibitors were determined according to previous studies (Cohen et al., 2007; Miki et al., 2007; Suleman et al., 2008) and confirmed by detecting the phosphorylation of ERK or Akt.

Determination of infarct size

At the end of reperfusion, the left anterior descending coronary artery was re-occluded and 0.25% Evans blue was injected to differentiate the area at risk (AAR) from the normal area which was stained blue. The heart was frozen at −80 °C for 20 min and cut into 2 mm slices along the longitudinal heart axis. The slices were placed in separate vials and incubated for 10 min with 1.0% triphenyltetrazolium chloride (TTC) (Sigma, USA) stain in phosphate buffer (pH 7.4) at 37 °C. Then tissues were stored in vials of 10% formaldehyde overnight to enhance the contrast of the stain, infarct size (IS), which was white, and AAR, which was brick red. The volumes of the left and right ventricles (LV + RV),