Ischemic Preconditioning Activates Mitochondrial but not Sarcolemmal ATP-sensitive Potassium Channels in Intact Rat Hearts: ^{87}Rb 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 (K_{ATP}) in IPC. There are two types of K_{ATP}: sarcolemmal (Sl) [1] and mitochondrial (M) [2]. M K_{ATP} are proposed to be activated during IPC, because M-specific K_{ATP} opener, diazoxide (Diaz), mimics IPC and M-specific K_{ATP} blocker, 5-hydroxydecanoate (5HD) abolishes the cardioprotective effects of IPC [3,4]. However, direct demonstration of the link between IPC and activation of M K_{ATP} in intact hearts is lacking. The aim of this study was to compare activation of Sl and M K_{ATP} by IPC in perfused rat hearts and to study signaling cascade activated by IPC by using ^{87}Rb 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 $NaHCO_3$, 4.7 KCl, 1.75 $CaCl_2$, 1.2 $MgSO_4$, 11.0 glucose, 0.5 EDTA aerated with 95% O_2/5% CO_2(constant flow of ~15 ml/min). The hearts were loaded for 30 min with a K^+ tracer, Rb^+, by substituting 50% of K^+ with Rb^+ in KHB. Rb^+ washout was initiated by switching to KHB. Rb^+ efflux was monitored using ^{87}Rb MRS. Rb^+ washout kinetics was analyzed to obtain Rb^+ efflux rate constants (x10^{-3}, min^{-1}). Sl Rb^+efflux was measured at 36^{o}C when cytoplasmic (C) and M pools of Rb^+ are kinetically indistinguishable [5]. M efflux was measured at 20^{o}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 ± STDEV for n=3-9 are presented.

NMR spectroscopy. ^{87}Rb spectra were acquired every 2 min (90^o pulse, 10 ms recycle time) at 117.8 MHz on a Bruker AM 360 spectrometer using a broadband probe (Morris Instruments). Memory size was 512 data points, a line broadening factor, 150 Hz. To minimize the signal from extracellular ^{87}Rb, the hearts were perfused in a dry mode by placing the suction line at the bottom of the NMR tube. A capillary containing 10 µl of 1 M RbCl + 5 M KI was used as chemical shift and concentration reference.

Results

At 36^{o}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^{o}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 K_{ATP} 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 K_{ATP} inhibitors Glib and 5HD reversed this stimulation, confirming that M K_{ATP} were activated (Table 2). To investigate signaling pathway involved in M K_{ATP} 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 K_{ATP} 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 K_{ATP} 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 K_{ATP}. Indeed, Diaz activated M but not Sl K_{ATP}. Selective activation of Rb^+ efflux by Diaz at 20^{o}C strongly suggests that we measured activity of M K_{ATP}.

It is thought that protective effect of IPC involves activation of K_{ATP}. Initially Sl K_{ATP} were implicated in this phenomenon, however later focus shifted towards M K_{ATP}. However, evidence was 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 directly demonstrated that IPC stimulated M but not Sl K_{ATP}. Glib and 5HD antagonized stimulating effect of IPC but not Diaz stimulation, indicating that activation of M K_{ATP} by IPC and by Diaz involves different mechanisms. Diaz directly activates M K_{ATP} by binding to a specific site. Inhibitory effects of K_{ATP} 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 K_{ATP} as the end-effectors [3,4]. Recently, it was found that in isolated myocytes, activation of PKC by phorbol ester potentiates opening of M K_{ATP} 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, SPT, reversed the effect of IPC. Thus, we concluded that in intact rat hearts, IPC activates M K_{ATP} in adenosine receptor- and PKC-dependent manner.

References

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

control	13.1±1.7
Diaz	17.2±3.1*
Diaz + Glib	17.2±3.9*
Diaz + 5HD	17.1±3.3*

Table 2. Effect of IPC on M Rb^+ efflux rate constant at 20^{o}C (*P≤ 0.05)

control	13.1±1.7
IPC	15.7±1.4*
IPC + Glib	11.3±2.8
IPC + 5HD	12.5±3.1
IPC + Chel	11.2±1.6
IPC + SPT	10.5±1.8