Ischemic Preconditioning Activates Mitochondrial but not Sarcolemmal ATP-sensitive Potassium Channels in Intact Rat Hearts: 82Rb NMR Study.

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Introduction

Ischemic preconditioning (IPC) consists of brief repetitive ischemic periods and induces protection against subsequent prolonged ischemia. Several studies have implicated ATP-sensitive K+ channels (KATP) in IPC. There are two types of KATP: sarcolemmal (Sl) [1] and mitochondrial (M) [2]. M KATP are proposed to be activated during IPC, because M-specific KATP opener, diazoxide (Diaz), mimics IPC and M-specific KATP blocker, 5-hydroxydecanoate (5HD) abolishes the cardioprotective effects of IPC [3,4]. However, direct demonstration of the link between IPC and activation of M KATP in intact hearts is lacking. The aim of this study was to compare activation of Sl and M KATP by IPC in perfused rat hearts and to study signaling cascade activated by IPC by using 82Rb MRS.

Methods

Heart perfusion and experimental protocols. Isolated hearts of Sprague-Dawley rats (330-400 g) were perfused retrogradely with Krebs-Henseleit buffer (KHB) containing (in mM) 118 NaCl, 25 NaHCO3, 4.7 KCl, 1.75 CaCl2, 1.2 MgSO4, 11.0 glucose, 0.5 EDTA aerated with 95% O2/5% CO2 (constant flow of ~15 ml/min). The hearts were loaded for 30 min with a K+ tracer, 82Rb+, by substituting 50% of K+ with Rb+ in KHB. Rb+ washout was initiated by switching to KHB. Rb+ efflux was monitored using 82Rb MRS. Rb+ washout kinetics was analyzed to obtain Rb+ efflux rate constants (τ×10^-3, min^-1). Sl K+ efflux was calculated at 36°C when cytoplasmic (C) and M pools of Rb+ are kinetically indistinguishable [5]. M efflux was measured at 20°C following removal of C pool, which leaves myocytes faster than M pool at this temperature [5]. IPC was applied prior to Rb+ washout and included three 4-min periods of no-flow ischemia interrupted by 6-min periods of reperfusion. Drugs were infused for 20 min. Means ± SD for n=3-9 are presented.

NMR spectroscopy. 82Rb spectra were acquired every 2 min (90° pulse, 10 ms recycle time) at 117.8 MHz on a Bruker AM 360 spectrometer. Rb+ washout was initiated by switching to KHB. Rb+ efflux was monitored using 82Rb MRS. Rb+ washout kinetics was analyzed to obtain Rb+ efflux rate constants (τ×10^-3, min^-1). Sl Rb+ efflux was measured at 36°C when cytoplasmic (C) and M pools of Rb+ are kinetically indistinguishable [5]. M efflux was measured at 20°C following removal of C pool, which leaves myocytes faster than M pool at this temperature [5]. IPC was applied prior to Rb+ washout and included three 4-min periods of no-flow ischemia interrupted by 6-min periods of reperfusion. Drugs were infused for 20 min. Means ± SD for n=3-9 are presented.

Results

At 36°C, Rb+ efflux kinetics was monoexponential indicating that sarcolemma was the limiting factor and Sl and M Rb+ pools could not be distinguished [5]. Sl rate constant determined under these conditions was 39.6 ± 0.9. At 20°C, Rb+ efflux had two phases: C Rb+ left myocytes fast while M Rb+ remained sealed inside M and left slowly (k = 13.1 ± 1.7), which is in excellent agreement with the previously published data [5]. Diaz (0.1 mM) did not affect Sl efflux: k = 40.6± 4.9. However, after 8 min of infusion, Diaz activated M Rb+ efflux by 30% (Table 1). Neither M-specific 5HD (0.2 mM), nor non-specific KATP blocker, glibenclamide (Glib, 0.005 mM) affected this stimulation (Table 1). IPC had no effect on Sl Rb+ efflux: k = 41.6± 3.5. However, IPC stimulated M efflux by 17% (Table 2). Both M KATP inhibitors Glib and 5HD reversed this stimulation, confirming that M KATP were activated (Table 2). To investigate signaling pathway involved in M KATP activation by IPC, we infused protein kinase C (PKC) inhibitor, chelerythrine (Chel, 0.005 mM) or adenosine receptor antagonist, 8-(p-sulfophenyl) theophylline (SPT, 0.05 mM) during IPC. Inhibition of PKC directly, by Chel, or indirectly, by preventing adenosine receptor activation, abolished stimulation of M efflux by IPC (Table 2).

Discussion

Under normal metabolic conditions, Sl and M KATP are inhibited by high concentration of intracellular ATP. In beating hearts, Sl Rb+ efflux is mediated mainly by voltage-gated K+ channels and possibly K+/anion co-transporters. Basal M Rb+ efflux can be mediated by K+/H+ exchanger, which extrudes K+ or Rb+ from M and brings H+ into the matrix. Opening of M KATP shifts the balance between K+ import and Rb+ export, until the efflux of Rb+ compensates for the increased influx of K+. Diaz is considered to be a specific opener for M KATP. Indeed, Diaz activated M but not Sl KATP. Selective activation of Rb+ efflux by Diaz at 20°C strongly suggests that we measured activity of M KATP.

It is thought that protective effect of IPC involves activation of KATP. Initially Sl KATP were implicated in this phenomenon, however later focus shifted towards M KATP. However, evidence is indirect and based on the inhibition of cardioprotective IPC effect by Glib and 5HD as well as IPC-like effects of Diaz [3,4]. In this study, we demonstrated that IPC stimulated M but not Sl KATP. Glib and 5HD antagonized stimulating effect of IPC but not Diaz stimulation, indicating that activation of M KATP by IPC and by Diaz involves different mechanisms. Diaz directly activates M KATP by binding to a specific site. Inhibitory effects of KATP blockers on this binding site depend on the assay system [6]. In contrast, IPC can activate a specific intracellular signaling pathway, which has been proposed to include M KATP as the end-effectors [3,4]. Recently, it was found that in isolated myocytes, activation of PKC by phorbol ester potentiates opening of M KATP presumably as a result of PKC phosphorylation [7]. We could not test the effect of phorbol ester because it produced powerful vasoconstriction of the isolated hearts, which made the perfusion problematic. In our experiments, PKC inhibitor, chelerythrine, and adenosine receptor antagonist, SFT, reversed the effect of IPC. Thus, we concluded that in intact rat hearts, IPC activates M KATP in adenosine receptor- and PKC-dependent manner.

References


Table 1. Effect of Diaz, Diaz + Glib, and Diaz + 5HD on M Rb+ efflux rate constant at 20°C (*P≤0.05)

<table>
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<th>control</th>
<th>Diaz</th>
<th>Diaz + Glib</th>
<th>Diaz + 5HD</th>
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<tr>
<td>Rb+ efflux rate constant at 20°C</td>
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<td>17.2±3.1*</td>
<td>17.2±3.9*</td>
<td>17.1±3.3*</td>
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Table 2. Effect of IPC on M Rb+ efflux rate constant at 20°C (*P≤0.05)

<table>
<thead>
<tr>
<th></th>
<th>control</th>
<th>IPC</th>
<th>IPC + Glib</th>
<th>IPC + 5HD</th>
<th>IPC + Chel</th>
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<td>IPC rate constant at 20°C</td>
<td>13.1±1.7</td>
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<td>11.3±2.8</td>
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