

# Mechanisms of protection afforded by preconditioning to endothelial function against ischemic injury

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Mechanisms of protection afforded by preconditioning to endothelial function against ischemic injury. *Am. J. Physiol.* 271 (*Heart Circ. Physiol.* 40): H1801–H1806, 1996.—The aim of this study was to assess whether the cardioprotective effect of ischemic preconditioning (IPC) on endothelial function in resistance coronary arteries of the rat involves adenosine and/or activation of ATP-sensitive K<sup>+</sup> channels (K<sub>ATP</sub> channels). Isolated rat hearts perfused under constant-flow conditions were exposed to 30 min of partial ischemia (flow rate 1 ml/min) followed by 20 min of reperfusion. Preconditioning was performed with 5 min of ischemia and 10 min of reperfusion before the 30-min ischemia. After the 20-min reperfusion period, coronary arteries were precontracted with U-46619 (0.1 μM), and the coronary response to the endothelium-dependent vasodilator serotonin (5-HT; 10 μM) was compared with that of the endothelium-independent vasodilator sodium nitroprusside (SNP; 3 μM). K<sub>ATP</sub> channels or adenosine receptors were blocked with perfusion of either glibenclamide (0.3 μM) or 8-phenyltheophylline (8-PT; 5 μM), respectively, starting 15 min before IPC or a corresponding sham period. In untreated hearts, ischemia selectively diminished 5-HT-induced vasodilation, compared with sham hearts (without ischemia). The vasodilation by SNP was unaffected after ischemia and reperfusion. Preconditioning in untreated hearts preserved the vasodilation produced by 5-HT. Treatment of hearts with either glibenclamide or 8-PT halved the vasodilation produced by both 5-HT and SNP in sham hearts. Glibenclamide reduced by one-half, whereas 8-PT completely blocked, the protective effect of IPC on endothelium-dependent vasodilation. These results suggest that IPC affords protection to endothelial function in resistance coronary arteries of the rat partially by activation of K<sub>ATP</sub> channels. Adenosine plays a major role in that protection.

coronary circulation; rat; serotonin; sodium nitroprusside

SHORT PERIODS OF ISCHEMIA, single or repetitive, render the heart more resistant to a subsequent longer ischemia. This phenomenon, called ischemic preconditioning (IPC), reduces myocardial infarct size (4, 20), decreases the number and severity of reperfusion-induced arrhythmias (8, 32), and improves systolic ventricular function recovery (4). The cardioprotective effect of IPC has been documented in various species, including pigs (31), dogs (20), rabbits (4), rats (14, 34), and humans (35).

Recent studies have demonstrated that the beneficial effect of IPC is not limited to the cardiomyocytes but can be observed in endothelial cells in various experimental models, including dog resistance coronary arteries in vivo (5) and conduit coronary arteries of the rat in vitro (28). Some studies have demonstrated that ischemia-reperfusion attenuated endothelial function in large coronary vessels (24, 33) and in coronary microvessels (5). Little is known about the effect of IPC on

endothelial function in intact rat coronary circulation, and the mechanisms involved in this endothelial protection remain unknown.

The first aim of this study was therefore to evaluate whether IPC affords protection against ischemic injury to the endothelium of coronary vessels in the isolated rat heart. The second aim was to identify the mechanisms whereby IPC provides this protection. Two mechanisms were postulated to explain this protective effect of IPC: 1) activation of adenosine receptors by adenosine released during IPC and 2) participation of ATP-sensitive potassium channels (K<sub>ATP</sub> channels).

## **METHODS**

*Preparation of hearts.* The investigation was performed in accordance with guidelines of the Canadian Council on Animal Care. Male Sprague-Dawley rats (300–350 g) were narcotized with CO<sub>2</sub> until a complete loss of consciousness and then rapidly decapitated. The thorax was rapidly opened, and the heart was excised and immersed in ice-cold heparinized buffer (10 IU/ml). The heart was immediately mounted on the experimental setup and perfused at constant flow by means of a digital roller pump. A 20-ml compliance chamber along the perfusion line ensured a continuous flow. The flow rate was adjusted during the stabilization period to obtain a coronary perfusion pressure of ~75 mmHg and was held constant, with the exception of the ischemic periods during which flow was either stopped (zero-flow ischemia) or reduced to 1 ml/min (low-flow ischemia). A second adjustment of the flow rate was made at the end of the long reperfusion period, before the perfusion of U-46619, to correct any deviation of the coronary perfusion pressure from 75 mmHg, and flow rate was held constant thereafter. Flow rate was measured throughout the experiment with an in-line ultrasonic flow probe and meter (Transonic Systems, model T106). Perfusion pressure was monitored to calculate coronary resistance. The normal perfusion solution consisted of a modified Krebs-Henseleit buffer containing (in mM) 118 NaCl, 4 KCl, 2.5 CaCl<sub>2</sub>, 1.2 KH<sub>2</sub>PO<sub>4</sub>, 1 MgSO<sub>4</sub>, 24 NaHCO<sub>3</sub>, 5 D-glucose, and 2 sodium pyruvate. The perfusate was gassed with 95% O<sub>2</sub>-5% CO<sub>2</sub> (pH 7.4) and kept at a constant temperature of 37°C. All drugs were administered through a Y connector in the aortic cannula with syringe pumps (Harvard Apparatus, model 11) at 1% of the coronary flow rate. Adequate mixing of the drugs was ensured by the turbulent flow created in the reverse drop-shaped aortic cannula. All concentrations mentioned refer to the final concentration after mixing. Coronary perfusion pressure was measured with a pressure transducer connected to a sidearm of the aortic perfusion cannula. Isovolumetric left ventricular pressure and its first derivative (dp/dt) was measured by a fluid-filled latex balloon inserted into the left ventricle through a pulmonary vein and connected to a second pressure transducer. The volume of the balloon was adjusted to obtain a diastolic pressure between 5 and 10 mmHg. Heart rate was derived from the left ventricular pressure trace by a tachograph. Data were recorded on a polygraph system (Grass model 79 polygraph).

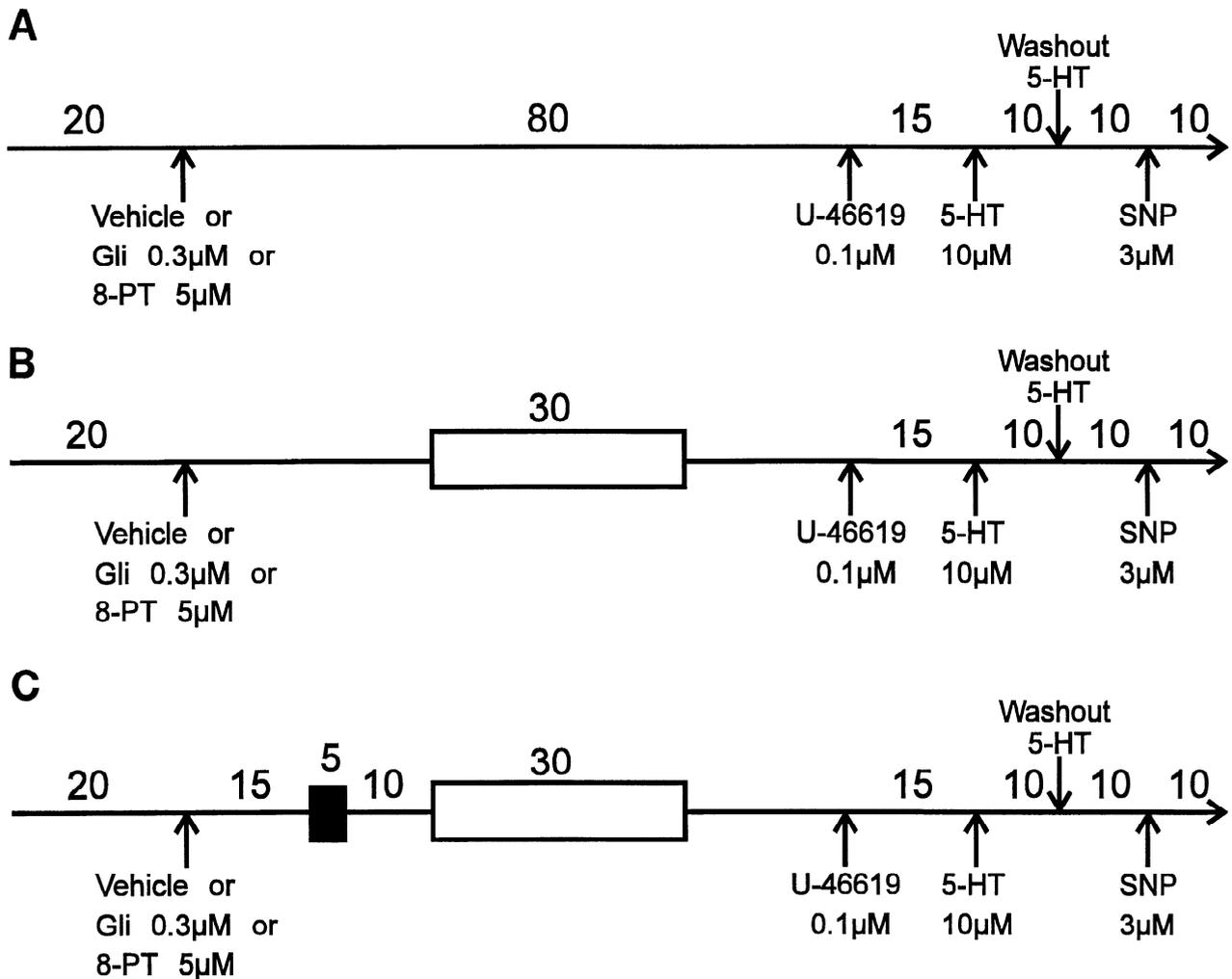


Fig. 1. Diagrams showing the different experimental protocols. Each experiment started with a 20-min stabilization period, followed by infusion of either glibenclamide (Gli; 0.3  $\mu$ M), 8-phenyltheophylline (8-PT; 5  $\mu$ M), or vehicle. **A**: hearts in sham protocol were used as time-matched controls. **B**: hearts in ischemia protocol underwent 30 min of low-flow (1 ml/min) ischemia (open box) and 20 min of reperfusion, after an additional 30-min stabilization period. **C**: hearts in IPC (ischemic preconditioning) + ischemia protocol were subjected to a preconditioning 5-min zero-flow ischemia (solid box) and a 10-min reperfusion before the 30-min low-flow ischemia (open box). For each group, endothelial and smooth muscle function was tested after the 20-min reperfusion period. Coronary arteries were then precontracted by continuous infusion of 0.1  $\mu$ M U-46619. After 15 min, a 10-min infusion of serotonin (5-HT; 10  $\mu$ M) was started. A washout period of 10 min was allowed between 5-HT and a 10-min infusion of sodium nitroprusside (SNP; 3  $\mu$ M). Numbers above bars represent time (min).

**Experimental protocols.** The animals were randomized into nine groups (Fig. 1). The hearts in all groups were subjected to a 20-min stabilization period. Drug or vehicle infusion was then started, followed by an additional 15-min perfusion period. The ischemic groups were subjected to a 15-min sham perfusion, followed by 30 min of partial ischemia (flow rate, 1 ml/min) prior to a 20-min reperfusion period. In the preconditioned groups (IPC + ischemia), the hearts were exposed to 5 min of global ischemia (zero flow) plus 10 min of reperfusion before the 30-min ischemia and 20-min reperfusion periods. The sham groups were not exposed to ischemia-reperfusion, but to a time-matched normal perfusion. After these periods, coronary arteries were precontracted with 0.1  $\mu$ M U-46619 administered until the end of the experiment. Fifteen minutes after the beginning of U-46619 infusion, the endothelial function was evaluated by the vasodilation produced by 10  $\mu$ M serotonin (5-HT), whereas coronary smooth muscle function was evaluated with 3  $\mu$ M sodium nitroprusside (SNP). These infusions were maintained for 10 min, which was long enough to reach a steady state. A washout period of 10 min

was allowed between each infusion. Vasodilation was evaluated by computing percent changes in coronary resistance (coronary perfusion pressure divided by coronary flow), measured immediately before each drug infusion and after a new steady state. The concentrations of 5-HT and SNP were determined in preliminary dose-response experiments to produce near-maximal vasodilation.

Sham, ischemia, and IPC + ischemia hearts were treated with either 0.3  $\mu$ M glibenclamide, 5  $\mu$ M 8-phenyltheophylline (8-PT), or vehicle, starting after the 20-min stabilization period and lasting throughout the experiments.

**Statistical analysis.** Values represent means  $\pm$  SE. Statistical significance of differences between means was evaluated by a two-way analysis of variance with Scheffé's post hoc test. In the case of an interaction between groups, one-way analysis of variance was used for each group. Commercially available software (Systat for Windows) was used.  $P < 0.05$  was considered to be statistically significant.

**Drugs.** U-46619 was a kind gift of Upjohn Canada. All other drugs were obtained from Sigma. A stock solution of gliben-

clamide (10 mM) was prepared in 100% dimethyl sulfoxide (DMSO). This stock solution was added directly to Krebs-Henseleit buffer. U-46619 (28.5 mM) was dissolved in 100% ethanol and diluted with 0.9% NaCl solution to obtain the desired final concentration. A stock solution of 8-PT (0.5 mM) was prepared in a 10 mM NaOH solution with DMSO (2%). This stock solution was not added directly to Krebs-Henseleit buffer but was administered through the Y connector with a syringe pump at 1% of the flow rate. Ethanol (0.003%), DMSO (0.02%), and NaOH (0.1 mM), at the concentrations obtained in the final dilutions, had no effect on any of the hemodynamic variables studied or on the dilator responses to 5-HT and SNP. All the other drugs were dissolved in 0.9% NaCl solution.

## RESULTS

**Untreated groups.** Coronary resistance measured just before 0.1  $\mu\text{M}$  U-46619 perfusion ( $n = 24$ ) was  $5.92 \pm 0.29 \text{ mmHg} \cdot \text{min} \cdot \text{ml}^{-1}$ , for a coronary flow rate of  $6.72 \pm 0.22 \text{ ml} \cdot \text{min}^{-1} \cdot \text{g}^{-1}$  (mean heart wt,  $1.90 \pm 0.05 \text{ g}$ ). Infusion of U-46619 (0.1  $\mu\text{M}$ ;  $n = 24$ ) induced a significant ( $P < 0.05$ ) vasoconstriction in all untreated groups (sham, ischemia and IPC + ischemia; Table 1). Vasodilation produced by 10  $\mu\text{M}$  5-HT in sham hearts was  $-25.2 \pm 3.3\%$ . Ischemia significantly diminished the vasodilation by more than one-half (Fig. 2). IPC prevented the deleterious effect of ischemia on endothelium-dependent vasodilation: the vasodilation produced by 5-HT in preconditioned hearts was comparable to that in sham hearts (Fig. 2). Endothelium-independent vasodilation to 3  $\mu\text{M}$  SNP was comparable in the three untreated groups (sham, ischemia, and IPC + ischemia; Fig. 3). The values of coronary flow, perfusion pressure, and coronary resistance, before and during infusion of 5-HT and SNP in all groups, are shown in Table 2.

**Glibenclamide-treated groups.** Blockade of  $\text{K}_{\text{ATP}}$  channels with glibenclamide (0.3  $\mu\text{M}$ ) was accompanied by significant increases in coronary resistance when measured just before 0.1  $\mu\text{M}$  U-46619 perfusion (glibenclamide-treated vs. untreated hearts,  $P < 0.05$ ; Table

Table 1. Effect of 0.1  $\mu\text{M}$  U-46619 infusion on coronary resistance

	<i>n</i>	Coronary Resistance, $\text{mmHg} \cdot \text{min} \cdot \text{ml}^{-1}$	
		Before U-46619	After U-46619
<b>Untreated</b>			
Sham	8	$5.87 \pm 0.35$	$10.10 \pm 0.82^*$
Ischemia	8	$5.96 \pm 0.82$	$11.53 \pm 0.75^*$
IPC + ischemia	8	$5.94 \pm 0.31$	$11.58 \pm 0.55^*$
<b>With Gli†</b>			
Sham	8	$10.00 \pm 0.50$	$13.86 \pm 0.62^*$
Ischemia	8	$8.15 \pm 0.69$	$12.84 \pm 0.95^*$
IPC + ischemia	8	$8.52 \pm 0.68$	$14.16 \pm 0.68^*$
<b>With 8-PT†</b>			
Sham	7	$12.79 \pm 1.50$	$16.21 \pm 1.25^*$
Ischemia	7	$9.64 \pm 0.46$	$14.32 \pm 0.70^*$
IPC + ischemia	7	$8.59 \pm 0.66$	$13.37 \pm 0.96^*$

Values are means  $\pm$  SE; *n*, no. of hearts. Gli, glibenclamide; 8-PT, 8-phenyltheophylline; IPC, ischemic preconditioning. Coronary resistance was calculated as coronary perfusion pressure ( $\text{mmHg}$ )/coronary perfusion flow ( $\text{ml}/\text{min}$ ).  $*P < 0.05$  compared with corresponding "before U-46619" value;  $\dagger P < 0.05$  compared with corresponding untreated value.

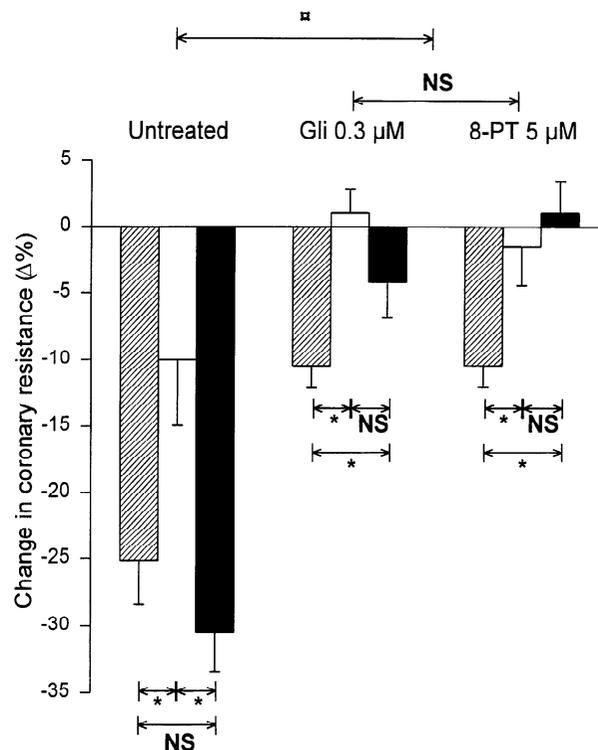


Fig. 2. Change in coronary resistance produced by 10  $\mu\text{M}$  5-HT in untreated hearts (left) and in hearts treated with either 0.3  $\mu\text{M}$  Gli (center) or 5  $\mu\text{M}$  8-PT (right). Hatched, open, and solid columns represent sham, 30-min ischemia, and IPC + ischemia hearts, respectively.  $*P < 0.05$ ; NS, nonsignificant;  $\square \square P < 0.05$ , untreated vs. Gli and 8-PT for sham hearts and IPC + ischemia hearts;  $n = 7$ –8/column.

1). The perfusion rate was  $5.30 \pm 0.13 \text{ ml} \cdot \text{min}^{-1} \cdot \text{g}^{-1}$  (mean heart wt,  $1.94 \pm 0.04 \text{ g}$ ). Infusion of U-46619 (0.1  $\mu\text{M}$ ;  $n = 24$ ) induced a significant ( $P < 0.05$ ) vasoconstriction in all glibenclamide-treated groups (Table 1). Vasodilation produced by 10  $\mu\text{M}$  5-HT ( $-10.6 \pm 1.6\%$  in sham hearts;  $n = 8$ ) was totally abolished in the ischemic group (Fig. 2). IPC in glibenclamide-treated hearts failed to prevent completely the deleterious effect of ischemia on 5-HT-induced vasodilation (Fig. 2). Vasodilation to 3  $\mu\text{M}$  SNP was comparable in the three glibenclamide-treated groups (sham, ischemia, and IPC + ischemia, Fig. 3). The vasodilation produced by SNP (Fig. 3) and 5-HT (Fig. 2) in glibenclamide-treated groups was significantly less than in the corresponding untreated groups.

**8-Phenyltheophylline-treated groups.** Blockade of adenosine receptors with 8-PT was accompanied by significant increases in coronary resistance when measured just before 0.1  $\mu\text{M}$  U-46619 perfusion (8-PT-treated vs. untreated hearts,  $P < 0.05$ ; Table 1). The perfusion rate was  $5.06 \pm 0.13 \text{ ml} \cdot \text{min}^{-1} \cdot \text{g}^{-1}$  (mean heart wt,  $2.02 \pm 0.04 \text{ g}$ ). Infusion of U-46619 (0.1  $\mu\text{M}$ ;  $n = 21$ ) induced a significant ( $P < 0.05$ ) vasoconstriction in all 8-PT-treated groups (Table 1). Vasodilation produced by 10  $\mu\text{M}$  5-HT ( $-10.5 \pm 1.6\%$  in sham hearts;  $n = 7$ ) was practically abolished in the ischemic group (Fig. 2). IPC in 8-PT-treated hearts was unable to prevent the deleterious effect of ischemia on 5-HT-induced vasodilation (Fig. 2). Vasodilation to 3  $\mu\text{M}$  SNP

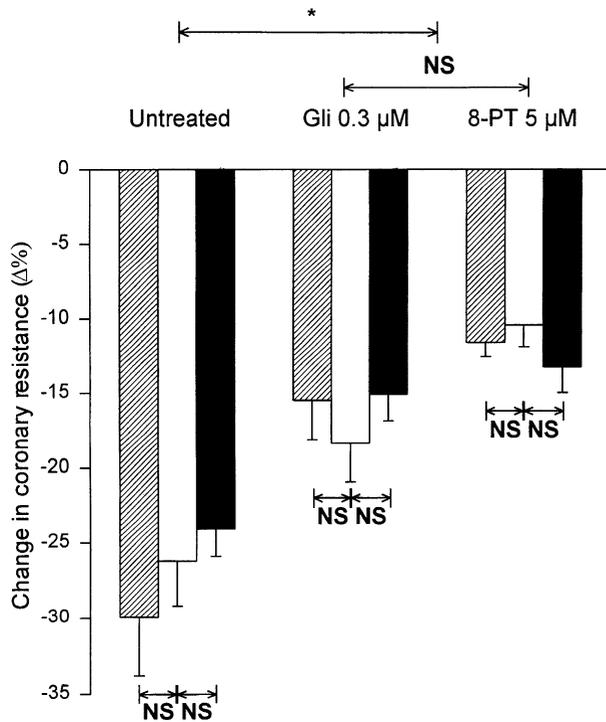


Fig. 3. Change in coronary resistance produced by 3  $\mu$ M SNP in untreated hearts (left) and in hearts treated with either 0.3  $\mu$ M Gli (center) or 5  $\mu$ M 8-PT (right). Hatched, open, and solid columns represent sham, ischemia, and IPC + ischemia hearts, respectively. \* $P < 0.05$ ;  $n = 7-8$ /column.

was comparable in the three 8-PT-treated groups (sham, ischemia, and IPC + ischemia; Fig. 3). The vasodilation produced by SNP (Fig. 3) and 5-HT (Fig. 2) in 8-PT-treated groups was significantly different from that in the corresponding untreated groups but was not significantly different from that in the corresponding glibenclamide-treated groups.

## DISCUSSION

In the present study, we evaluated whether IPC, in addition to its well-known protective effect on myocardial function, could prevent endothelial cell dysfunction

induced by ischemia-reperfusion injury in the coronary circulation. The contributions of adenosine and  $K_{ATP}$  activation to that protection were also evaluated. The major findings of this study were that 1) IPC by a single short period of ischemia prevented endothelial dysfunction produced by ischemia-reperfusion and 2)  $K_{ATP}$  channel activation partially explains that protection, whereas 3) adenosine is a major contributor to the beneficial role of IPC.

**Effect of preconditioning on endothelial dysfunction.** In the present study, the vasodilation to 5-HT was used as an index of endothelial function. 5-HT has been shown to be an endothelium-dependent vasodilator in several isolated vessel preparations (10) as well as in isolated rat hearts (18). In isolated hearts, the coronary vasodilation to 5-HT is blocked after treatment with nitric oxide synthase inhibitors (18). Thus the vasodilatory response to 5-HT is indicative of the ability of endothelial cells to generate and release nitric oxide. Our data show that endothelium-dependent vasodilation of coronary arteries to 5-HT is drastically decreased after ischemia-reperfusion injury. However, the same vessels retained the ability to dilate to SNP, an endothelium-independent vasodilator. This indicates that, under our experimental conditions, ischemia-reperfusion selectively altered the functionality of the endothelium without affecting that of smooth muscle cells. IPC prevented the reduction in vasodilation to 5-HT, suggesting that IPC can protect endothelial function in coronary arteries against the deleterious effect of ischemia-reperfusion. A similar protective effect of IPC was observed in endothelium-dependent acetylcholine-induced relaxation of epicardial coronary arteries in dogs (5) and in rat isolated left coronary arteries in vitro (28). In contrast, in an anesthetized open-chest canine model, IPC could not prevent the reduction in both endothelium-dependent and -independent dilator responses observed after 1 h of coronary occlusion and 4 h of reperfusion (2). However, it is currently unclear whether this discrepancy is species related or due to a different severity of ischemic insult.

Table 2. Effects of 10  $\mu$ M 5-HT and 3  $\mu$ M SNP on perfusion pressure and coronary resistance

	Coronary Flow, $n \text{ ml} \cdot \text{min}^{-1} \cdot \text{g}^{-1}$	Perfusion Pressure, mmHg				Coronary Resistance, $\text{mmHg} \cdot \text{min} \cdot \text{ml}^{-1}$				
		Before 5-HT	After 5-HT	Before SNP	After SNP	Before 5-HT	After 5-HT	Before SNP	After SNP	
Untreated										
Sham	8	6.64 $\pm$ 0.41	129.22 $\pm$ 4.81	96.88 $\pm$ 6.03*	126.25 $\pm$ 7.23	90.00 $\pm$ 7.63*	10.09 $\pm$ 0.85	7.64 $\pm$ 0.90*	9.92 $\pm$ 1.02	6.97 $\pm$ 0.69*
Ischemia	8	6.17 $\pm$ 0.38	143.13 $\pm$ 4.11	128.44 $\pm$ 7.30*	153.59 $\pm$ 5.79	112.97 $\pm$ 5.06*	11.58 $\pm$ 0.73	10.55 $\pm$ 1.09	12.45 $\pm$ 0.91	9.15 $\pm$ 0.69*
IPC + ischemia	8	7.36 $\pm$ 0.23	140.16 $\pm$ 3.03	97.34 $\pm$ 4.56*	146.88 $\pm$ 4.28	111.25 $\pm$ 2.95*	11.72 $\pm$ 0.57	8.08 $\pm$ 0.41*	12.29 $\pm$ 0.66	9.28 $\pm$ 0.40*
With Gli										
Sham	8	5.06 $\pm$ 0.21 <sup>†</sup>	139.06 $\pm$ 8.69	125.00 $\pm$ 9.39*	142.81 $\pm$ 9.05	120.94 $\pm$ 9.07*	13.91 $\pm$ 0.87 <sup>†</sup>	12.50 $\pm$ 0.94* <sup>†</sup>	14.28 $\pm$ 0.91 <sup>†</sup>	12.09 $\pm$ 0.91* <sup>†</sup>
Ischemia	8	5.24 $\pm$ 0.20	129.56 $\pm$ 7.02	130.63 $\pm$ 7.02	143.78 $\pm$ 6.74	116.88 $\pm$ 5.42*	12.55 $\pm$ 0.79	12.66 $\pm$ 0.81 <sup>†</sup>	13.94 $\pm$ 0.83	11.33 $\pm$ 0.65*
IPC + ischemia	8	5.70 $\pm$ 0.24 <sup>†</sup>	150.47 $\pm$ 6.07	144.38 $\pm$ 7.55 <sup>†</sup>	154.84 $\pm$ 6.20	131.41 $\pm$ 5.76*	14.54 $\pm$ 0.60 <sup>†</sup>	13.96 $\pm$ 0.76 <sup>†</sup>	14.97 $\pm$ 0.63	12.72 $\pm$ 0.64*
With 8-PT										
Sham	7	5.04 $\pm$ 0.22 <sup>†</sup>	165.49 $\pm$ 11.78 <sup>†</sup>	148.00 $\pm$ 9.33* <sup>†</sup>	172.86 $\pm$ 10.38 <sup>†</sup>	152.68 $\pm$ 9.13* <sup>†</sup>	16.59 $\pm$ 1.18 <sup>†</sup>	14.80 $\pm$ 0.93* <sup>†</sup>	17.29 $\pm$ 1.04 <sup>†</sup>	15.27 $\pm$ 0.97* <sup>†</sup>
Ischemia	7	4.84 $\pm$ 0.16 <sup>†</sup>	145.89 $\pm$ 7.25	142.86 $\pm$ 5.47	159.82 $\pm$ 8.50	143.21 $\pm$ 8.24* <sup>†</sup>	14.59 $\pm$ 0.72 <sup>†</sup>	14.29 $\pm$ 0.55 <sup>†</sup>	15.98 $\pm$ 0.85 <sup>†</sup>	14.32 $\pm$ 0.82* <sup>†</sup>
IPC + ischemia	7	5.31 $\pm$ 0.27 <sup>†</sup>	143.57 $\pm$ 7.99	145.36 $\pm$ 9.58 <sup>†</sup>	162.50 $\pm$ 12.90	141.07 $\pm$ 11.07* <sup>†</sup>	13.90 $\pm$ 1.02	14.11 $\pm$ 1.20 <sup>†</sup>	15.79 $\pm$ 1.53	13.73 $\pm$ 1.38* <sup>†</sup>

Values are means  $\pm$  SE;  $n$ , no. of hearts. 5-HT, serotonin; SNP, sodium nitroprusside. \* $P < 0.05$  compared with corresponding "before" value; <sup>†</sup> $P < 0.05$  compared with corresponding untreated value.

*Role of  $K_{ATP}$  channels in IPC.* In the glibenclamide-treated groups, the protective effect of IPC on endothelial function was diminished by one-half, as reflected by the reduction of endothelium-dependent vasodilation to 5-HT in the preconditioning group, compared with the glibenclamide-treated sham group. The unaltered dilator response to SNP in all glibenclamide-treated groups implies that the decreased vasodilation to 5-HT in the glibenclamide-treated preconditioned group was not the result of a nonspecific smooth muscle dysfunction but rather a specific diminution of the protective effect of IPC on endothelium-mediated dilation. These data suggest that  $K_{ATP}$  channel activation is one of the mechanisms involved in the protection afforded by IPC against endothelial dysfunction observed with ischemia-reperfusion.

*Role of adenosine receptors in IPC.* In the 8-PT-treated preconditioned group, the vasodilation to 5-HT was completely abolished, whereas the vasodilation to SNP was not significantly different from that observed in the sham group. These data suggest that adenosine, acting on its receptors, plays a predominant role in the endothelial protection afforded by IPC.

The protective role of IPC against endothelial dysfunction and the mechanisms whereby it produces this effect seem to share similarities with those observed in cardiomyocytes. It is widely recognized that IPC limits infarct size (20–22, 27), reduces the risk of ischemia-reperfusion arrhythmias (8, 25, 32), improves recovery of ventricular function (3, 13), reduces catabolite accumulation, and slows ischemic metabolism (21, 27).  $K_{ATP}$  channels (6, 23) and adenosine receptors (16, 19) are mechanisms that have been proposed to explain the protective effect of IPC on these different end points. The contribution of adenosine or  $K_{ATP}$  channels can differ depending on the animal species or the end points used. For example, there is evidence in the dog, rabbit, and pig, but not in the rat, to support the hypothesis that  $K_{ATP}$  channel activation may contribute to the beneficial effect of IPC on infarct size (23).

The intracellular mechanisms by which IPC protects the myocardium or the coronary endothelium from the ischemic damage are not completely understood, but some hypotheses have been proposed. In the myocardium, activation of  $K_{ATP}$  channels may inhibit ischemic depolarization, which could reduce  $Ca^{2+}$  entry via voltage-gated channels, resulting in a reduction in intracellular  $Ca^{2+}$  levels and decreased myocardial contractility (6). Furthermore, recent studies have shown the presence of  $K_{ATP}$  channels in mitochondrial membranes. The role of these channels remains unknown, but they may maintain membrane polarity or even control mitochondrial  $Ca^{2+}$  concentration (6). This control of intracellular  $Ca^{2+}$  concentration via  $K_{ATP}$  channel activation can prevent mitochondrial  $Ca^{2+}$  overload, a key factor in myocardial ischemic damage. Some evidence indicates that activation of  $K_{ATP}$  channels during ischemia can also preserve the myocardial energy status (7, 9). Although the presence of  $K_{ATP}$  channels in the endothelium of rat coronary arteries has not been directly demonstrated, patch-clamp stud-

ies have shown the presence of  $K_{ATP}$  channels on rat aorta and brain microvascular endothelial cells (11). In endothelial cells, activation of  $K_{ATP}$  channels also produces hyperpolarization. Because endothelial cells lack voltage-gated  $Ca^{2+}$  channels, hyperpolarization will have an unusual effect on  $Ca^{2+}$  influx in these cells: it will increase the electrochemical gradient and facilitate  $Ca^{2+}$  entry (1, 11), which will enhance nitric oxide release from endothelial cells (17). The contribution of nitric oxide to the protection of the endothelium afforded by IPC remains unknown.

Adenosine has often been reported to be a mediator of protection against ischemia afforded by IPC (16, 19). Adenosine, released from ischemic tissues and acting on its  $A_1$  receptors, can activate  $K_{ATP}$  channels via a G protein (12) and produce effects similar to those described for  $K_{ATP}$  channels. A recent study has reported adenosine  $A_2$  receptors on guinea pig coronary endothelial cells (30). However, adenosine 3',5'-cyclic monophosphate production was used to rank the different agonists: since  $A_1$  receptors may be coupled to effectors other than adenylate cyclase, the presence of  $A_1$  receptors might have been overlooked. Recently, Liu et al. (16) have reported that adenosine acting through  $A_3$  receptors may mediate the protection afforded by preconditioning. The physiological role of the  $A_3$  receptor is still poorly characterized, but it has recently been implicated as an activator of mast cells (26). According to this hypothesis, mast cells would release mediators (histamine, leukotrienes, free radicals, thromboxanes, cytokines) during the preconditioning period (transient ischemia); the mediators would produce little or no damage to myocytes, being washed away too rapidly. During the subsequent prolonged ischemic insult, depleted mast cells could no longer release these deleterious mediators, resulting in reduced damage (15). It remains to be established whether these mechanisms can also explain the protective effect of IPC on endothelial function.

It has been reported that IPC attenuates reperfusion-induced myocardial edema (29). This reduction in myocardial edema could presumably improve coronary perfusion through a reduction in external compression of coronary microvessels. However, in the present study, vasodilation to SNP was unaffected by ischemia-reperfusion, suggesting that myocardial edema under our conditions was not severe enough to affect vasodilator function. On the other hand, we cannot rule out the possibility that IPC attenuates edema of endothelial cells, which could improve endothelium-dependent vasodilation.

Treatment with either glibenclamide or 8-PT reduced the vasodilation produced by 5-HT and SNP, compared with the untreated groups. This effect is probably due to the increase in coronary resistance observed in all treated hearts. It is important to note that, for each treatment, coronary resistance measured before infusion of vasodilators in sham, ischemia, and IPC + ischemia hearts was comparable.

In conclusion, these data suggest that IPC affords protection to endothelial function against subsequent

ischemic injury in the intact coronary circulation of the rat. The reduced protective effect of IPC in the presence of glibenclamide suggests that this protection may be mediated partially by  $K_{ATP}$  channel activation. The complete inhibitory action of 8-PT on IPC suggests that adenosine may play a major role in the protection of endothelial function.

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## REFERENCES

- Adams, D. J., J. Barakeh, R. Laskey, and C. van Breemen. Ion channels and regulation of intracellular calcium in vascular endothelial cells. *FASEB J.* 3: 2389–2400, 1989.
- Bauer, B., B. Z. Simkhovich, R. A. Kloner, and K. Przyklenk. Does preconditioning protect the coronary vasculature from subsequent ischemia/reperfusion injury? *Circulation* 88: 659–672, 1993.
- Cave, A. C., and D. J. Hearse. Ischaemic preconditioning and contractile function: studies with normothermic and hypothermic global ischaemia. *J. Mol. Cell. Cardiol.* 24: 1113–1123, 1992.
- Cohen, M. V., G. S. Liu, and J. M. Downey. Preconditioning causes improved wall motion as well as smaller infarcts after transient coronary occlusion in rabbits. *Circulation* 84: 341–349, 1991.
- DeFily, D. V., and W. M. Chilian. Preconditioning protects coronary arteriolar endothelium from ischemia-reperfusion injury. *Am. J. Physiol.* 265 (Heart Circ. Physiol. 34): H700–H706, 1993.
- Grover, G. J. Protective effects of ATP sensitive potassium channel openers in models of myocardial ischemia. *Cardiovasc. Res.* 28: 778–782, 1994.
- Grover, G. J., J. Newburger, P. G. Sleph, S. Dzwonczyk, S. C. Taylor, S. Z. Ahmed, and K. S. Atwal. Cardioprotective effects of the potassium channel opener cromakalim: stereoselectivity and effects on myocardial adenine nucleotides. *J. Pharmacol. Exp. Ther.* 257: 156–162, 1991.
- Hagar, J. M., S. L. Hale, and R. A. Kloner. Effects of preconditioning ischemia on reperfusion arrhythmias after coronary artery occlusion and reperfusion in the rat. *Circ. Res.* 68: 61–68, 1991.
- Hosoda, H., M. Sunamori, and A. Suzuki. Effect of pinacidil on rat hearts undergoing hypothermic cardioplegia. *Ann. Thorac. Surg.* 58: 1631–1636, 1994.
- Hoyer, D., D. E. Clarke, J. R. Fozard, P. R. Hartig, G. R. Martin, E. J. Mylecharane, P. R. Saxena, and P. A. Humphrey. International Union of Pharmacology classification of receptors for 5-hydroxytryptamine (serotonin). *Pharmacol. Rev.* 46: 157–193, 1994.
- Janigro, D., G. A. West, E. L. Gordon, and H. R. Winn. ATP-sensitive  $K^+$  channels in rat aorta and brain microvascular endothelial cells. *Am. J. Physiol.* 265 (Cell Physiol. 34): C812–C821, 1993.
- Kirsch, G. E., J. Codina, L. Birnbaumer, and A. M. Brown. Coupling of ATP-sensitive  $K^+$  channels to  $A_1$  receptors by G proteins in rat ventricular myocytes. *Am. J. Physiol.* 259 (Heart Circ. Physiol. 28): H820–H826, 1990.
- Lasley, R. D., G. M. Anderson, and R. M. Mentzer, Jr. Ischaemic and hypoxic preconditioning enhance postischaemic recovery of function in the rat heart. *Cardiovasc. Res.* 27: 565–570, 1993.
- Li, Y., and R. A. Kloner. Cardioprotective effects of ischaemic preconditioning are not mediated by prostanoids. *Cardiovasc. Res.* 26: 226–231, 1992.
- Linden, J. Cloned adenosine  $A_3$  receptors: pharmacological properties, species differences and receptor functions. *Trends Pharmacol. Sci.* 15: 298–306, 1994.
- Liu, G. S., S. C. Richards, R. A. Olsson, K. Mullane, R. S. Walsh, and J. M. Downey. Evidence that the adenosine  $A_3$  receptor may mediate the protection afforded by preconditioning in the isolated rabbit heart. *Cardiovasc. Res.* 28: 1057–1061, 1994.
- Lückhoff, A., and R. Busse. Calcium influx into endothelial cells and formation of endothelium-derived relaxing factor is controlled by the membrane potential. *Pfluegers Arch.* 416: 305–311, 1990.
- Mankad, P. S., A. H. Chester, and M. H. Yacoub. 5-Hydroxytryptamine mediates endothelium dependent coronary vasodilatation in the isolated rat heart by the release of nitric oxide. *Cardiovasc. Res.* 25: 244–248, 1991.
- Miura, T., and O. Iimura. Infarct size limitation by preconditioning: its phenomenological features and the key role of adenosine. *Cardiovasc. Res.* 27: 36–42, 1993.
- Murry, C. E., R. B. Jennings, and K. A. Reimer. Preconditioning with ischemia: a delay of lethal cell injury in ischemic myocardium. *Circulation* 74: 1124–1136, 1986.
- Murry, C. E., V. J. Richard, K. A. Reimer, and R. B. Jennings. Ischemic preconditioning slows energy metabolism and delays ultrastructural damage during a sustained ischemic episode. *Circ. Res.* 66: 913–931, 1990.
- Parratt, J. R. Protection of the heart by ischaemic preconditioning: mechanisms and possibilities for pharmacological exploitation. *Trends Pharmacol. Sci.* 15: 19–25, 1994.
- Parratt, J. R., and K. A. Kane.  $K_{ATP}$  channels in ischaemic preconditioning. *Cardiovasc. Res.* 28: 783–787, 1994.
- Pearson, P. J., H. V. Schaff, and P. M. Vanhoutte. Acute impairment of endothelium-dependent relaxations to aggregating platelets following reperfusion injury in canine coronary arteries. *Circ. Res.* 67: 385–393, 1990.
- Piacentini, L., C. L. Wainwright, and J. R. Parratt. The antiarrhythmic effect of ischaemic preconditioning in isolated rat heart involves a pertussis toxin sensitive mechanism. *Cardiovasc. Res.* 27: 674–680, 1993.
- Ramkumar, V., G. L. Stiles, M. A. Beaven, and H. Ali. The  $A_3$  adenosine receptor is the unique adenosine receptor which facilitates release of allergic mediators in mast cells. *J. Biol. Chem.* 268: 16887–16890, 1993.
- Reimer, K. A., R. S. Vander Heide, and R. B. Jennings. Ischemic preconditioning slows ischemic metabolism and limits myocardial infarct size. *Ann. NY Acad. Sci.* 723: 99–115, 1994.
- Richard, V., N. Kaeffer, C. Tron, and C. Thuillez. Ischemic preconditioning protects against coronary endothelial dysfunction induced by ischemia and reperfusion. *Circulation* 89: 1254–1261, 1994.
- Sanz, E., D. Garcia Dorado, J. Oliveras, J. A. Barrabés, M. A. Gonzalez, M. Ruiz-Meana, J. Solares, M. J. Carreras, A. Garcia-Lafuente, M. Desco, and J. Soler-Soler. Dissociation between anti-infarct effect and anti-edema effect of ischemic preconditioning. *Am. J. Physiol.* 268 (Heart Circ. Physiol. 37): H233–H241, 1995.
- Schiele, J. O., and U. Schwabe. Characterization of the adenosine receptor in microvascular coronary endothelial cells. *Eur. J. Pharmacol.* 269: 51–58, 1994.
- Schwarz, E. R., M. Mohri, S. Sack, and M. Arras. Duration of infarct size limiting effect of ischemic preconditioning in the pig (Abstract). *Circulation* 84, Suppl. II: II-432, 1991.
- Shiki, K., and D. J. Hearse. Preconditioning of ischemic myocardium: reperfusion-induced arrhythmias. *Am. J. Physiol.* 253 (Heart Circ. Physiol. 22): H1470–H1476, 1987.
- Van Benthuyzen, K. M., I. F. McMurtry, and L. D. Horwitz. Reperfusion after acute coronary occlusion in dogs impairs endothelium-dependent relaxation to acetylcholine and augments contractile reactivity in vitro. *J. Clin. Invest.* 79: 265–274, 1987.
- Yellon, D. M., A. M. Alkhulaifi, E. E. Browne, and W. B. Pugsley. Ischaemic preconditioning limits infarct size in the rat heart. *Cardiovasc. Res.* 26: 983–987, 1992.
- Yellon, D. M., A. M. Alkhulaifi, and W. B. Pugsley. Preconditioning the human myocardium. *Lancet* 342: 276–277, 1993.