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Short communication

The K_{ATP} blocker sodium 5-hydroxydecanoate does not abolish preconditioning in isolated rat hearts

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Abstract

Blockers of ATP-sensitive K^+ channels (K_{ATP}) abolish preconditioning in several species. Glyburide does not abolish preconditioning in rat hearts, but this may be due to a loss of its activity during ischemia. We determined the effect of a K_{ATP} blocker, which is more active during ischemia (sodium 5-hydroxydecanoate, 5-HD), on preconditioning in isolated rat hearts. Rat hearts were subjected to 4 periods of 5 min global ischemia followed by 30 min of global ischemia and reperfusion. Preconditioning significantly enhanced post-ischemic recovery of function and reduced lactate dehydrogenase (LDH) release vs. sham. 5-HD (100 μM) did not abolish preconditioning. Cromakalim (20 μM) was protective in this ischemic model and this was abolished by 5-HD. This is further evidence that K_{ATP} opening is not the mechanism of preconditioning in rats.

Keywords: Myocardial ischemia; K^+ channel, ATP-sensitive; Preconditioning

1. Introduction

The cardioprotective mechanism of preconditioning is still not clear. In most models, adenosine A_1 receptor activation appears to be involved with preconditioning (see review by Downey et al., 1993). Other mechanisms have been suggested, indicating the possibility of multiple pathways depending on experimental conditions or species of animal tested (Banerjee et al., 1993). Work from several laboratories showed that opening of ATP-sensitive K^+ channels (K_{ATP}) may mediate preconditioning (Auchampach et al., 1992; Toombs et al., 1993a) and that A_1 activation and K_{ATP} opening are linked (Yao and Gross, 1993).

Unfortunately, neither adenosine A_1 receptor antagonists nor the K_{ATP} blocker glyburide appear to abolish preconditioning in rat hearts (Grover et al., 1993; Li and Kloner, 1993). This is interesting because both K_{ATP} openers and adenosine A_1 receptor agonists protect ischemic rat hearts and K_{ATP} blockers abolish the effects of K_{ATP} openers in rats (Grover et al., 1989; Lasely et al., 1990). Glyburide loses efficacy under ischemic conditions (Venkatesh et al., 1991) and may not block K_{ATP} during preconditioning. The purpose of this study was to determine the effect of a structurally dissimilar K_{ATP} blocker, sodium 5-hydroxydecanoate (5-HD), which may not lose its efficacy during ischemia (McCullough et al., 1991), on preconditioning in isolated rat hearts.

2. Materials and methods

Male Sprague-Dawley rats (400–500 g) were anesthetized with 100 mg/kg sodium pentobarbital (i.p.). The trachea was intubated and the jugular vein was injected with heparin (1000 U/kg). While being mechanically ventilated, the hearts were perfused in situ via retrograde cannulation of the aorta, excised and quickly moved to a Langendorff apparatus where they were perfused with oxygenated Krebs-Henseleit solution containing (in mM): 112 NaCl, 25 $NaHCO_3$, 5 KCl, 1.2 $MgSO_4$, 1 KH_2PO_4, 1.2 $CaCl_2$, 11.5 glucose at a constant perfusion pressure (85 mm Hg). A water-filled latex balloon was inserted into the left ventricle and connected to a Statham pressure transducer for measurement of left ventricular pressure. The hearts were allowed to equilibrate at which time end diastolic pressure was adjusted to 5 mm Hg and this balloon

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volume was maintained. Baseline function and coronary flow (extracorporeal electromagnetic flow probe, Carolina Medical Electronics, King, NC, USA) were then measured. End diastolic pressure was subtracted from left ventricular peak systolic pressure, giving left ventricular developed pressure. Cardiac temperature was maintained throughout the experiment by submerging the hearts in 37°C buffer.

In hearts to be preconditioned, a protocol of 4 periods of 5 min of global ischemia was initiated, each separated by 5 min of reperfusion. Ischemia was initiated by completely shutting off perfusate flow. Sham hearts were perfused with the buffer solution for an equivalent period of time. After preconditioning or sham, the hearts were subjected to 30 min of global ischemia (again completely shutting off perfusate flow) and 30 min reperfusion. Starting 10 min before preconditioning and during the reperfusion periods separating the individual preconditioning episodes, either vehicle ($n = 8$) or 100 μM 5-HD ($n = 6$) was given. Vehicle ($n = 6$, 0.04% dimethyl sulfoxide) or 100 μM 5-HD ($n = 6$) was also infused for an equivalent period of time in sham hearts. Severity of ischemia or reperfusion injury was determined from the recovery of contractile function at 30 min into reperfusion, and lactate dehydrogenase (LDH) release into the reperfusate as previously described (McCullough et al., 1991). This was measured as the cumulative LDH released per g of heart over the reperfusion period.

Unlike our previous work on the pharmacologic effects of K_{ATP} modulators in ischemic rat hearts, the preconditioning studies were performed without pyruvate because it increases the threshold for preconditioning (Sargent et al., 1994). We have not determined whether K_{ATP} openers can protect ischemic rat hearts which are not perfused with pyruvate and if 5-HD can abolish this effect. Rat hearts were prepared as described above and were subjected to 10 min of the following treatments: vehicle ($n = 10$, 0.04% dimethyl sulfoxide), 20 μM cromakalim ($n = 10$), 100 μM 5-HD ($n = 8$), 100 μM 5-HD + 20 μM cromakalim ($n = 8$). The hearts were then rendered globally ischemic for 30 min followed by 30 min of reperfusion. Recovery of contractile function and LDH release were determined as described above.

3. Results

Baseline contractile function and coronary flow were similar for all groups in the preconditioning study (Table 1). The data are shown under baseline conditions (Control), 5 min after the last preconditioning period (1 min before the final, 30 min ischemia, Post-Prec) and at 30 min into reperfusion (Reper). Preconditioning significantly reduced left ventricular developed pressure before the longer ischemic period compared to sham treated hearts and 5-HD did not affect this observation. Reperfusion left ventricular developed pressure was significantly reduced in vehicle treated sham hearts and preconditioning significantly

Table 1
The effect of 100 μM sodium 5-hydroxydecanoate (5-HD) on cardiac function and coronary flow in sham or preconditioned rat hearts

	Control	Post-Prec	Reper 30 min
LVDP (mm Hg)			
Vehicle-Sham	125 ± 4	123 ±6	9 ±2 [a]
Vehicle-Precond	117 ± 2	77 ±4 [a,b]	49 ±6 [a,b]
5-HD-Sham	117 ± 4	121 ±5	11 ±5 [a]
5-HD-Precond	119 ±11	88 ±7 [a,b]	41 ±6 [a,b]
Coronary flow (ml / min / g)			
Vehicle-Sham	21.1 ± 1.3	18.6 ± 1.4	10.7 ± 1.2 [a]
Vehicle-Precond	22.0 ± 1.6	19.6 ± 1.5	22.4 ± 5.0 [b]
5-HD-Sham	20.5 ± 1.9	21.9 ± 3.1	11.1 ± 2.5 [a]
5-HD-Precond	19.8 ± 1.2	21.9 ± 1.5	13.8 ± 2.3 [a]
LDH release (U / g)			
Vehicle-Sham	–	–	33 ±5
Vehicle-Precond	–	–	13 ±2 [b]
5-HD-Sham	–	–	32 ±6
5-HD-Precond	–	–	15 ±4 [b]

All values are mean ± S.E.; Post-Prec = after final preconditioning period (before 30 min ischemia); Reper = at the 30 min time point; Precond = preconditioned hearts; 5-HD = 100 μM sodium 5-hydroxydecanoate. [a] Significantly different from its respective, paired control value ($P < 0.05$). [b] Significantly different from its respective sham group value ($P < 0.05$).

Table 2
The effect of 100 μM sodium 5-hydroxydecanoate (5-HD) on the protective effect of cromakalim on cardiac function in ischemic/reperfused rat hearts

	Pre-drug	Post-drug	Reper 30 min
LVDP (mm Hg)			
Vehicle	121 ±2	119 ±3	9 ±2 [a]
20 μM cromakalim	114 ±2	97 ±2 [a,b]	35 ±4 [a,b]
100 μM 5-HD	116 ±5	112 ±7	7 ±5 [a]
5-HD + cromakalim	119 ±5	100 ±3 [a,b]	9 ±4 [a]
Coronary flow (ml / min / g)			
Vehicle	21.0 ± 1.8	18.3 ± 0.9	11.7 ± 0.5 [a]
20 μM cromakalim	20.6 ± 1.1	24.6 ± 0.9 [a,b]	22.4 ± 3.0 [b]
100 μM 5-HD	19.5 ± 1.8	20.1 ± 0.9	11.2 ± 2.1 [a]
5-HD + cromakalim	18.8 ± 1.2	23.9 ± 0.8 [a,b]	10.3 ± 1.0 [a]
LDH release (U / g)			
Vehicle	–	–	27 ±2
20 μM cromakalim	–	–	14 ±2 [b]
100 μM 5-HD	–	–	28 ±3
5-HD + cromakalim	–	–	28 ±4

All values are mean ± S.E.; 5-HD = sodium 5-hydroxydecanoate. [a] Significantly different from its respective predrug value ($P < 0.05$). [b] Significantly different from its respective vehicle group value ($P < 0.05$).

enhanced its recovery. 5-HD did not alter the protective effect of preconditioning on the recovery of function. While we did not show the data for all time points, preconditioning enhanced the rate of recovery during the 30 min reperfusion. Vehicle treated, preconditioned hearts had significantly enhanced reflow, an effect which was blocked by 5-HD. Preconditioning also significantly reduced reperfusion LDH release and this was not altered by 5-HD. Heart rate was not altered by any treatment (data not shown, vehicle baseline heart rate = 298 ± 9).

We needed to be sure that the concentration of 5-HD used was sufficient to block K_{ATP}. Before ischemia, cromakalim significantly increased coronary flow and slightly reduced left ventricular developed pressure (LVDP), effects which were not abolished by 5-HD (Table 2). Cromakalim significantly enhanced the post-ischemic recovery of left ventricular developed pressure as well as post-ischemic reflow. 5-HD completely abolished the reperfusion recovery of left ventricular developed pressure as well as the increased reflow. Cromakalim significantly reduced reperfusion LDH release and this was abolished by 5-HD. Heart rate was slightly reduced during reperfusion in all groups (data not shown).

4. Discussion

Cardiac preconditioning has been universally found to exert cardioprotective effects. Several studies suggested an interaction between adenosine A_1 receptor activation and K_{ATP}. One hypothesis is that adenosine A_1 receptor activation increases the open probability of K_{ATP} indicated by the demonstration that K_{ATP} blockers abolish the protective effects of adenosine A_1 receptor agonists and K_{ATP} openers (Yao and Gross, 1993; Toombs et al., 1993b; McCullough et al., 1991). An important role for K_{ATP} in preconditioning exists in most mammalian species, including man (Tomai et al., 1994), with one notable exception. Neither adenosine A_1 receptors nor K_{ATP} blockers abolish the protective effects of preconditioning in rats (Grover et al., 1993; Li and Kloner, 1993). This is interesting as both adenosine A_1 receptor agonists and K_{ATP} openers are cardioprotective in ischemic/reperfused rat hearts (Grover et al., 1989; Lasely et al., 1990).

These rat studies were performed using the K_{ATP} blocker glyburide. It has been shown that glyburide loses efficacy during ischemia (Venkatesh et al., 1991). Thus, ischemic preconditioning may reduce the ability of glyburide to block K_{ATP}. 5-HD has a different pharmacologic profile vs. glyburide as its blocking activity appears to be seen only during ischemia (McCullough et al., 1991). Thus, 5-HD offers a unique tool for determining the role of K_{ATP} in preconditioning in rat hearts. Previous work has shown that 5-HD abolishes preconditioning in some species (Auchampach et al., 1992). 5-HD abolishes the cardioprotective effects of K_{ATP} openers in rats, although this was shown in our isolated rat heart model where the hearts were perfused with pyruvate (McCullough et al., 1991).

Pyruvate increases the threshold for preconditioning in isolated rat hearts (Sargent et al., 1994) and thus we do not include pyruvate in our perfusate in our rat preconditioning model. We have not previously determined whether K_{ATP} openers can protect ischemic rat hearts not perfused with pyruvate. 5-HD abolished the protective effects of cromakalim without affecting its pre-ischemic coronary dilator activity, as expected because of its lack of activity under nonischemic conditions (McCullough et al., 1991). Both K_{ATP} openers and 5-HD appear to work in a similar manner to that observed in pyruvate perfused rat hearts. 5-HD was used at 100 μM as this is the highest concentration which blocks K_{ATP} without exerting pro-ischemic effects on its own and thus cloud interpretation of the data. Nevertheless, 5-HD had no effect on the protective activity of preconditioning. This is further evidence that K_{ATP} opening is not involved in preconditioning in rats. It was interesting that 5-HD abolished the hyperemic response observed with preconditioning; however, this did not alter the protective effect of preconditioning of myocardial function and enzyme release. K_{ATP} blockers have been shown to attenuate reactive hyperemia under some circumstances (Daut et al., 1990), but our results indicate that the reactive hyperemia is not responsible for preconditioning.

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