Ischemic preconditioning attenuates acidosis and postischemic dysfunction in isolated rat heart

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Asimakis, Gregory K., Karen Inners-McBride, Glen Medellin, and Vincent R. Conti. Ischemic preconditioning attenuates acidosis and postischemic dysfunction in isolated rat heart. *Am. J. Physiol.* 263 (*Heart Circ. Physiol.* 32): H887–H894, 1992.—The hypothesis that brief ischemia (preconditioning) protects the isolated heart from prolonged global ischemia was tested. Isovolumic rat hearts were preconditioned with either 5 min of ischemia followed by 5 min of perfusion (P1) or two 5-min episodes of ischemia separated by 5 min of perfusion (P2). Control hearts received no preconditioning. All hearts received 40 min of sustained ischemia and 30 min of reperfusion. Preconditioning (P1 or P2) significantly ($P < 0.0005$) improved recovery of the rate-pressure product; percentage recoveries were 17.8 ± 3.2 ($n = 14$), 59.9 ± 5.5 ($n = 6$), and 46.4 ± 4.7 ($n = 8$) for control, P1, and P2, respectively. Improved functional recovery of preconditioned hearts was associated with reduced end-diastolic pressure and improved myocardial perfusion. During the 40-min ischemic period, myocardial pH decreased from ~7.4 to 6.3 ± 0.1 ($n = 7$) in the control hearts and to 6.7 ± 0.1 ($n = 7$) in the preconditioned hearts ($P < 0.01$). Also during the 40-min ischemic period, myocardial lactate (expressed as nmol/mg protein) increased to 146 ± 11 ($n = 7$) and 101 ± 12 ($n = 8$) in control and preconditioned hearts, respectively ($P < 0.02$). The results demonstrate that a brief episode of ischemia can protect the isolated rat heart from a prolonged period of ischemia. This protection is associated with decreased tissue acidosis and anaerobic glycolysis during the sustained ischemic period.

ischemic contracture; reperfusion; pH

PROGRESSIVE CELL INJURY occurs during myocardial ischemia leading eventually to irreversible injury and cell death. Therefore the extent of irreversible damage is dependent on the degree and duration of ischemia at the time of reperfusion. Using a model of regional ischemia in the intact canine heart, Murry et al. (26) reported the seemingly paradoxical finding that development of irreversible damage (necrosis) is significantly reduced if the area at risk is subjected to multiple brief episodes of ischemia and reperfusion immediately before the sustained ischemic period. This phenomenon has been termed "ischemic preconditioning." Since this initial report (26), other investigators have reported improved myocardial salvage after ischemic preconditioning in models of regional ischemia in the dog (21), pig (30), and rabbit (14) hearts.

Although these important studies describe a potent experimental intervention that protects the heart from ischemic (or reperfusion) injury, the mechanism of myocardial protection by ischemic preconditioning remains unknown. It is known that the protection is not due to increased collateral blood flow during the sustained ischemia period (14, 21, 26, 30). However, some flow to the affected tissue may be required for the preconditioning effect. Neely and Grotyohann (29) reported that a brief period (10–15 min) of anoxic perfusion before ischemia resulted in a significant improvement in postischemic contractile function of the isolated rat heart. However, little is known about the effects of ischemic preconditioning in a model of total global ischemia. The purpose of the present study was to characterize the myocardial protective effect of ischemic preconditioning in a model of prolonged global ischemia using the isolated perfused rat heart.

METHODS

Isolated Perfused Rat Heart

Adult male Sprague-Dawley rats weighing 225–300 g each were injected intraperitoneally with 5 mg of heparin. After 30 min, the rats were anesthetized with an intraperitoneal injection of 25–30 mg of pentobarbital sodium. The hearts were quickly excised and placed in ice-cold Krebs-Henseleit bicarbonate buffer (KHB).

The aorta was quickly cannulated upon removal, and the heart was perfused with KHB using a Langendorff nonrecirculating preparation. KHB consisted of 4.7 mM KCl, 2.5 mM $CaCl_2$, 1.25 mM $MgCl_2$, 1.25 mM KH_2PO_4, 0.5 mM EDTA, 25 mM $NaHCO_3$, 118 mM NaCl, and 5 mM glucose. KHB was filtered through a 0.45-μm cellulose filter to remove any particulate matter. The buffer was kept at 37°C in a water-jacketed column and gassed continuously with 95% O_2-5% CO_2. The hearts were perfused at a constant perfusion pressure of 120 cmH_2O.

A small incision was made in the center of the left atrium. A 14-gauge needle was used to make an apical stab to vent the left ventricle. A latex balloon was inserted through the left atrium and into the left ventricle. The balloon was tied securely into place and was filled with water to give an end-diastolic pressure of 5–10 mmHg. The fluid-filled balloon was interfaced to a pressure transducer (Camino Laboratories, San Diego, CA), which in turn was interfaced to a Tangent 386 computer. The left ventricular pressure and heart rate were recorded throughout the experiment using Cardiology Data Acquisition Program (version 1.05, Symbolic Logic, Dallas, TX). Rate-pressure products were determined by multiplying heart rate × left ventricular developed pressure (systolic − diastolic).

Experimental Protocol

In the first set of experiments, three groups of hearts were used to determine the effect of ischemic preconditioning on postischemic mechanical function. Groups were distinguished by the following treatments.

Controls. Controls were perfused for 35 min followed by 40 min of ischemia (aortic inflow line clamped) and then 30 min of reperfusion.

Preconditioned × 1 (P1). P1 hearts were perfused for 25 min followed by 5 min of ischemia, 5 min of reflow, 40 min of ischemia, and then 30 min of reperfusion.

Preconditioned × 2 (P2). P2 hearts were perfused for 25 min followed by 5 min of ischemia, 5 min of reflow, 5 min of ischemia, 5 min of reflow, 40 min of ischemia, and then 30 min of reperfusion.

Coronary flow rates were determined after the initial perfusion period and after 15 and 30 min of reperfusion by collecting effluents for 1 min in graduated cylinders. In all groups, during ischemia, the hearts were immersed in KHB in a water-jacketed chamber maintained at 37°C. The hearts were allowed to beat spontaneously throughout the experiments. Hearts were not used if during the initial perfusion period heart rate was <250 beats/min, systolic pressure was <100 mmHg, or diastolic pressure increased >20%. Reperfusion arrhythmias occurred, particularly during the first 10 min of reperfusion. In some cases, the hearts experienced transient ventricular fibrillation; these hearts either spontaneously converted to normal rhythm or were converted simply by tapping the ventricle. The number of hearts undergoing transient fibrillation for each group were control (3 of 14), P1 (5 of 6), and P2 (1 of 8).

After 30 min of reperfusion, some of the hearts were injected with undiluted 0.3% pathalocyanine pigment (Monastral blue B suspension) through a sidearm of the aortic cannula until the dye was seen in the coronary effluent, at which time the aortic inflow line was clamped. The hearts were then removed from the cannula and rinsed well in ice-cold saline solution. Hearts were placed in formaldehyde solution for 24 h. A transverse cut was made in each heart midway between the base and the apex, and the cross sections were photographed. The areas of no flow (absence of dye) were delineated and cut out with scissors. The weight of the total cross-sectional area and the no-flow area were determined. The area of no reflow was expressed as percentage of the total cross-sectional area. Areas of no reflow were determined in control and P1 hearts only.

In the second set of experiments, the effect of ischemic preconditioning on changes in tissue metabolites during ischemia was determined. Control and P1 hearts were freeze-clamped with Wollenberger tongs (cooled in liquid nitrogen) immediately before initiation of the 40-min ischemic period (baseline), after 15 min of ischemia, and after 40 min of ischemia. The balloons were deflated and removed immediately before freeze-clamping. Myocardial pH was continuously monitored in the hearts that were freeze-clamped after 40 min of ischemia; a pH microelectrode (Microelectrodes, Londonderry, NH) was inserted into the midmyocardium of the left ventricular wall.

Frozen Heart Extraction and Analysis

Each frozen heart was ground to a fine powder in liquid nitrogen with a mortar and pestle. Three milliliters of 1 N perchloric acid (PCA) were added to the powdered sample, which was still in liquid nitrogen. After the liquid nitrogen boiled off, the PCA suspension was allowed to thaw and then kept on ice. The extracts were centrifuged to remove the denatured protein. The protein pellet was extracted a second time in 3 ml of ice-cold 1 N PCA, and centrifugation was repeated. The two supernatants were combined. Three milliliters of the extracts were neutralized (pH 7.0–7.2) with 6 N KOH. The neutralized extracts were analyzed for lactate, ATP, and creatine phosphate (CrP) by enzymatic analysis (8, 20). When glycogen was to be determined, the remainder of the unneutralized extracts (~3.5 ml) was added back to the respective protein pellets. The pellets were homogenized with a Polytron tissue processor at high speed for ~5 s. The homogenates (0.2 ml) were treated and analyzed for glycogen content by the method of Keppler and Decker (15). The remainder of the homogenates were centrifuged. The pellets were suspended in 6 N KOH and incubated at 30°C for 6 h. The dissolved protein pellets were diluted with water and assayed for protein content (24). Myocardial metabolite contents were expressed as nanomoles per milligram of myocardial protein.

Statistical Analysis

Analysis of variance (ANOVA) was used to determine whether there were any significant differences between any of the groups. If indicated by ANOVA, the Student's *t* test with the Bonferroni correction was used to determine which groups differed significantly. A value of $P < 0.05$ was considered significant.

Table 1. *Hemodynamic properties of control and preconditioned groups*

Group	Baseline	Post-preconditioning	Postischemia	
			15 min	30 min
Control (n = 14)				
Heart rate, beats/min	306±6		195±34	262±90
Systolic LVP, mmHg	137±7		91±4	100±4
Diastolic LVP, mmHg	7±1		80±5	74±6
RPP × 10^{-3}, mmHg/min	37.5±1.3		2.4±0.8	6.8±1.3
Coronary flow, ml/min	19.2±0.7		8.3±0.6	8.8±0.7
P1 (n = 6)				
Heart rate, beats/min	312±13	315±9	243±28	263±14
Systolic LVP, mmHg	148±5	116±6	113±7*	125±4†
Diastolic LVP, mmHg	6±1	5±1	41±5†	26±4†
RPP × 10^{-3}, mmHg/min	44.8±2.3*	34.5±2.3	16.3±1.4†	25.7±1.1†
Coronary flow, ml/min	19.9±0.8	ND	14.2±1.6†	14.5±1.6†
P2 (n = 8)				
Heart rate, beats/min	310±10	306±10	253±17	239±18
Systolic LVP, mmHg	137±5	109±4	102±6	118±8
Diastolic LVP, mmHg	8±1	11±1‡	49±6†	39±6†
RPP × 10^{-3}, mmHg/min	39.9±1.7	30.1±1.8	13.3±2.1†	19±2†
Coronary flow, ml/min	18.8±0.9	ND	12.5±0.8†	13.0±1.0†

Values are means ± SE; n, no. of hearts in each group. LVP, left ventricular pressure; RPP, rate-pressure product; ND, not determined. Baseline values for controls are immediately before initiation of 40-min sustained ischemic period. Baseline values for preconditioned hearts are immediately before 1st preconditioning episode. Postpreconditioning values are immediately before initiation of 40-min sustained ischemic period. Postischemic values are after 15 and 30 min of reperfusion after 40-min ischemic period. * $P < 0.015$ vs. control. † $P < 0.005$ vs. control. ‡ $P < 0.005$ vs. P1.

RESULTS

Postischemic Recovery of Hemodynamic Functions

The hemodynamic properties of control, P1, and P2 groups are shown in Table 1. There were no significant differences between groups for any of the baseline values, with the exception that the rate-pressure product of the P1 group was ~20% higher than the control group. Preconditioning with one or two episodes of ischemia resulted in reduced contractile function as seen by the ~20% lower values (compared with baseline values $P < 0.001$) for systolic pressure and the rate-pressure product (post-preconditioning, Table 1). There were no post-preconditioning differences between the P1 and P2 groups with the exception that the diastolic pressure was higher in the P2 group. Postischemic (15 and 30 min of reperfusion) heart rates were not statistically different between any of the groups. For the P1 group, the systolic pressure, rate-pressure product, and coronary flow rate were significantly higher than control values, whereas the left ventricular diastolic pressure was significantly lower than control values at both 15 and 30 min of reperfusion. Similar differences were seen between the P2 and control groups with the exception that the systolic pressures were not statistically different. Figure 1 shows the postischemic recovery (expressed as percentage of baseline) of the rate-pressure product after 30 min of reperfusion. The preconditioned groups recovered approximately threefold greater than the control group. Although the recovery of the P2 group tended to be less than the P1 group, the difference was not statistically significant.

Figure 2 shows the left ventricular pressure development throughout the experimental protocol for the control, P1, and P2 groups. Values were taken every 2 min. Within 10 min after the onset of the 40-min ischemic period, contracture (rigor) began in all three groups. Although all three groups showed hypercontraction (increased resting pressure) upon reperfusion (after 40 min of ischemia), the hypercontracture was much less severe in the preconditioned hearts. Control hearts began to develop systolic pressure (as indicated by a separation of systolic and diastolic pressures) 5–10 min after reperfusion, whereas the preconditioned hearts began to develop systolic pressure within 3–5 min of reperfusion. Pressure development progressively improved throughout the reperfusion period. In the control hearts, the improving pressure development was due primarily to improving diastolic function. In the preconditioned hearts, the improving pressure development was due both to improving systolic and diastolic function.

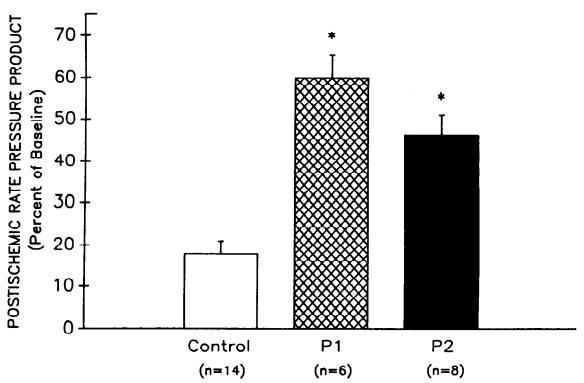

Fig. 1. Effect of ischemic preconditioning on postischemic recovery of contractile function (rate-pressure product). Rate-pressure products (heart rate × developed pressure) were determined for baseline and after 30 min of reperfusion from data shown in Table 1. Values are means ± SE for rate-pressure products (expressed as % of baseline) after 30 min of reperfusion. Preconditioned groups recovered significantly better than control group (* $P < 0.0005$). Two episodes of preconditioning did not enhance myocardial protection beyond that observed with one episode of preconditioning.

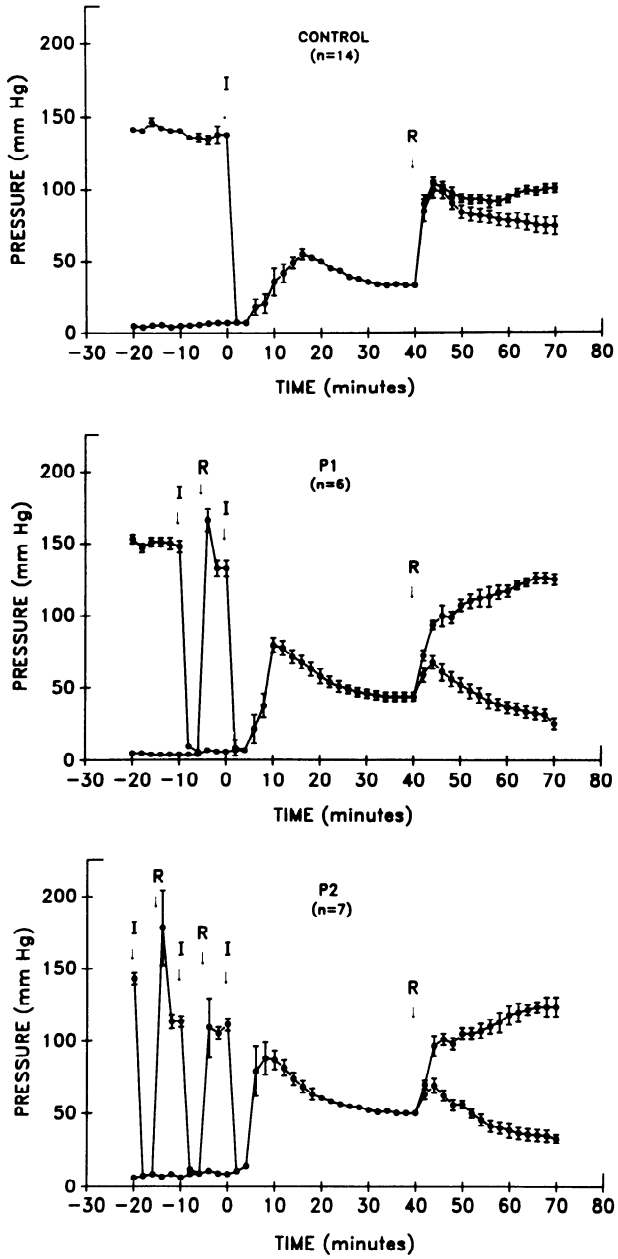

Fig. 2. Left ventricular pressure development in control, P1, and P2 experimental groups. Mean ± SE systolic (*top*) and diastolic (*bottom*) pressures are shown for control and preconditioned groups. Ischemia (I) and reflow (R) are indicated by arrows. In all groups, 40-min sustained ischemia began at *time 0* and 30 min of reperfusion began at *time 40*. Values shown were taken every 2 min. When arrhythmias occurred at time of measurements, values were determined immediately after return to normal sinus rhythm.

Table 2. *Effect of preconditioning on contracture*

Group	n	Time to Onset of Contracture, s	Peak Pressure During Contracture, mmHg	End-Ischemic Pressure, mmHg	Max Diastolic Pressure During Reflow, mmHg
Control	14	481±41	68.8±5.6	35.6±2.3	106.9±6.7
P1	6	336±24	82.2±6.5	43.0±3.0	69.0±5.8*
P2	8	232±14†‡	98.5±6.9*	54.7±6.5*	79.6±7.1*

Values are means ± SE; n, no. of hearts in each group. Time to contracture is time after initiation of sustained ischemia that left ventricular pressure development increased 2 mmHg above baseline (see Fig. 2). End-ischemic pressure is left ventricular pressure immediately before beginning 30-min reperfusion period. Max diastolic was obtained during 30-min reperfusion period; this occurred 3–5 min after onset of reperfusion. * $P < 0.015$ vs. control. † $P < 0.001$ vs. control. ‡ $P < 0.01$ vs. P1.

Collectively, the results demonstrate that a brief episode of global ischemia and reperfusion protects the isolated perfused rat heart during a subsequent sustained period of ischemia and is associated with improved functional recovery. Moreover, two episodes of ischemic preconditioning does not further enhance protection above that observed with a single preconditioning episode.

Effect of Ischemic Preconditioning on Ischemic Contracture and No-Reflow Phenomenon

Table 2 shows the effects of preconditioning (P1 and P2) on time to onset of contracture, maximum ventricular pressure developed during contracture, ventricular pressure at the end of the sustained ischemic period, and maximum diastolic pressure (as an index of hypercontraction) during reperfusion. Preconditioning with two ischemic episodes (P2) decreased the time to onset of contracture by ~50% compared with the control group. In the P2 group, peak contracture pressure and end-ischemic pressure were elevated over control values by 43 and 53%, respectively. During ischemia the values for the P1 group were intermediate between the control and P2 groups. Thus preconditioning with one ischemic episode tended to accelerate the development and extent of contracture during ischemia; however, the differences compared with the control group were not statistically significant. During reperfusion (after 40-min sustained ischemic period), the maximum diastolic pressure developed in the P1 and P2 groups were lower than the control value by 35 and 25%, respectively. The preconditioned hearts had significantly lower diastolic pressures (compared with the control group) throughout the reperfusion period. As previously mentioned, coronary flow during reperfusion was enhanced by ischemic preconditioning (Table 1). To better understand the effect of ischemic preconditioning on the myocardial distribution of the coronary flow during reperfusion, several control and P1 hearts were infused with a blue dye after 30 min of reperfusion. Figure 3*A* shows cross sections of representative control and preconditioned hearts. The areas of no reflow expressed as percentages of the total cross-sectional area were 22.6 ± 2.7 and 2.5 ± 0.6 % for control and preconditioned hearts, respectively (Fig. 4*B*). The area of no reflow was limited to the subendocardium. Therefore the reduced coronary flow in the postischemic control hearts appears to be due to restriction of flow to the subendocardium.

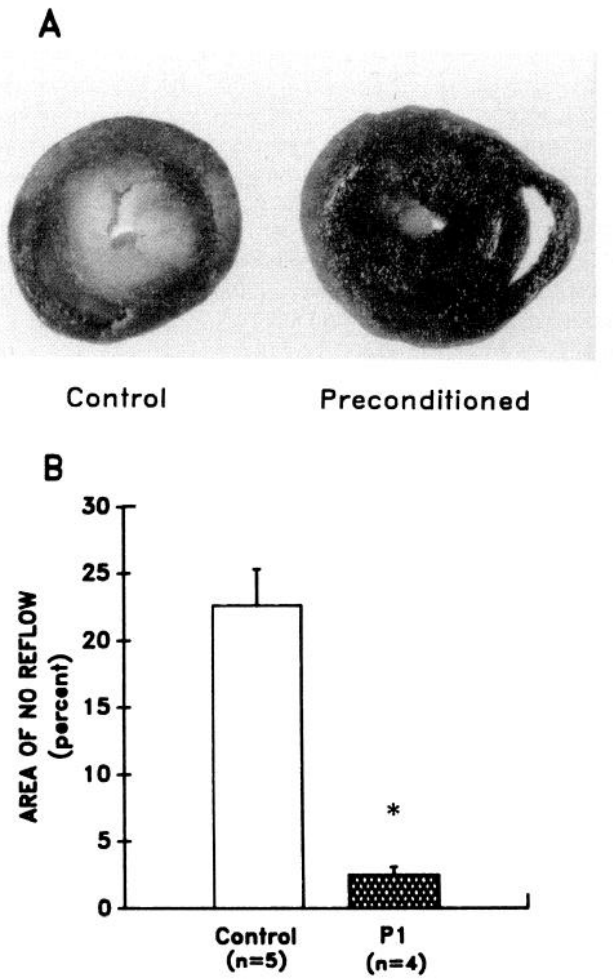

Fig. 3. Effect of preconditioning on no-reflow phenomenon. *A*: photograph shows cross sections of representative control and preconditioned (P1) hearts. At end of 30 min of reperfusion, hearts were perfused with Monastral blue dye. Unstained (light) regions show area of no-reflow, particularly in subendocardium of control hearts. *B*: quantification of areas of no reflow. Preconditioning significantly (* $P < 0.0005$) reduced area of no-flow. Area of no reflow is expressed as percentage of total ventricular area of cross sections of heart. Values are means ± SE.

Myocardial Metabolite Changes During Ischemia: Effect of Preconditioning

In this experiment, control and P1 hearts were treated as described in METHODS with the exception that the hearts were freeze-clamped either at the point just before the onset of sustained ischemia (initial), after 15 min of sustained ischemia, or after 40 min of sustained ischemia.

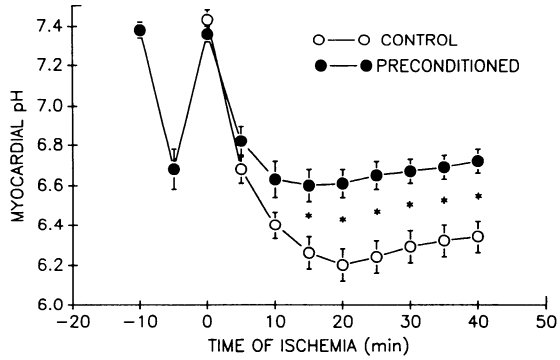

Fig. 4. Effect of preconditioning on tissue acidosis during ischemia. Serial changes in myocardial pH during sustained ischemia in control and preconditioned hearts. Values are means ± SE for 7 hearts in each group. In preconditioned group, pH declined during 5-min ischemic period (*time −5*) but returned to baseline upon reflow (*time 0*) during preconditioned period. Although both groups showed a significant reduction in pH during 40-min sustained period of ischemia, pH of preconditioned hearts was significantly higher ($P < 0.01$) than control hearts after 15 min of ischemia. This difference was maintained throughout remainder of 40-min ischemic period.

Baseline heart rates, systolic pressure, diastolic pressure, and rate-pressure products were not significantly different between control and preconditioned hearts (data not shown). Ischemic preconditioning had no significant effect on the initial myocardial ATP and lactate values (Table 3). However, the CrP levels were ~40% higher after preconditioning; this is consistent with many reports that have shown a CrP overshoot after reperfusion following a brief ischemic period. Both control and preconditioned hearts lost 80–90% of the initial ATP and CrP levels during sustained ischemia; most of this loss occurred during the first 15 min (Table 3). After 15 min of ischemia, the preconditioned hearts had slightly but significantly ($P < 0.01$) lower ATP and CrP levels.

Although lactate accumulated during ischemia in both the control and preconditioned hearts, the levels were ~30% lower in the preconditioned hearts after 15 and 40 min of ischemia (Table 3). The difference in lactate accumulation may be due to a direct effect on the glycolytic enzymes (which may affect the rate of glycolysis) or to partial loss of myocardial glycogen (which may affect the extent of anaerobic glycolysis). In fact, glycogen levels were ~30% lower after preconditioning (before the sustained ischemic period). The glycogen values before prolonged ischemia were 104 ± 13 ($n = 8$) and 68 ± 6 ($n = 7$) glycosyl U/mg protein for the control and preconditioned hearts, respectively; the difference was statistically significant ($P < 0.05$). In both groups, lactate levels increased between 15 and 40 min of ischemia, indicating a continual glycolytic flux (though slower than the initial flux) after 15 min of ischemia.

Table 3. *Values for ATP, CrP, and lactate after ischemia*

Group	*n*	ATP, nmol/mg protein	CrP, nmol/mg protein	Lactate, nmol/mg protein
Initial				
CN	8	28.1±1.3	31.6±2.3	1.4±0.3
PC	7	27.7±1.6	45.0±5.2*	2.8±5.2*
Ischemia (15 min)				
CN	8	4.6±0.5	4.9±0.2	96.9±4.1
PC	8	3.0±0.2†	4.3±0.1†	63.7±5.3‡
Ischemia (40 min)				
CN	7	3.4±0.2	5.7±0.4	145.5±10.6
PC	8	2.7±0.3	4.8±0.3	100.7±11.6§

Values are means ± SE; *n*, no. of hearts. CrP, creatine phosphate. Hearts were freeze-clamped *1*) after 35 min initial perfusion (CN) or *2*) after 25 min initial perfusion, 5 min ischemia, and 5 min reflow (PC). Values significantly different from CN: * $P < 0.05$; † $P < 0.01$; ‡ $P < 0.0005$; § $P < 0.02$.

Tissue acidosis during ischemia was significantly inhibited by ischemic preconditioning (Fig. 4). Although the myocardial pH declined during the 5-min ischemic episode during preconditioning, the pH returned to baseline after 5 min of perfusion before the 40-min sustained ischemic period. During sustained ischemia, the pH fell (from ~7.4) to 6.2 and 6.6 in the control and preconditioned hearts, respectively. In control hearts, the decrease in pH ceased after ~20 min, whereas in the preconditioned hearts the decrease in pH ceased between 10 and 15 min of ischemia. The difference in pH between the two groups was statistically significant after 15 min of ischemia, and the difference was maintained throughout the remainder of the ischemic period.

DISCUSSION

Several reports have confirmed that brief episodes of ischemia can reduce infarct size after a sustained period (40–60 min) of regional ischemia in the intact heart (3, 14, 21, 23, 26, 30). In addition, two groups of investigators (3, 6) reported that ischemic preconditioning improved postischemic wall motion of the involved region. The present study shows that a brief episode of ischemia offers substantial protection to the isolated rat heart from damage due to sustained global ischemia. This protection is evidenced by markedly improved postischemic perfusion and contractile function. A similar preconditioning effect on postischemic contractile function of the globally ischemic isolated rat heart has been reported in abstract form (2). We also found that preconditioning with a single episode of brief ischemia protected the heart as effectively as two episodes.

Mechanism of Improved Postischemic Perfusion by Ischemic Preconditioning

Ischemic preconditioning in our study improved postischemic myocardial perfusion and coronary flow rates. In the normal beating heart, coronary flow is restricted during systole due to intramyocardial pressure (5, 17). Upon reperfusion of the global ischemic rat heart, the extent to which the myocardium is initially perfused following ischemia will be dependent on *1*) the degree of intramyocardial pressure resulting from ischemic contracture and *2*) the perfusion pressure at the onset of reperfusion (1, 13, 22). Because the intraventricular pressure at the end of ischemia was greater in the preconditioned hearts compared with the control hearts (Table 2), resistance to flow would be expected to be greater in the preconditioned hearts at the onset of reperfusion. However, reperfusion was associated with hypercontracture

(as indicated by the elevated end-diastolic pressures; see Fig. 2, Table 1), which was significantly greater in the control hearts. Although we cannot eliminate the possibility that improved postischemic perfusion in the preconditioned hearts was due to a significant vascular effect, Humphrey et al. (12) reported that perfusion with the natural vasodilator, adenosine, did not lessen the no-reflow phenomenon in the isolated rat heart. Therefore the prevention of the no-reflow phenomenon by ischemic preconditioning is probably explained by an effect on the myocyte that lessens postischemic myocardial contracture.

Although increased ventricular pressure associated with ischemic contracture is primarily due to ATP depletion (9, 10, 19, 33), the increase in resting tension upon reperfusion has been shown to be dependent on the Ca^{2+} concentration of the perfusate (32). Using the isolated rat heart, Tani and Neely (34) reported that the rate of myocardial Ca^{2+} uptake during reperfusion correlated with elevation of diastolic pressure, reduced developed pressure, and decreased postischemic recovery ventricular function. These investigators (24) suggest that excessive production of H^+ during ischemia results in intracellular accumulation of Na^+ slowly during ischemia and rapidly during reperfusion; the excess intracellular Na^+ causes (by Na^+-Ca^{2+} exchange) excessive Ca^{2+} uptake during reperfusion. Besides causing postischemic hypercontracture, excessive Ca^{2+} overloading may cause irreversible damage at several intracellular sites (31). Therefore the degree of postischemic hypercontracture observed in our study may reflect the extent of Ca^{2+} overload and myocardial damage.

In our study, the ventricular balloon was left inflated throughout the experiment in all study groups. Therefore contracture was seen as developed resting tension and not fiber shortening. Humphrey et al. (13) reported that isovolumic contracture prevents the no-reflow phenomenon in the isolated heart, whereas we found large areas of no reflow in our control hearts. This apparent discrepancy may be due to our shorter ischemic period (40 vs. 60 min). We determined ventricular perfusion (dye marker) after 30 min of reperfusion, whereas they perfused the heart with dye marker at the end of the ischemic period, when transmural flow has been shown by others to occur upon initial reperfusion (1). In our study, the balloon was inflated when we injected the dye to determine myocardial perfusion, whereas they (13) deflated the balloon at the point of dye injection. Nevertheless, although pressure from the inflated balloon may have contributed to restriction of flow to the subendocardium, preconditioning greatly limited the development of no reflow.

Mechanism of Cardioprotection by Ischemic Preconditioning

Preconditioning lessened the degree of acidosis during sustained ischemia (Fig. 4). Based on the previously mentioned reports (32, 34), the higher intracellular pH during ischemia may result in less intracellular Ca^{2+} overload and less intracellular damage during ischemia and reperfusion of the preconditioned hearts. Supportive of this hypothesis, VanderHeide et al. (35) reported in a recent abstract that ischemic preconditioning lessens the rise in intracellular Ca^{2+} during ischemia in the globally ischemic rat heart. A similar mechanism of protection may occur in models of regional ischemia; Kida et al. (16) reported that ischemic preconditioning decreased the rate of acidosis during sustained regional ischemia in the pig heart. The possible role of H^+ accumulation in ischemic injury in models of regional and global ischemia is also suggested by observations that maintenance of extracellular acidosis during early reperfusion improves postischemic mechanical function, presumably by attenuating Na^+-Ca^{2+} exchange (10, 18).

During the early stage of myocardial ischemia, acidosis is mainly due to ATP hydrolysis and CO_2 production and retention (4). The slower rate of lactate accumulation during ischemia in the preconditioned hearts (Table 3) is indicative of a slower glycolytic flux, possibly resulting from a decreased rate of ATP hydrolysis. Another possible factor contributing to pH preservation during early ischemia is the elevated CrP levels after preconditioning, since the transfer of phosphate from CrP to ADP consumes a proton (4). Also, the partial glycogen depletion we found associated with preconditioning may play a role in the reduced myocardial acidosis as suggested by Garlick et al. (7).

In our model, ischemic preconditioning resulted in depressed myocardial function before the 40-min sustained ischemic period. (Table 1, Fig. 2). The reduced contractile state after preconditioning may reduce ATP utilization during ischemia; this may provide a protective effect by reducing acidosis (as discussed above) or by conserving high-energy phosphates. Our data do not support the hypothesis that the protective effect of ischemic preconditioning is due to conservation of ATP (Table 3). Conservation of ATP may have occurred during the early ischemic period (<15 min). However, this is not likely, since a protective mechanism involving preservation of tissue ATP would be expected to prolong time to ischemic contracture, whereas we found that time to contracture was actually decreased by preconditioning (Table 2). Similarly, Neely and Grotyohann (29) reported that postischemic contractile dysfunction in the isolated perfused rat heart does not correlate with low tissue levels of ATP or CrP during ischemia. In models of regional ischemia, ischemic preconditioning has been reported to transiently conserve ATP (18, 28) and CrP (18) levels during sustained ischemia, but the degree of conservation of the high energy phosphate was considerably less than the degree of infarct-size limitation (18, 28). Moreover, two recent reports demonstrated that the infarct size-limiting effect of ischemic preconditioning in regional ischemia models did not correlate with the extent of reduced wall motion (stunning) after the preconditioning period (25, 27). It is therefore unlikely that myocardial stunning or conservation of high energy phosphates are sufficient to explain the protective effects of ischemic preconditioning.

In comparing the results of the present study to other

investigations, one must be aware of possible species differences in the mechanisms of ischemic injury and protection by preconditioning. Moreover, differences in models of ischemia, i.e., regional (in situ) vs. global ischemia (denervated), in the isolated heart should be considered. Furthermore, with ischemic preconditioning, different mechanisms may be responsible for improved postischemic contractile recovery and for infarct size limitation. In our model of global ischemia and preconditioning, postischemic contractile dysfunction of control hearts was associated with no reflow, whereas in a model regional ischemia, postischemic myocardial blood flow was near normal and did not differ between control and preconditioned groups (30). The differences may be due to greater ischemic injury in the global ischemic model (since any protective effect of collateral flow is eliminated) or to the mechanical stretching (which may limit contracture upon reperfusion) of the affected region of the beating heart in the regional ischemia model. We do not know to what degree improved postischemic perfusion of the preconditioned hearts contributed to enhanced postischemic contractile function in our study. Although the improved perfusion likely enhanced myocardial salvage, this may be unique to the isolated perfused heart. However, the present study demonstrates a cardioprotective effect of a short ischemic episode that cannot be explained by modulation of circulating systemic agents, autonomic neuronal activity, or neutrophil-mediated injury.

In summary, the results of this study show that ischemic preconditioning improves contractile function and myocardial perfusion and decreases postischemic diastolic dysfunction after prolonged global ischemia in the isolated perfused rat heart. This enhanced tolerance to ischemia was not associated with conservation of ATP and CrP but was associated with decreased tissue acidosis and anaerobic glycolysis during the sustained ischemic episode.

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