European Journal of Pharmacology 737 (2014) 125–132

Contents lists available at ScienceDirect

European Journal of Pharmacology

journal homepage: www.elsevier.com/locate/ejphar

Cardiovascular pharmacology

Cardioprotective efficacy against reperfusion injury of EMD-87580: Comparison to ischemic postconditioning

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ARTICLE INFO

Article history:
Received 17 March 2014
Received in revised form
5 May 2014
Accepted 7 May 2014
Available online 22 May 2014

Keywords:
Infarct size
NHE-1 blockade
Ischemic postconditioning
Reactive oxygen species
P-GSK-3β

ABSTRACT

Previous results show that prolonged treatment with EMD-87580 (EMD) NHE-1 blocker attenuates and reverses postinfarction remodelling. Our aim was to evaluate the effects of the treatment of EMD compared to ischemic postconditioning (IPO) in a model of regional ischemia. Isolated hearts were subjected to 40-min coronary occlusion followed by 60-min reperfusion (IC). Other hearts were treated with EMD 5 μM during the first 10 min of reperfusion or submitted to one cycle of 2 min of reperfusion and 2 min of ischemia as IPO protocol.

Infarct sizes (IS), postischemic myocardial and vascular functions were assessed. The concentration of thiobarbituric reactive substances (TBARS), reduced glutathione (GSH) and expression of phosphorylated forms of ERK1/2, Akt, GSK-3β, eNOS were analyzed. MnSOD cytosolic activity – as an index of mitochondrial permeability – was also measured. EMD treatment and IPO decreased IS∼50% and significantly improved the postischemic recovery of contractility and coronary perfusion. TBARS decreased and GSH increased after interventions compared to the values observed in IC hearts. MnSOD cytosolic activity increased in IC group and was significantly attenuated by EMD and abolished in IPO hearts. The content of P-ERK1/2 increased whereas P-Akt, P-GSK-3β and P-eNOS decreased in IC hearts. EMD treatment and IPO reversed these changes.

The present data show that EMD treatment at the beginning of reperfusion-similarly to IPO- limited infarct size and attenuated the postischemic impairment of myocardial function through reactive oxygen species-mediated ERK1/2/Akt/GSK-3β/eNOS pathways.

1. Introduction

Activation of the cardiac isoform of Na^+/H^+ exchanger (NHE-1) is an important regulator of intracellular pH myocardium during ischemia (Piper et al., 1996) extruding H^+ and causing intracellular Na^+ overload (Lazdunski et al., 1985), which leads to intracellular Ca^{2+} overload by activation of reverse mode Na^+/Ca^{2+} exchanger (NCX) (Tani and Neely, 1989). Reactive oxygen species increase at the beginning of reperfusion and together with Ca^{2+} overload are two mechanisms proposed to explain the postischemic cellular damage (Webster, 2012; Murphy and Steenbergen, 2008). It is difficult to separate cause and effect between these two variables since reactive oxygen species mediate the activation of upstream kinases that promote NHE-1 activation (Sabri et al., 1998), and consequently increase of intracellular Ca^{2+} concentration. Moreover, increases of Ca^{2+} and reactive oxygen species are regulators of the formation and/or opening of mitochondrial permeability transition pore (mPTP), a key step in the process of reperfusion injury (Halestrap et al., 2004; Javadov and Karmazyn, 2007). Studies using different experimental models show that NHE-1 inhibition performed before or after ischemia improves the postischemic recovery of myocardial function and limits the infarct size (Mosca and Cingolani, 2008; Scholz et al., 1993; Karmazyn et al., 2001; Avkiran and Marber, 2002; An et al., 2001; Hurtado and Pierce, 2000). It has also been reported that the cardioprotection afforded by the NHE-1 blockers cariporide and KR-32560 (Fantinelli et al., 2006; Jung et al., 2010) is associated to an attenuation of oxidative stress. This beneficial effect appears to be mediated by mitochondrial actions (Jung et al., 2010; Pérez Nuñez et al., 2011; Javadov et al., 2008). However, the effects of NHE-1 specific inhibitor EMD-87580 (2-methyl-4,5-di-(methylsulfonyl-benzoyl)-guanidine), during ischemia and reperfusion are still unclear.

Interesting results from our (Fantinelli and Mosca, 2007) and other laboratories (Zhao et al., 2003; Yang et al., 2004; Iliodromitis et al., 2006) show that the application of brief episodes of

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http://dx.doi.org/10.1016/j.ejphar.2014.05.010
0014-2999/

ischemia-reperfusion at the onset of reperfusion intervention called "ischemic postconditioning" (IPO) decreases postischemic alterations. Several kinases such as phosphatidylinositol 3-kinase PI3K/Akt and GSK-3β, ERK1/2 MAPKs have been involved in the mechanism of IPO-mediated cardioprotection (Zhu et al., 2006; Miura and Miki, 2009). However, the participation of those kinases in the actions of NHE-1 blockers during ischemia and reperfusion has not been clearly defined.

The purpose of the present study was to assess the effects of EMD-87580 NHE-1-selective inhibitor applied at the onset of reperfusion compared to IPO on myocardial infarct size and tissue oxidative stress in a model of regional ischemia in rats analyzing the pathways involved.

2. Material and methods

2.1. Isolated heart preparation

All procedures followed during the present investigation conform to the Guide for the Care and Use of Laboratory Animals published by the US National Institutes of Health (NIH Publication No. 85-23, revised 1996) and to the guidelines laid down by the Animal Welfare Committee of La Plata School of Medicine.

Hearts from male Wistar rats were quickly isolated and perfused with Ringer's solution containing (in mmol/L) 118 NaCl, 5.9 KCl, 1.2 $MgSO_4$, 1.35 $CaCl_2$, 20 $NaCO_3H$ and 11.0 glucose (gassed with 95% O2–5% CO2, pH 7.4, 37 °C) through the Langendorff technique and using the perfusion pump Masterflex Model 7016-21 (Cole–Parmer). The conductive tissue in the atrial septum was damaged with a fine needle to achieve atrioventricular block, and the right ventricle was paced at 280 ± 10 beat/min. A latex balloon tied to the end of a polyethylene tube was passed into the left ventricle through the mitral valve; the opposite end of the tube was then connected to a Statham P23XL pressure transducer. The balloon was filled with water to provide a left ventricular end-diastolic pressure (LVEDP) of 8–12 mmHg, and this volume remained unchanged for the rest of the experiment. Coronary perfusion pressure (CPP) was monitored at the point of cannulation of the aorta and was adjusted to approximately 60–70 mmHg. Coronary flow (CF), which was controlled with a peristaltic pump, was 11 ± 2 mL/min. Left ventricular pressure (LVP) and its first derivative (dP/dt) were recorded with a direct writing recorder.

2.2. Experimental protocols

After 20-min stabilization, the following experimental protocols were performed (Fig. 1):

Non-ischemic control hearts (NIC; $n=8$): Hearts were perfused for 120 min without any treatment.

Ischemic control hearts (IC, $n=9$): Hearts were subjected to 40 min of occlusion of the left anterior descending (LAD) artery followed by 60 min of reperfusion.

EMD-87580 (EMD, $n=8$): EMD was a gift from Merck KGaA, Darmstadt, Germany. Hearts were treated during 10 min at the beginning of reperfusion with a dose of 5 μM of EMD NHE-1 blocker. This dose was found to completely inhibit NHE-1 activity in myocytes in which intracellular acidosis was induced by ammonium chloride pulsing technique (Gan et al., 2010).

Ischemic postconditioning (IPO, $n=8$): One cycle of ischemia-reperfusion 2 min each was applied at the onset of reperfusion.

The effects of EMD-87580 in non-ischemic hearts (NIC+EMD, $n=4$) administered at the same time as the EMD group were assessed.

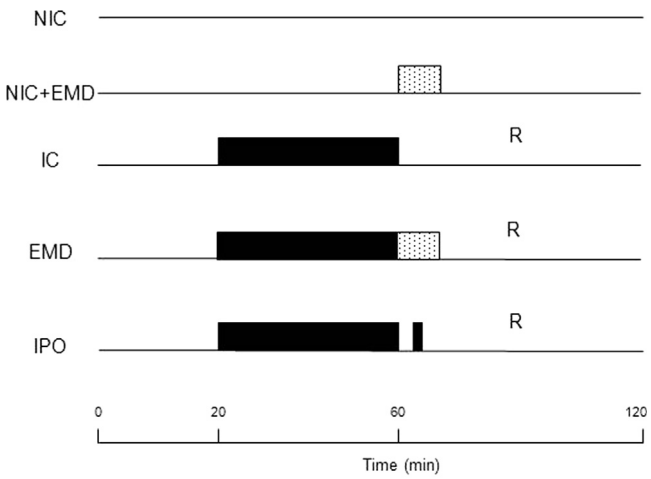

Fig. 1. Scheme of the different experimental groups. NIC: Non-ischemic control; NIC+EMD: treatment with the NHE-1 blocker EMD-87580; IC: Ischemic control; EMD: treatment with EMD-87580 and IPO: ischemic postconditioning.

2.3. Infarct size determination

At the end of reperfusion, the LAD was occluded again and the myocardium was perfused for 1 min with a 0.1% solution of Evans blue. This procedure delineated the non-ischemic myocardium as dark blue. After staining, the hearts were frozen and cut into six transverse slices, which were incubated for 15 min at 37 °C in 1% solution of tripenyltetrazoliumchloride (TTC). All atrial and right ventricular tissues were excised. To measure myocardial infarction the slices were weighed and scanned. The infarcted (pale), viable ischemic/reperfused (red), and non-ischemic (blue) areas were measured by computed planimetry (Scion Image 1.62; Scion Corp., Frederick, Maryland, USA). By TTC technique non-infarcted viable myocardium containing dehydrogenase stained brick red; whereas the infarcted tissue remained unstained because of the lack of the enzyme. The area at risk (AAR) was the portion of the left ventricle supplied by the previously occluded coronary artery identified by the absence of blue dye. Infarct weights were calculated as $(A1 \times W1)+(A2 \times W2)+(A3 \times W3)+(A4 \times W4)+(A5 \times W5)+(A6 \times W6)$, where A is the area of infarct in the slice and W is the weight of the respective section. The weight of the AAR was calculated in a similar fashion. IS was expressed as a percentage of AAR (Fantinelli et al., 2013).

2.4. Systolic and diastolic function

The systolic function was assessed by the left ventricular developed pressure (LVDP) – calculated by subtracting left ventricular end diastolic pressure (LVEDP) from the left ventricular peak pressure values – and the maximal velocity of rise of LVP ($+dP/dt_{max}$). The diastolic function was evaluated through the maximal velocity of relaxation ($-dP/dt_{max}$) and LVEDP values.

2.5. Coronary resistance (CR) determination

CR was calculated as a quotient between CPP and CF and expressed as difference between the values obtained at the end of reperfusion period and that observed in the preischemic period.

2.6. Preparation of tissue homogenate

At the end of reperfusion a portion of left ventricle (LV) was homogenized in 5 volumes of PO_4KH_2 25 mM and ClK 140 mM at pH=7.4 with a polytron homogenizer. Aliquots of homogenate were used for assessing reduced glutathione content (GSH) and