Phenylephrine produces late pharmacological preconditioning in the isolated rat heart

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1. Introduction

Brief periods of ischemia lead to a reduced severity of cardiac injury following a sustained period of ischemia. This cardioprotective effect has been termed ischemic preconditioning (Tonkovic-Capin et al., 2002). The cardioprotective effect of preconditioning occurs in two phases. The early phase lasts for 1–3 h while the late phase of protection occurs at least 12 h following the initial sublethal ischemic insult and has been shown to last up to 72 h (Zaugg et al., 2003).

Certain pharmacological agents can mimic the cardioprotective effects of ischemic preconditioning (Tonkovic-Capin et al., 2002). Although the triggers and mediators of preconditioning are still not well understood, some studies have shown that stimulation of α1-adrenoceptors is one of the essential triggers of the early phase of ischemic preconditioning (Banerjee et al., 1993). In addition, recent studies have indicated that activation of α1-adrenoceptors can exert both early (Salvi, 2001; Imani et al., 2008; Rojas et al., 2008) and late preconditioning (Kudej et al., 2006; Tejero-Taldo et al., 2002).

It has been shown that mitochondrial ATP-sensitive K channels (mKATP) have a greater influence on cardioprotection afforded by preconditioning (Tonkovic-Capin et al., 2002; Rajesh et al., 2004). In this manner, diazoxide, as a specific opener of the mKATP channels, can mimic early ischemic preconditioning (Shen et al., 2004) and application of 5HD (5-hydroxydecanoate), as a putatively specific mKATP channel blocker, prevents cardioprotective effect of early ischemic preconditioning (Tsukamoto et al., 2005) or pharmacological preconditioning (Obal et al., 2005). However, it has been suggested that the mKATP channels are involved as a subcellular mediator in early preconditioning afforded by α1-adrenoceptor activation (Gao et al., 2007; Cohen et al., 2001), but the role of this channel in phenylephrine-induced late preconditioning is still unknown.

Therefore, the present study was designed to evaluate the possibility of phenylephrine-induced late cardioprotection in the isolated rat heart and examined the potential involvement of the mKATP channels.

2. Materials and methods

2.1. Preparation of isolated hearts

Male wistar rats (200–250 g) were housed in an air-conditioned colony room on a light/dark cycle at 21–23 °C with free access to food and water. The animals were anesthetized with sodium pentobarbital (60 mg/kg, i.p) and given heparin sodium (500 IU). Hearts were...
rapidly excised and placed in an ice-cold buffer, and mounted on a constant pressure (80 mmHg) Langendorf-perfusion apparatus. All experiments were conducted in accordance with the institutional guidelines of Tehran University of Medical Sciences (Tehran, Iran) and the National Institutes of Health guidelines for the care and use of laboratory animals.

Hearts were perfused retrogradely with modified Krebs–Henseleit bicarbonate buffer containing (in mmol/l): NaHCO3 25; KCl 4.7; NaCl 118.5; MgSO4 1.2; KH2PO4 1.2; glucose 11; CaCl2 2.5 gassed with 95% O2/5% CO2 (pH 7.35–7.45 at 37 °C). A latex, fluid-filled, isovolumic balloon was introduced into the left ventricle through the left atrial appendage and inflated to give a preload of 8 to 10 mmHg and connected to a pressure transducer (Harvard). Two thin stainless steel electrodes fixed at the ventricular apex and right atrium were employed to record ECG for monitoring heart rate.

A surgical needle was passed under the origin of the left anterior descending coronary artery, and the ends of the suture were passed through a pipette tip to form a snare. Regional ischemia was induced by tightening the snare and reperfusion was performed by releasing the ends of the suture. The perfusion apparatus was water-jacketed to maintain constant perfusion temperatures of 37 °C. Hearts were allowed to beat spontaneously throughout the experiments. Hemodynamic parameters [left ventricular developed pressure (the difference between left ventricular systolic and diastolic pressure) and heart rate] were monitored with a homemade program (Ossillo Graph Monitor, Biomed). Left ventricular function was assessed by left ventricular parameters [left ventricular developed pressure (the difference between left ventricular systolic and diastolic pressure) and heart rate]

2.2. Experimental protocol

After heart isolation and prior to baseline period, all hearts were perfused and allowed to stabilize for 30 min in which heart rate and left ventricular developed pressure were maintained at the same level of pressure transducer (Harvard). Two thin stainless steel electrodes fixed at the ventricular apex and right atrium were employed to record ECG for monitoring heart rate.

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3. Results

3.1. Hemodynamic function

Since heart rate and left ventricular developed pressure may recover to different degrees, rate pressure product was calculated by multiplying heart rate by left ventricular developed pressure and

2.3. Infarct size measurement

After completion of the reperfusion period, the left coronary artery was reoccluded, and Evans blue dye was infused via the aorta to differentiate the ischemic zone from the non ischemic zone. Hearts were frozen overnight and then, sliced into 2-mm transverse sections from apex to base. Slices were then incubated with 1% triphenyltetrazolium chloride (TTC in 0.1 M phosphate buffer, pH 7.4) for a period of 20 min at 37 °C.

TTC reacts with viable tissue, producing a red formazan derivative, which is distinct from the white necrotic tissue once fixed in 10% formalin for 24 h. The areas of the left ventricle and infracted tissues were measured by way of a planimetry from the scanned hearts by using Photoshop program. Volumes were obtained by multiplying the area by the thickness of the slices. Area at risk was expressed as a percentage of left ventricular volume for each heart. The infarct size was determined by using computer-aided planimetry and expressed as a percentage of area at risk.

2.4. Measurement of creatine kinase-MB (CK-MB)

The level of CK-MB was calculated in coronary effluent samples at 5 min and 60 min of reperfusion with a specific CK-MB Kit (Pars Azmoon), using an autoanalyzer (Roche Hitachi Modular DP Systems, Mannheim, Germany).

2.5. Chemicals

Phenylephrine, 5HD and triphenyltetrazolium chloride (TTC) were obtained from Sigma-Aldrich (Deisinhofen, Germany) and general laboratory chemicals were acquired from Merck (Darmstadt, Germany). Stock solutions of phenylephrine and 5HD were diluted with distilled water and added to the Krebs’–Henseleit bicarbonate (KHB) buffer and equilibrated with O2 (95%)-CO2 (5%) (pH 7.4 at 37 °C).

2.6. Statistical analyses

Data are expressed as means ± S.E.M. Statistical comparison of means between groups was made by one-way ANOVA and a subsequent Tukey test. Significant differences were determined as P<0.05.

Fig. 1. Schematic illustration of experimental groups. 5HD, 5-hydroxydecanoate; PE, phenylephrine.
presented as a reliable left ventricular function parameter for the isolated heart.

No differences were found between the experimental groups for rate pressure product at baseline period (Table 1a). Rate pressure product (as a percentage of an individual baseline) decreased during regional ischemia period, and recovered partially throughout reperfusion period (Table 1a and 1b).

Compared to ischemia/reperfusion group, pretreatment with phenylephrine in both early and late phases increased the recovery of rate pressure product (70.9% and 68.2% vs. 47% in IR group, \( P < 0.001 \)) at the end of reperfusion period. Administration of 5HD significantly abolished the recovery effect of phenylephrine on rate pressure product in early and late phases (52.7% and 51.7% respectively, \( P < 0.001 \)) and increased this parameter to the level of IR group as seen in Fig. 2. Administration of 5HD alone prior to ischemia/reperfusion had no effect on hemodynamic function compared to ischemia/reperfusion group.

### 3.2. Infarct size and area at risk

Fig. 3a shows the original pictures of TTC staining heart and Fig. 3b illustrates the ratio of area at risk to total left ventricular area and ratio of infarct size to area at risk. There were no significant differences in the ratio of area at risk to total left ventricular area between the hearts in all experimental groups. The ratio of infarct size to area at risk decreased considerably from 37.8±0.7 in ischemia/reperfusion group to 8.8±0.3 and 8.8±0.3 in phenylephrine-early preconditioning and phenylephrine-late preconditioning groups respectively. Phenylephrine-induced infarct limitation was inhibited by administration of 5HD in early and late phase, where the ratio of infarct size to area at risk increased to 36.1±1.6 and 35.9±2.1, respectively. Pretreatment with 5HD before ischemia/reperfusion had no effect on area at risk and infarct size compared to ischemia/reperfusion group.

### Table 1a

Hemodynamic parameters.

<table>
<thead>
<tr>
<th>Group</th>
<th>Baseline</th>
<th>End of ischemia</th>
</tr>
</thead>
<tbody>
<tr>
<td></td>
<td>HR (bpm)</td>
<td>LVDP (mmHg)</td>
</tr>
<tr>
<td>Con</td>
<td>289±3.8</td>
<td>90±3.0</td>
</tr>
<tr>
<td>IR</td>
<td>282±4.4</td>
<td>88±3.5</td>
</tr>
<tr>
<td>PE-Early</td>
<td>283.5±5.7</td>
<td>92±4.36</td>
</tr>
<tr>
<td>5HD-PE Early</td>
<td>260±7.2</td>
<td>95±4.7</td>
</tr>
<tr>
<td>PE Late</td>
<td>267.2±7.2</td>
<td>86±5.4</td>
</tr>
<tr>
<td>PE Late-5HD</td>
<td>275±10.11</td>
<td>84±3.1</td>
</tr>
<tr>
<td>5HD-IR</td>
<td>278±8</td>
<td>81±4.5</td>
</tr>
</tbody>
</table>

LVDP, left ventricular developed pressure; CF, coronary flow; HR, heart rate; bpm, beat per minute; in Control (Con), ischemia/reperfusion (IR), phenylephrine-Early preconditioning (PE-Early), phenylephrine-Late preconditioning (PE-Late), phenylephrine Early preconditioning-5HD (5HD-PE Early), phenylephrine Late preconditioning-5HD (PE Late-5HD), phenylephrine-Late preconditioning (PE-Late-5HD), 5HD-phenylephrine Early preconditioning (5HD-PE Early), 5HD-phenylephrine Late preconditioning (5HD-PE Late), 5HD-phenylephrine Early preconditioning (5HD-PE Early), 5HD-phenylephrine Late preconditioning (5HD-PE Late). Data are presented as Mean±S.E.M.

### Table 1b

Hemodynamic parameters.

<table>
<thead>
<tr>
<th>Group</th>
<th>15 min of reperfusion</th>
<th>60 min of reperfusion</th>
</tr>
</thead>
<tbody>
<tr>
<td></td>
<td>HR (b.min)</td>
<td>LVDP %</td>
</tr>
<tr>
<td>Con</td>
<td>271±3.2</td>
<td>88±3.2</td>
</tr>
<tr>
<td>IR</td>
<td>268±2.3</td>
<td>90±2.8</td>
</tr>
<tr>
<td>PE-Early</td>
<td>258±2.3</td>
<td>150±5.8</td>
</tr>
<tr>
<td>5HD-PE Early</td>
<td>248±2.4</td>
<td>85±2.5</td>
</tr>
<tr>
<td>PE Late</td>
<td>267±1.2</td>
<td>168±7.3</td>
</tr>
<tr>
<td>PE Late-5HD</td>
<td>263±3.2</td>
<td>74±1.5</td>
</tr>
<tr>
<td>5HD-IR</td>
<td>261±4.5</td>
<td>87±4</td>
</tr>
</tbody>
</table>

LVDP, left ventricular developed pressure; CF, coronary flow; HR, heart rate; bpm, beat per minute; in Control (Con), ischemia/reperfusion (IR), phenylephrine Early preconditioning (PE-Early), phenylephrine Late preconditioning (PE-Late), phenylephrine Early preconditioning-5HD (5HD-PE Early), phenylephrine Late preconditioning-5HD (PE Late-5HD), 5HD-phenylephrine Early preconditioning (5HD-PE Early), 5HD-phenylephrine Late preconditioning (5HD-PE Late), 5HD-phenylephrine Early preconditioning (5HD-PE Early), 5HD-phenylephrine Late preconditioning (5HD-PE Late). Data are presented as Mean±S.E.M.

* Significant difference with Con group \( P < 0.001 \).

** Significant difference with IR group \( P < 0.001 \).

### Fig. 2

Recovery of RPP (Rate Pressure Product, Basal value %) in Control (Con), ischemia/reperfusion (IR), phenylephrine-Early preconditioning (PE-Early), phenylephrine-Late preconditioning (PE-Late), 5HD-phenylephrine Early preconditioning (5HD-PE Early), phenylephrine Late preconditioning-5HD (PE Late-5HD), 5HD, 5 hydroxydecanoate. Data are presented as Mean±S.E.M. Significant difference with Con group \( P < 0.001 \). Significant difference with IR group \( P < 0.001 \).
3.3. CK-MB levels

CK-MB levels in coronary artery effluent markedly declined by phenylephrine-induced preconditioning in early and late compared with ischemia/reperfusion group in 5 min (30.3±15.8, 38.2±3 vs. 135±15 respectively) and 60 min (26.4±14.5, 28.5±3.3 vs.73±14 respectively) after reperfusion. Addition of 5HD restored the levels of CK-MB as seen in ischemia/reperfusion group in early and late phenylephrine-induced preconditioning either in 5 min (118.2±2.4 and 107.9±12.7) and 60 min (75.1±3.9 and 85±12.4) after reperfusion. Compared to ischemia/reperfusion group, administration of 5HD prior to ischemia/reperfusion didn’t change CK-MB level of coronary effluent (Fig. 4).

4. Discussion

Our study indicates that phenylephrine induces early and late cardioprotection in the ischemic myocardium, which is manifest as an increase in recovery of post-ischemic developed pressure was paralleled by a significant attenuation in CK-MB release from coronary effluent and reduction in infarct size. These cardioprotective effects of phenylephrine in early and late phases were inhibited when putatively specific mKATP channel blocker, 5HD, was administrated before ischemia. Therefore, we have demonstrated that phenylephrine confers early and late cardioprotection from ischemia and reperfusion injury via activation of the mKATP channels.

The role of α1-adrenoceptors has been studied extensively in the early preconditioning. Pharmacological activation of α1-adrenoceptors has been shown to mimic early preconditioning (Salvi, 2001; Imani et al., 2008; Rojas et al., 2008) and its blockade abolishes the cardioprotective effect of preconditioning (Piascik and Perez, 2001). Although α1-adrenoceptor stimulation mimics ischemic preconditioning, but it is not an essential component in the mechanism behind the protective effect of ischemic preconditioning in rat heart (Vasara et al., 2002). Similarly, studies examining the role of α1-adrenoceptors...
have found no effect on early preconditioning (Baghelai et al., 1999b). One study which utilized surgical sympathectomy failed to block early preconditioning (Ardell et al., 1996). Our study shows that pharmacological stimulation of the α1-adrenoceptors induced early cardioprotection in the isolated rat heart. Earlier studies have shown that stimulation of α1-adrenoceptor could produce early preconditioning in humans (Loubani et al., 2004), isolated rat heart (Ravingerova et al., 2002), and rabbit heart (Bankwala et al., 1994).

It has been shown that post-ischemic functional recovery is enhanced not only 30 min but also 24 h after norepinephrine treatment in the isolated perfused rat heart (Meng et al., 1999). Therefore, it seems that activation of α1-adrenoceptor can mimic ischemic preconditioning and induce late cardioprotection. In this manner, we have shown that stimulation of α1-adrenoceptors produces cardioprotection beginning 24 h after the in vivo treatment with norepinephrine in the isolated rat heart. Previously, it has been shown that phenylephrine induces late preconditioning in rabbit by a decrease in infarct size (Baghelai et al., 1999b), limitation of apoptosis and amplified bcl2/bax ratio (Baghelai et al., 1999a).

ATP-sensitive potassium (KATP) channels exist in the sarcolemmal membrane as well as the mitochondrial membrane of cardiomyocytes. It has been recently shown that preconditioning induces activation and trafficking of sarcolemmal KATP channels (Budas et al., 2004), which, in turn, decreases the duration of action membrane potential and Ca2+ influx, thus promoting cell survival during ischemia (Kane et al., 2005; Sukhodub et al., 2007).

Also, mitochondrial KATP channels play an important role in the preconditioning (Baghelai et al., 1999a). It has been shown that pretreatment with a specific opener of the mKATP channel, most notably diazoxide, produced an effective cardioprotection against ischemia/reperfusion injuries and this effect was prevented by putative specific blocker of this channel, 5HD (Garlid et al., 2003; Hanley and Daut, 2005).

Previously, it has been found that other pharmacological agents such as acetylimide, bradykinin, opioids and phenylephrine, but not adenosine, trigger early preconditioning by the opening of mKATP channels in the isolated rabbit heart (Cohen et al., 2001). Our study also has indicated that phenylephrine produced early cardioprotection through opening of mKATP channel in the isolated rat heart.

When hemodynamic performance of the heart is used to assess recovery after ischemia/reperfusion, the effect of 5HD to reverse the protective effects of diazoxide is not observed (Grover et al., 1995; Lim et al., 2002). Conversely, in our study addition of 5HD reversed the protective effect of phenylephrine on hemodynamic parameters in early and late phases.

Evidence documenting the importance of the KATP channels in the late preconditioning (Bernardo et al., 1999a; Ockaili et al., 1999) prompted us to test the role of mKATP channels in phenylephrine-induced late cardioprotection in the isolated rat heart. In this regard, it has been indicated that late preconditioning conferred by adenosine (Bernardo et al., 1999b), opioids (Fryer et al., 1999) and isoflurane (Tonkovic-Capin et al., 2002) was abolished with addition of 5HD and therefore, activation of mKATP channel was involved in late cardioprotection. Our results also show that administration of 5HD blocked phenylephrine-induced late preconditioning and it seems that phenylephrine induced late preconditioning via activation of mKATP channels in the isolated rat heart.

Although the mechanism by which phenylephrine leads to the opening of mKATP channels after 24 h and exerts late cardioprotection was not explored in this study, several hypotheses can be put forward to explain this effect.

However, 5-HD is generally assumed as a specific blocker of mKATP channel in preconditioning-induced cardioprotection, it has been reported that 5-HD blocks sarcolemmal KATP channels (Notsu et al., 1992) and also, 5-HD has a complex metabolic actions in cardiomyocytes (Hanley et al., 2005; Hanley et al., 2003). Therefore, it seems that 5-HD contributes in preconditioning through different mechanisms and its action isn’t only mKATP channels inhibition.

In contrast to early preconditioning, the late phase of preconditioning requires the de novo synthesis of cardioprotective proteins. It has been shown that the stimulation of α1-adrenoceptors by phenylephrine is the activating pathway to protein kinase C (Broadley and Penson, 2004) resulting in the activation of mitogen activated protein kinase and subsequent nuclear translocation of several transcription factors such as nuclear factor-κB (Tejero-Taldo et al., 2002). Activation of nuclear factor-κB can lead to the induction of the late preconditioning via iNOS gene translocation (Xuan et al., 1999). It is well-known that iNOS is one of the common mediators in late preconditioning-induced by pharmacological agent (Xi and Kukreja, 2000) and ischemic preconditioning (Guo et al., 1999). On the other hand, release of nitric oxide through opening of the mKATP channels can lead to cardioprotection (Sasakini et al., 2000).

In conclusion, this study shows that phenylephrine induced late preconditioning and application of 5-HD eliminated this cardioprotection in the isolated rat heart.

References


