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Cyclosporine-A mimicked the ischemic pre- and postconditioning-mediated cardioprotection in hypertensive rats: Role of PKCε

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ABSTRACT

Our aim was to assess the action of cyclosporine-A (CsA) against reperfusion injury in spontaneously hypertensive rats (SHR) compared to the effects of ischemic pre- (IP) and postconditioning (IPC), examining the role played by PKCε. Isolated hearts were submitted to the following protocols: IC: 45 min global ischemia (GI) and 1 h reperfusion (R); IP: a cycle of 5 min GI and 10 min of R prior to 45 min-GI; and IPC: three cycles of 30 s-GI/30 s-R at the start of R. Other hearts of the IC, IP and IPC groups received CsA (mitochondrial permeability transition pore inhibitor) or chelerythrine (Che, non-selective PKC inhibitor). Infarct size (IS) was assessed. TBARS and reduced glutathione (GSH) content — as parameters of oxidative damage, the expression of P-Akt, P-GSK-3β, P-PKCε and cytochrome c (Cyc) release — as an index of mitochondrial permeability and the response of isolated mitochondria to Ca^{2+} were also measured. IS similarly decreased in preconditioned, postconditioned and CsA treated heart showing the highest values in the combinations IP + CsA and IPC + CsA. TBARS decreased and GSH was partially preserved after all interventions. The content of P-Akt, P-GSK-3β and P-PKCε increased in cytosol and decreased in mitochondria after IP and IPC. In CsA treated hearts these enzymes increased in both fractions reaching the highest values. Cyc release was attenuated and the response of mitochondria to Ca^{2+} was improved by the interventions. The beneficial effects of IP and IPC were annulled when PKC was inhibited with Che. A PKCε/VDAC association was also detected. These data show that, in SHR, the CsA treatment mimicked and reinforced the cardioprotective action afforded by IP and IPC in which PKCε-mediated attenuation of mitochondrial permeability appears as the main mechanism involved.

1. Introduction

The left ventricular hypertrophy consequent to chronically elevated blood pressure has been frequently associated with postischemic contractile dysfunction (Friehs and del Nido, 2003). It was previously shown in stroke-prone spontaneously hypertensive rats (SHR-SPs) (Chen et al., 2000; Yano et al., 2011) and recently in spontaneously hypertensive rats (SHR) (Fantinelli et al., 2013) that hypertrophy aggravates the irreversible reperfusion injury. It was also demonstrated in our and in other laboratories that ischemic pre- (IP) and postconditioning (IPC) decrease myocardial dysfunction (Fantinelli et al., 2013; Fantinelli and Mosca, 2007) and limit the infarct size in hypertrophied hearts (Ferdinandy et al., 2007; Speechly-Dick et al., 1994).

It is well established that hypertension induces oxidative stress, but major sources of reactive oxygen species (ROS) are not absolutely certain, raising the possibility that NADPH oxidase, nitric oxide synthase, lipoxygenases, cyclo-oxygenases, xanthine oxidase, and cytochrome P450 enzymes, and the mitochondrial respiratory chain may be important ROS producers (Puddu et al., 2008; Lesnefsky et al., 2001). ROS are implicated in the mitochondrial permeability transition pore (mPTP) opening which plays a crucial role in the ischemia–reperfusion-induced cell death (Halestrap, 2009; Ong et al., 2015). Regardless of all controversies in resolving the molecular enigma of mPTP, a general consensus

Abbreviations: CsA, cyclosporine A; SHR, spontaneously hypertensive rats; IP, ischemic preconditioning; IPC, ischemic postconditioning; PKCε, protein kinase C ε; Che, chelerythrine; TBARS, thiobarbituric acid reactive substances; GSH, reduced glutathione; Akt, Serine/threonine-specific protein kinase; Akt, Serine/threonine-specific protein kinase; Cyc, cytochrome c; VDAC, voltage-dependent anion channels; mPTP, mitochondrial permeability transition pore; CyD, cyclophilin D; TTC, triphenyltetrazolium chloride; GAPDH, glyceraldehyde 3-phosphate dehydrogenase; GPCR, G protein-coupled receptors; GP, G-protein; CN, calcineurin; Akt, serine/threonine-specific protein kinase; ROS, radical oxygen species; ANT, adenine nucleotide translocator.

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exists on the role of cyclophilin D (CyD) as a regulator of mPTP (Ong et al., 2015). Indeed, the immunosuppressive agent, cyclosporine A (CsA) was effective in reducing infarct size in patients (Hausenloy et al., 2012; Piot et al., 2008) and in experimental studies performed in normotensive animals (Javadov and Kuznetsov, 2013; Nakagawa et al., 2005; Mewton et al., 2010). However, its effects in hypertensive animals have not yet been reported.

On the other hand, it was reported that PKC and specifically the ε isoform (PKCε) have an important role in IP triggering process and is a potential mediator of IPC (Budas and Mochly-Rosen, 2007; Baines et al., 2002; Yoshida et al., 1997; Inagaki et al., 2003) in normotensive animals. According to a previous paper (Johnsen et al., 2005) the expression of PKCε in hypertensive is higher – approximately 3 fold – than normotensive rats. In a previous work performed in our laboratory (Fantinelli and Mosca, 2007) the postischemic myocardial dysfunction of hearts from SHR was severely affected when PKC was inhibited indicating that this kinase plays a protective role against myocardial stunning. If PKC is also involved in cell death caused by a more prolonged ischemia has not been properly clarified.

Therefore, our objective was to compare the effects of CsA treatment with those obtained by IP and IPC on infarct size and oxidative stress in isolated hearts from SHR examining the role played by PKCε.

2. Materials and methods

An expanded 'Methods' section is available in Online Data Supplements.

2.1. Isolated rat heart

All procedures followed during this investigation were approved by the Institutional Animal Care and Use Committee (IACUC) of the Faculty of Medicine, University of La Plata following the Guide for the Care and Use of Laboratory Animals from the Institute for Laboratory Animal Research, National Research Council, Washington, D.C., National Academy Press, 2010 and/or European Union Directive for Animal Experiments 2010/63/UE.

Experiments were conducted in 5-month-old SHR, which were originally derived from Charles River Breeding Farms, Wilmington, Mass. Systolic blood pressure (SBP) was measured weekly using the methods indicated in Supplementary material online. Animals were anesthetized with an intraperitoneal injection of sodium pentobarbital (60 mg/kg body wt). The heart was rapidly excised and perfused by the non-recirculating Langendorff technique and it was paced at 280 ± 10 beats/min.

2.2. Experimental protocols

After 30 min of stabilization, hearts from SHR were assigned to the following experimental protocols (Fig 1): Non-ischemic control hearts (NIC; n = 6): Hearts were perfused for 135 min without any treatment and ischemic control hearts (IC; n = 8): Hearts were subjected to 45 min of normothermic global ischemia followed by 1 h of reperfusion. Global ischemia was induced by stopping the perfusate inflow line and the heart was placed in a saline bath held at 37 °C; ischemic preconditioning (IP, n = 8): One cycle of 5 min of ischemia and 10 min of reperfusion was previously applied to the 45-min ischemic period followed by 1-hour reperfusion; and ischemic postconditioning (IPC, n = 8): Three cycles of 30 s of ischemia and 30 s of reperfusion was applied early during reperfusion.

Chelerythrine (Che) treatment: Hearts were treated with 1 μM Che (PKC inhibitor), 10 min before 45-min ischemia (IC + Che, n = 4; IPC + Che, n = 5) or 5-min ischemia (IP + Che, n = 6).

Cyclosporine A (CsA) treatment: Hearts were treated with 0.5 μM CsA (mPTP inhibitor), 10 min before 45-min ischemia (IC + CsA, n = 4; IPC + CsA, n = 4) or 5-min ischemia (IP + CsA, n = 5).

In other hearts (n = 3) CsA was added to non-ischemic control hearts. Separated groups of hearts subjected to the same protocols (n = 6 for each one) were used for biochemical determinations. Additional hearts submitted to the different protocols (n = 4 for each one) were used for mitochondria isolation.

2.3. Infarct size determination

Infarct size was assessed by the widely validated triphenyltetrazolium chloride (TTC) staining technique and expressed as a percentage of area at risk.

2.4. Preparation of tissue homogenate

At the end of reperfusion a portion of left ventricle (LV) was homogenized in 5 volume of 25 mmol/L PO_4KH_2–140 mmol/L ClK at pH = 7.4

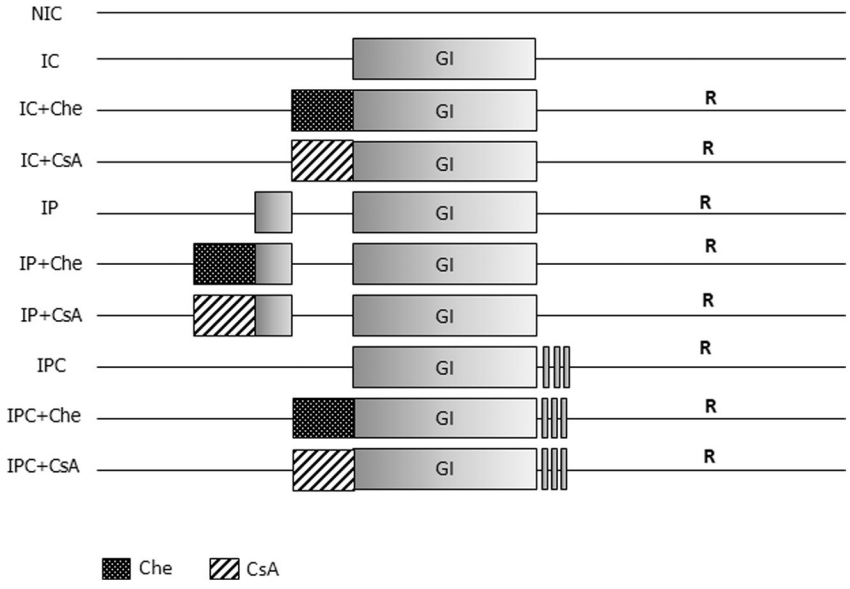

Fig. 1. Scheme of the experimental protocols. NIC: non-ischemic control; IC: ischemic control; IC + Che: ischemic control in the presence of chelerythrine (Che) inhibitor of PKC; IC + CsA: ischemic control in the presence of cyclosporine A (CsA), inhibitor of cyclophilin D; IP: ischemic preconditioning; IP + Che: ischemic preconditioning plus Che; IP + CsA: ischemic preconditioning plus CsA; IPC: ischemic postconditioning; IPC + Che: ischemic postconditioning plus Che; IPC + CsA: ischemic postconditioning plus CsA.