

Preconditioning with ischemia: a delay of lethal cell injury in ischemic myocardium

CHARLES E. MURRY, B.S., ROBERT B. JENNINGS, M.D., AND KEITH A. REIMER, M.D., PH.D.

ABSTRACT We have previously shown that a brief episode of ischemia slows the rate of ATP depletion during subsequent ischemic episodes. Additionally, intermittent reperfusion may be beneficial to the myocardium by washing out catabolites that have accumulated during ischemia.¹ Thus, we proposed that multiple brief ischemic episodes might actually protect the heart from a subsequent sustained ischemic insult. To test this hypothesis, two sets of experiments were performed. In the first set, one group of dogs ($n = 7$) was preconditioned with four 5 min circumflex occlusions, each separated by 5 min of reperfusion, followed by a sustained 40 min occlusion. The control group ($n = 5$) received a single 40 min occlusion. In the second study, an identical preconditioning protocol was followed, and animals ($n = 9$) then received a sustained 3 hr occlusion. Control animals ($n = 7$) received a single 3 hr occlusion. Animals were allowed 4 days of reperfusion thereafter. Histologic infarct size then was measured and was related to the major baseline predictors of infarct size, including the anatomic area at risk and collateral blood flow. In the 40 min study, preconditioning with ischemia paradoxically limited infarct size to 25% of that seen in the control group ($p < .001$). Collateral blood flows were not significantly different in the two groups. In the 3 hr study, there was no difference between infarct size in the preconditioned and control groups. The protective effect of preconditioning in the 40 min study may have been due to reduced ATP depletion and/or to reduced catabolite accumulation during the sustained occlusion. These results suggest that the multiple anginal episodes that often precede myocardial infarction in man may delay cell death after coronary occlusion, and thereby allow for greater salvage of myocardium through reperfusion therapy.

Circulation 74, No. 5, 1124–1136, 1986.

OCCLUSION of a major coronary artery in the dog induces injury that is reversible for the first 15 to 20 min, in that complete tissue recovery ensues after reperfusion. However, if the occlusion is maintained beyond this time, the injury becomes irreversible; some cells die despite reperfusion. Ischemic cell death begins first in the subendocardial region and then progresses with time toward the subepicardium.²

A previous study from our laboratory¹ has shown that repeated brief episodes of ischemia do not have a cumulative deleterious effect. Four 10 min occlusions produced no more ATP depletion than a single occlusion, and did not cause necrosis, although 40 min of sustained ischemia has been associated with severe

ATP depletion and cell death.^{2,3} Thus, intermittent reperfusion prevents the cumulative effects of repeated ischemic insults. The mechanism by which intermittent reperfusion prevents cumulative injury is not known with certainty. However, we found that the rate of ATP depletion was actually slowed in subsequent ischemic episodes compared with the first. Additionally, many potentially harmful catabolites, such as lactate, H^+ , NH_3 , etc., were washed out with each reperfusion.

Given these salutary effects of intermittent ischemia and reperfusion with respect to sustained ischemia, we postulated that multiple brief ischemic episodes might actually protect the myocardium during a subsequent sustained ischemic insult so that, in effect, we could exploit ischemia to protect the heart from ischemic injury. This experiment was designed to answer two basic questions: (1) Will preconditioning the myocardium with four 5 min episodes of ischemia result in less necrosis from a sustained 40 min coronary occlusion when compared with that in animals subjected to

From the Department of Pathology, Duke University Medical Center, Durham, NC 27710.

Supported in part by NIH grants 27416 and 23138.

Address for correspondence: Keith A. Reimer, M.D., Ph.D., Department of Pathology, Box 3712, Duke University Medical Center, Durham, NC 27710.

Received May 28, 1986; revision accepted Aug. 7, 1986.

only a single 40 min occlusion? (2) If so, will this protective effect extend through a 3 hr episode of sustained ischemia?

Materials and methods

Animal selection and surgical preparation. All experiments reported here conformed to the American Physiological Society's guidelines regarding the use of laboratory animals. Forty-four healthy adult mongrel dogs of either sex, weighing between 10 and 25 kg and having hematocrits of 35 or more, were fasted overnight and anesthetized with 30 to 40 mg/kg of sodium pentobarbital. Animals were intubated and ventilated, with use of a Harvard model 607 animal respirator, with 200 ml/kg-min room air supplemented with oxygen. Ventilatory rate and oxygen content were adjusted as needed to maintain arterial blood gas values within physiologic ranges. Penicillin, 750,000 U im, was given and an aseptic surgical technique was used throughout each experiment. Polyethylene catheters were placed in the right femoral artery and vein. The arterial catheter was used to measure blood pressure via a Statham P23 pressure transducer and to withdraw reference samples for measurement of blood flow by a microsphere technique (see below). The venous catheter was used to administer additional anesthetic, as needed, throughout the experiment.

A left thoracotomy was performed through the fourth intercostal space, and the heart was suspended in a pericardial cradle. The left atrial appendage was snared, and the left circumflex coronary artery was isolated distal to its atrial appendage branch but proximal to its first large marginal branch. A silk suture was passed around it. Occlusion later was accomplished by snaring the artery into a small plastic tube. Two catheters were placed in the left atrium via its appendage. One was used to monitor left atrial pressure, and the other to inject microspheres for measurement of myocardial blood flow. Tween 80 was administered (0.05%, 0.6 ml) to desensitize the animal before injection of microsphere suspensions containing this detergent. Left atrial pressure, arterial pressure, lead II of the standard electrocardiogram, and pericardial temperature were monitored throughout the experiment on a Gould model 2400 recorder. The animals were allowed at least 15 min after these surgical procedures and instrumentation to reach steady state.

Any animal that developed ventricular fibrillation during ischemia or reflow was cardioverted, if possible, with an MRL model 560 defibrillator and internal paddles. After completion of the experimental protocol, the chest wound was closed, air was evacuated from the chest, and the animals were allowed to survive for 4 days to delineate the necrotic muscle. At the completion of the experiment, animals were reanesthetized and given 5000 units of heparin to aid postmortem coronary perfusion. Hearts then were excised for postmortem analysis (see below).

Measurements of regional blood flow. Regional myocardial blood flow was measured before and after occlusion by the microsphere technique. At the times indicated in the experimental design section, 2 to 3 million radiolabeled $9 \pm 1 \mu\text{m}$ microspheres, labeled with ^{46}Sc , ^{113}Sn , or ^{153}Gd (New England Nuclear) and suspended in 0.05% Tween 80 and 10% dextran, were injected through the catheter in the left atrial appendage, followed by a 15 ml saline flush. Beginning just before and continuing for 2.5 min after injection, reference samples were withdrawn from the femoral artery at a rate of 7.75 ml/min. Sample radioactivity was measured on a Packard model 5912 gamma counter, with corrections made for overlap of isotope spectra. Myocardial blood flow was calculated according to the formula: tissue flow = (tissue counts) (reference flow)/refer-

ence counts and expressed in milliliters per minute per gram wet weight.

Postmortem studies. The primary experimental end point was histologic infarct size, which was assessed in relation to major baseline predictors of infarct size, namely the area at risk and collateral blood flow.^{2,4}

Area at risk. The left main and circumflex coronary arteries of the excised heart were cannulated and perfused simultaneously with dye solutions at 120 to 140 mm Hg pressure to define the interface between the previously occluded and unoccluded vascular beds. The perfusion fluid was sodium phosphate buffer ($8.8 \times 10^{-2}\text{M}$ dibasic and $1.8 \times 10^{-3}\text{M}$ monobasic sodium phosphate, pH 8.25 to 8.6) with 6% dextran (mol wt 82,000, Sigma Chemical Company) added to prevent edema during perfusion. Triphenyl tetrazolium chloride (TTC, 1%, Sigma Chemical Company) was perfused into the previously ischemic circumflex bed, while monastral blue dye (0.5%, DuPont) was perfused into the nonischemic vascular bed via the left main artery. The perfused hearts were incubated at 37° C in normal saline for 30 min, and were then fixed by immersion in a large volume of phosphate-buffered formalin (pH 7.0). The fixed left ventricles then were cut into eight transverse sections (figure 1) that were weighed, and the apical surfaces were then photographed. Enlarged projections of the ventricular cross sections were traced, including the boundaries of the previously ischemic vascular bed (area at risk of infarction). The area at risk then was measured by "cut and weight" techniques on photocopies of these tracings.²

Histologic analysis. The method of tissue sampling is shown in figure 1. The apical surfaces of slices 1a, 2a, 3a, 4a, and 4b were used to measure infarct size. Tissue sections were stained with Heidenhain's variant of Mallory's connective tissue stain. Enlarged projections of the histologic slides were traced to define the necrotic and viable areas, and infarct size was calculated by cut and weigh techniques on copies of these tracings. Infarct size was determined for each slice, as a percentage of the entire left ventricle, and as a percentage of the area at risk.

Regional blood flow. Flow measurements were made in the remaining portions of the first three ventricular slices (figure 1). The slices were divided into the nonischemic and central ischemic zones. The latter zone consists of the central 50% to 60% of the vascular bed at risk. The lateral and septal border zones were excluded to avoid possible misinterpretation of measurements from samples that contained a mixture of ischemic and nonischemic tissue. The nonischemic and central ischemic regions were subdivided into subendocardial, midmyocardial, and subepicardial thirds. Corrections were made for apparent microsphere loss in 13 dogs that had preocclusion ischemic region/nonischemic region flow ratios of less than 0.90. This allows for correction of artifactually low blood flows and high infarct sizes that occur secondary to edema, inflammatory cells, and hemorrhage within the region of the infarct.⁵ Data from four animals that had subendocardial blood flows greater than 0.15 ml/min-g wet weight during the sustained occlusion (see tables 2 and 3) were excluded from the group comparison of infarct size because they did not develop severe subendocardial ischemia. However, these data were included in the comparison of infarct size vs collateral blood flow.

Statistics. All measurements are expressed as group means $\pm$ SEM. A two-tailed unpaired Student t test was used to compare two different groups of animals. When data from the same animals at different times were compared, Student's paired t test was used. A p value $\leq .05$ was considered indicative of a statistically significant difference.

Experimental design. The experimental design is shown in figure 2. Animals were randomized into preconditioned and control groups in two studies. Each study compared the effects

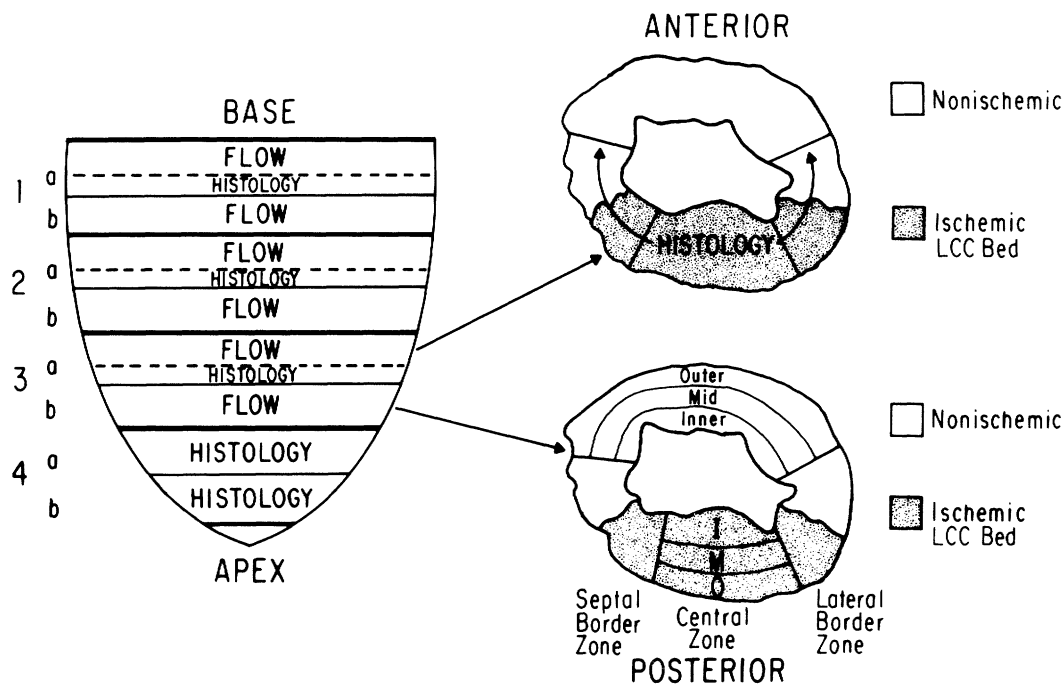


FIGURE 1. The postmortem technique for calculating the area at risk, histologic infarct size, and regional blood flow. The formalin-fixed left ventricle was isolated and sliced first into four slices and then into eight slices with a commercial meat slicer. These eight slices were used to determine the area at risk. Histologic slides were obtained from the apical surfaces of slices 1a, 2a, 3a, 4a, and 4b, and were assumed to represent the amount of infarction in slices 1, 2, 3, and 4, respectively. The remaining myocardium from slices 1 through 3 was used to calculate regional myocardial blood flow. Nonischemic and central ischemic regions were divided into inner, middle, and outer thirds for blood flow analysis. The lateral and septal samples were discarded to avoid use of samples that contained mixtures of ischemic and nonischemic tissue. This figure was reproduced from Reimer and Jennings.²²

of preconditioning with four 5 min occlusions, each separated by 5 min of reperfusion, on the viability of cells after a longer, sustained episode of ischemia. Thus, in the first study the preconditioned group ($n = 12$) underwent four 5 min episodes of ischemia, each separated by 5 min of reperfusion, followed by a sustained 40 min occlusion. The control group ($n = 9$) underwent a single 40 min occlusion. Myocardial blood flow was measured before occlusion and midway through the 40 min occlusion in both preconditioned and control groups. In addition, to test for an effect of preconditioning on collateral blood flow, blood flow was measured 2.5 min into the first preconditioning occlusion, and at 2.5 min into the 40 min occlusion in the control group.

In the second study the preconditioned group ($n = 13$) underwent four 5 min ischemic episodes, each separated by 5 min of reflow, followed by 180 min of sustained ischemia. The control group ($n = 9$) underwent a single 180 min occlusion. Myocardial blood flow was measured before occlusion and 105 min into the 180 min occlusion in both groups. As in the first study, to test for an effect of preconditioning on collateral blood flow, flow measurements were made 2.5 min into the first preconditioning occlusion and at 2.5 min into the 180 min occlusion in the control group. One additional control animal that was subjected to a single 180 min occlusion as part of a concurrent study was included in the control group reported here to make group sizes more comparable.

Results

General biology of preconditioning. Initially, this study was attempted with use of one 15 min occlusion for the

preconditioning period on the basis that we wanted to achieve reversible injury of maximum severity before exposing the heart to a later, prolonged episode of ischemia. However, preconditioning of this duration was associated with excessive mortality; approximately 75% of the animals developed intractable ventricular fibrillation. We then attempted two 10 min occlusions, but again found mortality to be high. The incidence of lethal arrhythmias was reduced greatly by switching to the 5 min occlusions used in this study, so that the mortality was essentially identical in the preconditioned and control groups (table 1). The use of four occlusions, while somewhat arbitrary, was chosen to enhance formation and subsequent washout of ischemic catabolites. Five minute reflow periods were chosen to ensure that the adenylate charge ($[ATP + \frac{1}{2}ADP]/[ATP + ADP + AMP]$) was restored and lactate was washed out.⁶

The first circumflex occlusion generally was accompanied by rapid development of epicardial cyanosis and by dramatic elevation of the ST segment of the electrocardiogram. However, in subsequent occlusions, these changes were slower to develop and frequently did not reach the magnitude seen during the

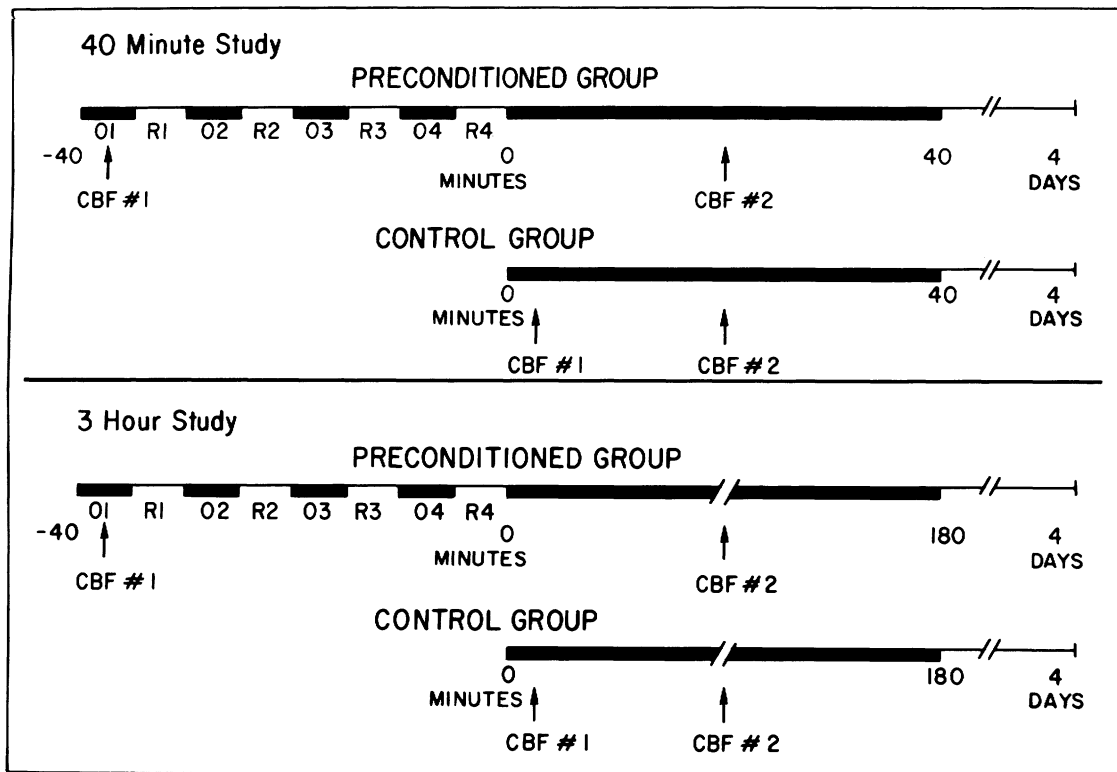


FIGURE 2. Experimental design. In the 40 min study, preconditioned animals underwent four 5 min circumflex coronary occlusions, each separated by 5 min of reperfusion, followed by a sustained 40 min occlusion. Control animals underwent a single 40 min occlusion. Collateral blood flow (CBF) in the preconditioned group was measured midway through the first 5 min occlusion and midway through the 40 min occlusion. CBF in the control group was measured 2.5 min into and midway through the 40 min occlusion. Both groups were allowed 4 days of reperfusion after the 40 min occlusion. In the 3 hr study, preconditioned animals underwent four 5 min circumflex coronary occlusions, each separated by 5 min of reperfusion, followed by a sustained 3 hr occlusion. Control animals underwent a single 3 hr occlusion. CBF in the preconditioned group was measured midway through the first 5 min occlusion and 105 min into the 3 hr occlusion. CBF in the control group was measured 2.5 min into and 105 min into the 3 hr occlusion. Both groups were allowed 4 days of reperfusion after the 3 hr occlusion. O = occlusion; R = reperfusion.

first occlusion. The mechanism behind this is unknown, but other investigators,⁷ using microelectrode-impaled cardiac myocytes, reported that repeated exposure to altered superfusion medium that mimicked ischemia (hypoxic, hyperkalemic, acidotic) resulted in

lesser electrical aberrations in later "ischemic" episodes than in the initial episode. Their findings may represent a cellular correlate to our findings after repeated occlusions.

To test for an effect of preconditioning on subsequent measurements of collateral blood flow, we used all animals in which early and late measurements of collateral flow were obtained, including some that were excluded from infarct size comparison because they died prematurely. Blood flow was measured accurately in the latter group, despite their unsuitability for inclusion in the comparison of infarct size. None of the groups exhibited an increase in subendocardial collateral flow from the early to late measurements. In control animals, flow to the subepicardial region tended to increase in the 40 min group (0.24 to 0.29 ml/min·g, $p = .06$) and showed a significant increase in the 180 min group (0.28 to 0.42 ml/min·g, $p < .005$). The preconditioned animals had increases in

TABLE 1
Mortality in 40 min and 3 hr studies

Group	No. of dogs enrolled	VF	Defibrillated	Late deaths	Survival (n/%)
40 min study					
Preconditioned	12	5	2	0	9/75
Control	9	2	0	1	6/67
3 hr study					
Preconditioned	12	2	1	1	10/83
Control	9	0	—	2	7/78

Two animals were excluded for technical reasons.

VF = ventricular fibrillation.

subepicardial flow from the first occlusion to midway through the sustained occlusion that were similar to those in control animals: 0.30 to 0.41 ml/min·g in the $5 \times 4 + 40$ group ($p < .05$) and 0.22 to 0.35 ml/min·g in the $5 \times 4 + 180$ group ($p < .005$). Because the increases in flow that occurred over time in the control animals were similar to those that occurred between the first and fifth occlusion in the preconditioned animals, we believe that preconditioning did not increase ultimate collateral flow. In any case, we used the measurement of collateral flow midway through the sustained occlusion as a baseline predictor of infarct size, thereby eliminating any effect of preconditioning-induced changes in collateral flow.

Forty minute study. Infarct size, blood flow, and hemodynamic data are shown for each dog in the 40 min study in table 2. Infarct size in control animals averaged $29.4 \pm 4.4\%$ of the anatomic area at risk. In sharp contrast, in animals that were preconditioned with four 5 min occlusions and then subjected to 40 min of sustained ischemia, infarct size averaged only $7.3 \pm 2.1\%$ of the area at risk (figure 3). Thus, despite the fact that they received 60 min of cumulative ischemia, the preconditioned animals had infarcts that were only one-fourth the size of those found in control animals undergoing 40 min of sustained ischemia.

Aside from the difference in infarct size, the morphologic characteristics of the infarcts were different

TABLE 2

Infarct size, blood flow, and hemodynamic data in preconditioned and control dogs (40 min study)

Group and dog No.	AAR %	Infarct size		Control LAD flow (ml/ min · g)	CBF 1 (ml/min · g)				CBF 2 (ml/min · g)				20 min hemodynamics			
		% LV	% AAR		I	M	O	T	I	M	O	T	HR	SBP	RPP/ 100	
Preconditioned																
1	35.5	1.6	4.4	0.83	0.01	0.01	0.08	0.04	0.03	0.05	0.11	0.06	134	131	175	
2	40.7	0.8	2.0	1.33	0.06	0.11	0.25	0.14	0.08	0.18	0.37	0.22	146	121	177	
3	32.1	1.2	3.7	0.98	0.00	0.02	0.05	0.03	0.02	0.04	0.05	0.04	168	141	237	
4	40.3	5.6	14.0	1.14	0.04	0.10	0.27	0.14	0.05	0.12	0.32	0.16	176	147	259	
5	41.7	6.1	14.5	1.04	0.01	0.03	0.11	0.05	0.02	0.04	0.11	0.05	158	115	182	
6	39.7	4.2	10.6	0.61	X	X	X	X	0.01	0.02	0.07	0.03	120	144	173	
7	46.9	1.0	2.2	1.72	0.05	0.12	0.33	0.17	0.13	0.25	0.51	0.30	140	136	190	
8	38.0	0.0	0.1	1.21	0.07	0.14	0.43	0.21	0.22	0.38	0.83	0.48	155	153	237	
9	39.6	0.1	0.3	0.77	0.34	0.52	0.88	0.59	0.71	0.86	1.01	0.86	154	150	231	
Mean	39.4	2.3	5.8	1.07	0.07	0.13	0.30	0.17	0.05	0.22	0.38	0.24	150	138	207	
± SEM	± 1.4	± 0.8	± 1.9	± 0.11	± 0.04	± 0.06	± 0.09	± 0.06	± 0.02	± 0.09	± 0.12	± 0.09	± 6	± 4	± 11	
Mean without dogs 8 and 9																
39.6	2.9	7.3	1.09	0.03	0.06	0.18	0.10	0.05	0.10	0.22	0.12	0.12	149	134	199	
± SEM	± 1.8	± 0.9	± 2.1	± 0.14	± 0.01	± 0.02	± 0.05	± 0.02	± 0.02	± 0.03	± 0.07	± 0.04	± 7	± 4	± 13	
Control																
1	42.3	11.1	26.3	0.90	0.03	0.07	0.24	0.12	0.03	0.09	0.29	0.14	180	176	317	
2	43.6	14.1	30.8	1.33	0.05	0.13	0.18	0.12	0.06	0.10	0.21	0.13	176	151	266	
3	41.4	19.2	43.4	1.56	0.01	0.01	0.15	0.07	0.00	0.02	0.17	0.08	168	176	296	
4	41.8	12.9	30.8	1.35	0.03	0.07	0.23	0.11	0.01	0.08	0.23	0.09	188	142	267	
5	32.9	5.5	15.8	1.44	0.03	0.14	0.34	0.18	0.07	0.19	0.44	0.24	126	120	151	
6	39.7	5.4	12.3	2.68	0.12	0.23	0.54	0.32	0.19	0.35	0.74	0.46	164	175	287	
Mean	40.3	11.4	26.6	1.54	0.04	0.11	0.28	0.15	0.03	0.14	0.35	0.19	167	157	264	
± SEM	± 1.6	± 2.2	± 4.6	± 0.24	± 0.02	± 0.03	± 0.06	± 0.04	± 0.01	± 0.05	± 0.09	± 0.06	± 9	± 9	± 24	
Mean without dog 6																
40.4	12.6	29.4	1.32	0.03	0.08	0.23	0.12	0.03	0.10	0.27	0.14	0.14	168	153	259	
± SEM	± 1.9	± 2.2	± 4.4	± 0.11	± 0.01	± 0.02	± 0.03	± 0.02	± 0.01	± 0.03	± 0.05	± 0.03	± 10	± 11	± 29	
p value ^A	NS	<.005	<.001	NS	NS	NS	NS	NS	NS	NS	NS	NS	NS	NS	NS	

AAR = anatomic area at risk; LV = left ventricle; Control LAD flow = transmural mean left anterior descending bed (nonischemic) flow before occlusion; CBFs 1 and 2 = collateral blood flows as in figure 2; I = inner (subendocardial) third; M = middle third; O = outer (subepicardial) third; T = transmural mean; HR = heart rate (beats/min); SBP = systolic blood pressure (mm Hg); RPP = rate-pressure product (mm Hg/min); X = no early collateral blood flow measurement.

^Ap values are result of unpaired t tests performed on group data from which findings in high-flow dogs (Nos. 8 and 9 in preconditioned, No. 6 in control group) have been excluded.

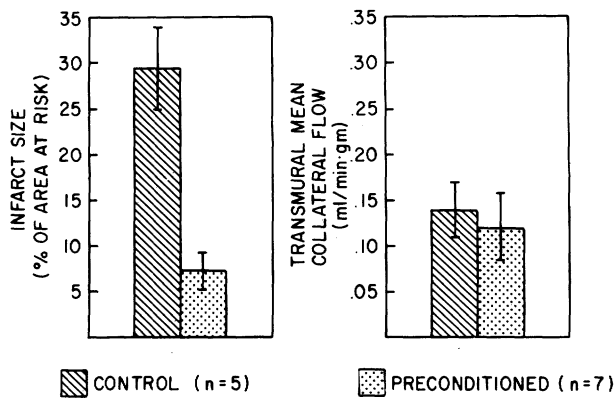


FIGURE 3. Infarct size and collateral blood flow in the 40 min study. *Left*, Infarct size, as a percentage of the anatomic area at risk, in the control (striped bar) and preconditioned (stippled bar) hearts. Infarct size in control animals averaged 29.4% of the area at risk. Infarct size in control hearts averaged only 7.3% of the area at risk (preconditioned vs control, $p < .001$). Transmural mean collateral blood flow (*right*) was not significantly different in the two groups. Thus, the protective effect of preconditioning was independent of the two major baseline predictors of infarct size, area at risk and collateral blood flow. Bars represent group means $\pm$ SEM.

in the two groups as well. The control animals generally had solid infarcts extending from one lateral margin of the ischemic bed to the other. Infarcts were located in the subendocardial myocardium, with peninsulas of viable myocardium projecting into the necrotic muscle. In the preconditioned animals, infarcts were rarely confluent in the lateral dimension, but rather consisted of multiple small foci of necrosis, scattered within predominantly viable myocardium. Moreover, within the necrotic foci, viable and necrotic cells were frequently interspersed. Because of this patchiness, some preconditioned infarcts were nearly undetectable grossly by the TTC method. This insensitivity of TTC macrochemistry was most likely caused by positive staining of a few viable cells, which could give an entire focus of patchy necrosis the appearance of viability.

Collateral blood flows to the subendocardium (zone of infarction) or as a transmural mean midway through the 40 min occlusion were not significantly different in the preconditioned and control groups (table 2; figure 3). Thus, the smaller infarcts in the preconditioned animals were not a result of greater collateral blood flow during the 40 min occlusion. Figure 4 shows a regression plot of infarct size, as a percentage of the area at risk, versus transmural mean collateral blood flow in preconditioned and control animals. We have included in this graphic analysis data from control animals in a concurrent study performed under exactly the same conditions to better define the relationship be-

tween flow and necrosis in control hearts. Although there was some scatter, data from control animals definitely reflected an inverse relationship between collateral blood flow and infarct size; i.e., animals with low flow had large infarcts and vice versa. However, infarct size in preconditioned animals was small, regardless of collateral flow. Thus, preconditioning limited infarct size and altered the relationship between necrosis and collateral flow.

Hemodynamic data from the 40 min study are shown in figure 5 and table 2. There were no significant differences between preconditioned and control groups with respect to any hemodynamic variables before the first coronary occlusion. After the preconditioning period, systolic pressure (and hence rate-pressure product) in preconditioned dogs was significantly lower than that observed in control dogs before the 40 min occlusion. In both groups, systolic pressure was modestly decreased during the 40 min occlusion compared with the respective initial values before the first occlusion. Although the systolic pressure and rate-pressure product midway through the 40 min occlusion tended to be higher in control vs preconditioned dogs, this difference did not reach statistical significance.

Three hour study. Infarct size, blood flow, and mid-occlusion hemodynamic data from the 3 hr study are shown in table 3. Infarct size in control animals undergoing a single 3 hr circumflex occlusion averaged $47.9 \pm 6.6\%$ of the anatomic area at risk (figure 6). This value agreed well with that from previous studies in our laboratory. Infarct size in preconditioned animals averaged $47.1 \pm 4.8\%$ of the area at risk, which was not significantly different from that in the control group.

In contrast to the 40 min study, animals that were preconditioned and then subjected to a 3 hr sustained occlusion developed infarcts that were confluent between the lateral margins of the ischemic vascular bed, similar to those in the 3 hr control group. The subepicardial regions of the preconditioned infarcts tended to be patchier than their control counterparts, but this qualitative difference was not nearly so pronounced as it was in the 40 min study.

Collateral blood flow (table 3; figure 6) midway through the 180 min occlusion was not significantly different in the two groups. This was true for both collateral flow to the subepicardium and as a transmural average. Figure 7 illustrates infarct size, as a percentage of the area at risk, plotted against mean transmural collateral flow midway through the 180 min occlusion. It is apparent that there is a general inverse relationship: animals with high collateral flow

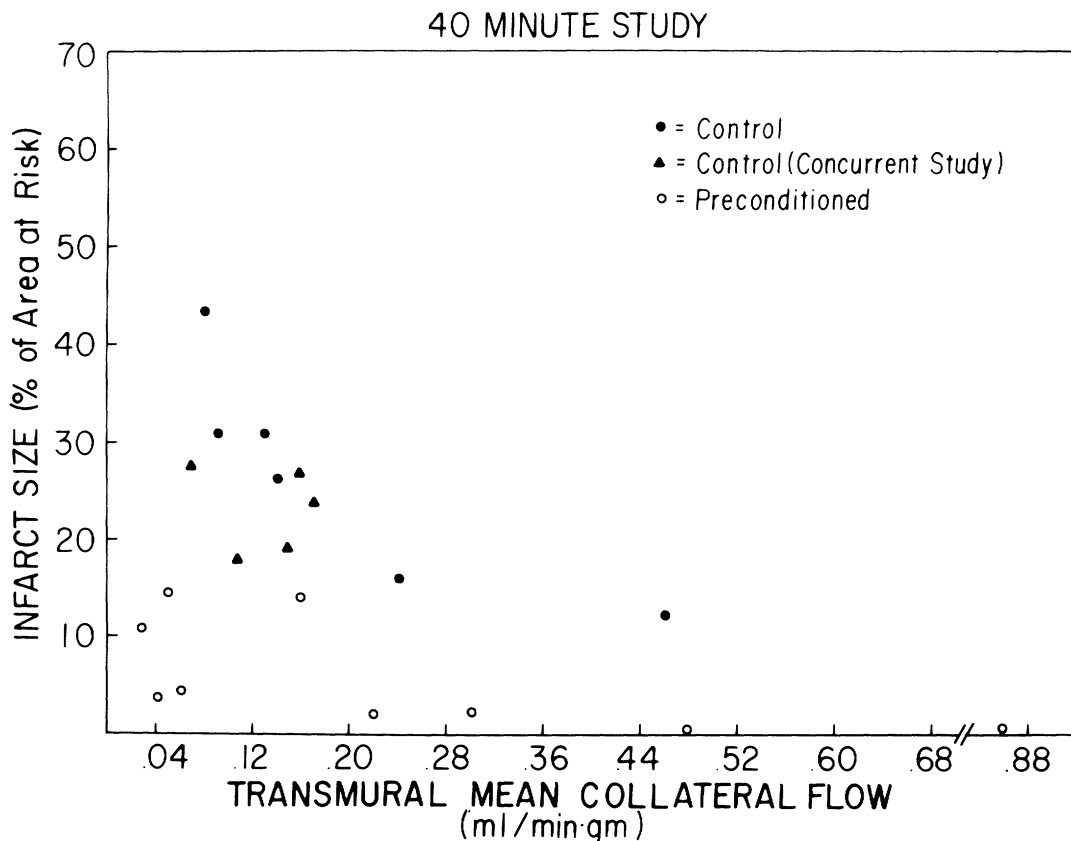


FIGURE 4. Infarct size vs collateral blood flow, 40 min study. The relationship between infarct size, normalized as a percentage of the area at risk, and transmural mean collateral blood measured at 20 min into the 40 min coronary occlusion is illustrated. In control dogs (closed circles and triangles), animals with greater collateral flow had smaller infarcts and vice versa. However, among preconditioned dogs (open circles), all animals had small infarcts, irrespective of collateral blood flow. Each point represents one animal. The closed triangles represent control animals from another study, treated identically, which are included to better define the relationship between flow and necrosis in control hearts.

had smaller infarcts, and those with low collateral flow had larger infarcts. Also apparent in this figure is the fact that there is no difference between preconditioned animals and control animals with respect to the relationship between infarct size and collateral blood flow; the lines are virtually superimposable. Thus, preconditioning with four 5 min episodes of ischemia failed to limit infarct size during a sustained 3 hr coronary occlusion.

Hemodynamic data from the 3 hr study are summarized in figure 8. Values were not significantly different between groups at a given time, or within groups at different times, except that heart rate midway through the 3 hr occlusion was significantly higher in the preconditioned animals than in the controls (165 ± 7 vs 136 ± 6 respectively, $p < .01$). This difference in heart rate led to a higher rate-pressure product in preconditioned animals during the 3 hr occlusion ($p < .05$).

Discussion

The data presented show that brief, intermittent episodes of ischemia have a protective effect on myocardium that is later subjected to a sustained bout of ischemia. Indeed, when animals were subjected to a sustained 40 min circumflex occlusion, those that were preconditioned suffered only one-quarter as much necrosis as that incurred by control "virgin" hearts. However, this protective effect served only to delay cell death; when the episode of sustained ischemia was extended to 3 hr, infarct size was not significantly different in control and preconditioned hearts.

Other investigators have studied the effects of repeated coronary occlusions on myocardial metabolism, structure, function, and viability. Several groups have shown that repeated brief episodes of ischemia do not produce cumulative deficits in myocardial adenine nucleotide content.^{1, 8, 9} Also, myocardial ultrastructure, tissue water, and electrolyte content do not show

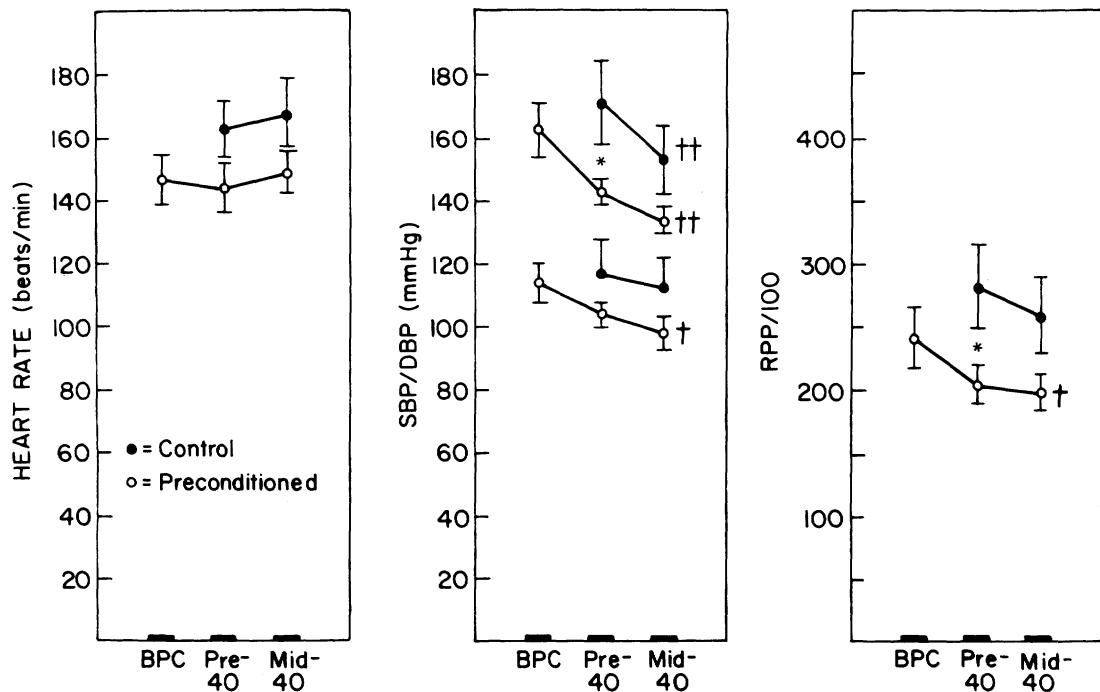


FIGURE 5. Hemodynamic values from the 40 min study. BPC = before preconditioning; Pre-40 = immediately before 40 min occlusion; Mid-40 = 20 min into the 40 min occlusion; SBP = systolic blood pressure; DBP = diastolic blood pressure; RPP = rate-pressure product (systolic blood pressure $\times$ heart rate). * $p < .05$ for preconditioned vs control groups at a given time; † and †† $p < .05$ and $p < .01$, respectively, for the first vs the second or third measurement in the same group. (No significant differences were observed between the second and third measurements for any hemodynamic variable in the preconditioned group). See text for additional explanation.

cumulative effects of four 10 min episodes of severe ischemia.¹⁰ Contractile deficits also are not cumulative. Lange et al.¹¹ have demonstrated that three 5 or 15 min coronary occlusions produce no greater impairment in regional myocardial function than that induced by a single occlusion.

With respect to cell death, Geft et al.¹² have reported that 18 brief periods of ischemia caused small subendocardial infarcts in a minority of dogs studied, while we have shown that four 10 min circumflex occlusions caused virtually no necrosis.¹ The reason for this difference may be the greater number of ischemic episodes induced in the former study. In any case, intermittent reperfusion appears to prevent or greatly attenuate the cumulative effects of brief ischemic episodes. The mechanism of this beneficial effect is unknown; however, washout of ischemic catabolites and/or restoration of the capacity of the myocyte to make ATP from phosphocreatine or anaerobic glycolysis are good possible explanations.

It is of interest that Selye et al.¹³ have reported that rats in which myocardial infarction was induced were protected against further cardiac necrosis induced by toxic doses of isoproterenol. This phenomenon was

termed myocardial resistance by Dusek et al.,¹⁴ who reported that the protective effect was sustained for a month beyond the time of coronary ligation. Whether resistance to isoproterenol-induced cardiotoxicity is related to the protective effect of preconditioning observed in the present study is unknown.

Critique of method. Any study that attempts to quantitate the effect of a particular treatment on myocardial viability after ischemic injury must first take into account the baseline predictors of infarct size. In the dog, these baseline variables have been shown to be the size of the ischemic vascular bed (area at risk), collateral blood flow to the ischemic region, and less importantly, myocardial oxygen demand as estimated by the rate-pressure product.^{2, 4, 15} Without accounting for these variables, unless very large numbers of animals are used, false results may be obtained as a result of variation in the characteristics of the animals included in the groups. In this study, infarct size has been reported as a percentage of the anatomic area at risk to correct for variation in vascular anatomy or occlusion site. Collateral blood flow was measured directly in each layer of the heart, and data from animals with high collateral flow were excluded from intergroup

TABLE 3

Infarct size, blood flow, and hemodynamic data in preconditioned and control dogs (3 hr study)

Group and dog No.	AAR %	Infarct size			Control LAD flow (ml/ min·g)	CBF 1 (ml/min·g)				CBF 2 (ml/min·g)				105 min hemodynamics		
		%	%	AAR		I	M	O	T	I	M	O	T	HR	SBP	RPP/ 100
		LV	LV	AAR												
Preconditioned																
1	33.5	8.1	22.7	0.84	0.08	0.15	0.30	0.18	0.14	0.26	0.65	0.36	158	168	265	
2	30.3	13.8	41.6	1.02	0.03	0.10	0.23	0.11	0.02	0.13	0.47	0.19	174	175	304	
3	40.4	19.3	47.8	0.90	0.06	0.10	0.22	0.13	0.03	0.07	0.28	0.14	138	168	232	
4	45.4	28.3	62.3	1.06	0.00	0.03	0.20	0.08	0.01	0.03	0.27	0.10	172	157	270	
5	31.7	15.1	45.3	3.37	0.04	0.11	0.18	0.12	0.03	0.12	0.48	0.24	170	145	246	
6	40.5	24.9	58.7	1.22	0.01	0.04	0.10	0.05	0.02	0.10	0.24	0.13	176	110	194	
7	37.0	20.7	55.8	1.10	0.02	0.05	0.13	0.07	0.02	0.05	0.19	0.09	168	168	282	
8	46.4	12.7	27.4	1.03	0.03	0.06	0.16	0.09	0.06	0.17	0.60	0.30	132	150	198	
9	47.0	30.5	62.4	1.34	0.02	0.04	0.13	0.06	0.01	0.04	0.19	0.09	198	125	247	
10	33.0	2.2	6.7	1.06	0.51	0.62	0.78	0.65	0.58	0.57	0.67	0.61	118	132	156	
Mean	38.5	17.6	43.1	1.29	0.08	0.13	0.24	0.15	0.09	0.15	0.40	0.22	160	150	239	
± SEM	± 2.0	± 2.8	± 5.9	± 0.24	± 0.05	± 0.06	± 0.06	± 0.06	± 0.06	± 0.05	± 0.06	± 0.05	± 7	± 6	± 13	
Mean without dog 10																
± SEM	± 1.8	± 2.5	± 4.8	± 0.26	± 0.01	± 0.01	± 0.02	± 0.01	± 0.01	± 0.02	± 0.06	± 0.03	± 7	± 7	± 12	
Control																
1	40.5	26.2	63.2	1.59	0.02	0.06	0.20	0.11	0.02	0.05	0.24	0.12	154	168	259	
2	37.8	25.2	60.4	1.35	0.01	0.01	0.10	0.04	0.01	0.01	0.16	0.06	144	155	223	
3	30.8	15.8	47.1	0.75	0.03	0.06	0.15	0.08	0.04	0.09	0.25	0.13	114	150	171	
4	41.3	17.0	39.8	1.13	0.01	0.04	0.16	0.08	0.05	0.17	0.48	0.26	134	138	185	
5	36.9	6.1	15.9	1.10	0.14	0.29	0.56	0.33	0.13	0.25	0.56	0.31	136	140	190	
6	47.0	33.2	66.3	1.11	0.01	0.02	0.06	0.03	0.01	0.01	0.17	0.07	158	135	213	
7	38.7	17.8	42.3	1.98	X	X	X	X	0.08	0.16	0.22	0.15	116	120	139	
8	33.1	5.5	16.2	1.14	0.16	0.30	0.66	0.38	0.42	0.74	1.04	0.74	188	140	263	
Mean	38.3	18.4	43.9	1.26	0.05	0.11	0.27	0.15	0.10	0.18	0.39	0.23	140	138	193	
± SEM	± 1.8	± 3.4	± 7.0	± 0.13	± 0.02	± 0.05	± 0.09	± 0.05	± 0.05	± 0.08	± 0.11	± 0.08	± 7	± 5	± 12	
Mean without dog 8																
± SEM	± 1.8	± 3.3	± 6.6	± 0.15	± 0.02	± 0.04	± 0.07	± 0.04	± 0.02	± 0.03	± 0.06	± 0.04	± 6	± 6	± 15	
p value ^A	NS	NS	NS	NS	NS	NS	NS	NS	NS	NS	NS	NS	<.01	NS	<.01	

Abbreviations and units are as in table 2.

^Ap values are result of unpaired t tests performed on group data from which findings in high-flow dogs (No. 10 preconditioned, No. 8 control group) have been excluded.

comparisons. Moreover, infarct size has been plotted against collateral blood flow to examine whether preconditioning altered this relationship between infarct size and collateral flow. Although the control animals in the 40 min study had significantly higher rate-pressure products than the preconditioned animals before the 40 min occlusion, there was no significant difference between the two groups midway through the 40 min occlusion. Differences in hemodynamic indexes of oxygen consumption have been shown to account for only a small amount of the variability in infarct size in pentobarbital-anesthetized dogs,^{2,4} and seem an unlikely explanation for the fourfold difference in infarct size observed in the 40 min study. Thus, the protection

seen with preconditioning in the 40 min study cannot be explained by random selection of groups with differing baseline characteristics.

Mechanism of protection. At present we do not know how preconditioning serves to protect the heart during a sustained period of ischemia. However, there are two general mechanisms that seem likely to be involved in this phenomenon: (1) slowing of ATP depletion, or (2) limitation of catabolite accumulation during the terminal episode of ischemia.

Depletion of ATP could be slowed by a reduction in energy demand during ischemia, or by an increase in the net availability of high-energy phosphates. A reduced ATP demand could result from less myosin

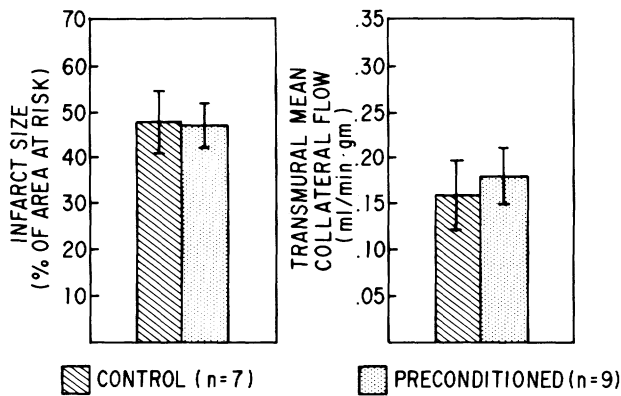


FIGURE 6. Infarct size and collateral blood flow in the 3 hr study. Infarct size in the control group (striped bar) averaged 47.9% of the area at risk. Infarct size in the preconditioned group (stippled bar) averaged 47.1% of the area at risk ($p = \text{NS}$). Transmural mean collateral blood flow was not significantly different in the two groups. Thus, preconditioning failed to limit infarct size after 3 hr of sustained ischemia. Bars represent group means $\pm$ SEM.

ATPase activity. Brief periods of ischemia are known to cause prolonged contractile dysfunction, the so-called "stunned myocardium."¹⁶ For example, Heyndrickx et al.¹⁷ reported that regional myocardial func-

tion remained depressed through more than 3 hr of reperfusion after a single 5 min coronary occlusion. Although contractile function during ischemia is impaired markedly, it seems likely that there is some persistent contractile effort in the ischemic region. If this is true, then preconditioning could effectively stun the myocardium before the sustained ischemic episode, and this in turn could reduce ATP utilization during the early phase of a sustained period of ischemia. Although possible, it seems unlikely that a reduced function of other ATPases, such as the Na^+/K^+ pump or the Ca^{++} -ATPases of the sarcolemma or sarcoplasmic reticulum, could be associated with increased cell viability.

Hypothetically, slowed ATP depletion also could result from increased ATP production from aerobic respiration, anaerobic glycolysis, or phosphocreatine reserves. However, aerobic ATP production cannot be the cause since collateral blood flows to the subendocardium were not different in preconditioned and control animals. It also is doubtful that anaerobic glycolysis was accelerated in the preconditioned hearts because when we studied repeated 10 min occlusions

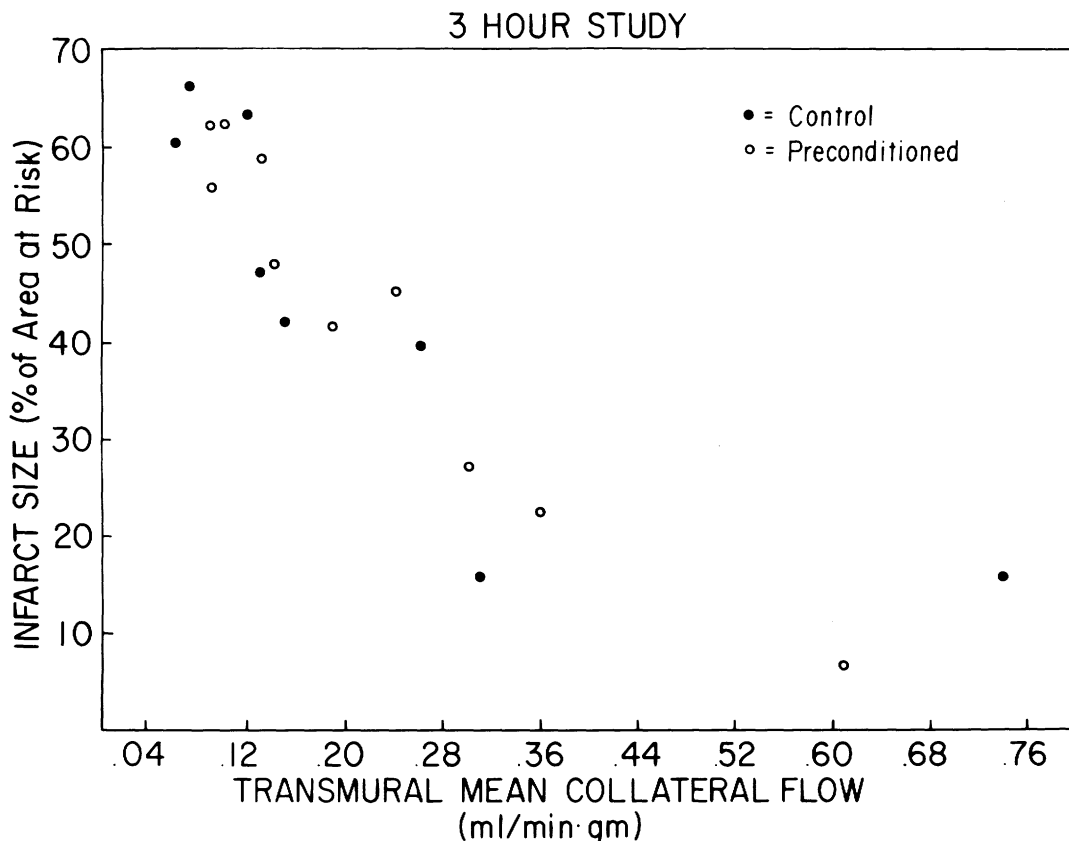


FIGURE 7. Infarct size vs collateral flow, 3 hr study. The relationship between infarct size, as a percentage of the area at risk, and subepicardial collateral blood flow measured 105 min into the 3 hr occlusion is illustrated. For both control (closed circles) and preconditioned (open circles) animals, there is a close inverse relationship between infarct size and collateral flow. Also, there is no difference between the preconditioned and control groups with respect to this relationship.

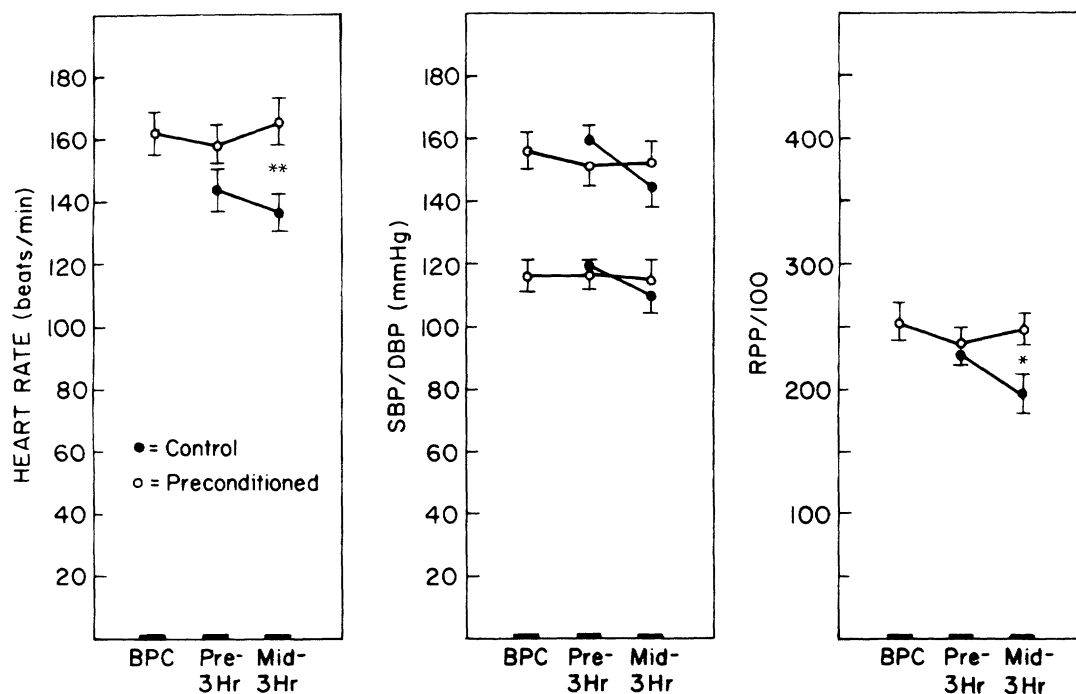


FIGURE 8. Hemodynamic values from the 3 hr study. BPC = before preconditioning; Pre-3Hr = immediately before the 3 hr occlusion; Mid-3Hr = 105 min into the 3 hr occlusion; SBP = systolic blood pressure; DBP = diastolic blood pressure; RPP = rate-pressure product (systolic blood pressure $\times$ heart rate). * and ** $p < .05$ and $p < .01$, respectively, for preconditioned vs control groups at a given time. (No significant differences were observed between the first and subsequent measurements for any hemodynamic variable within either group.) See text for additional explanation.

we found that lactate accumulation, and hence anaerobic glycolysis, was slowed in subsequent episodes of ischemia. Other investigators¹⁸ have reported a phosphocreatine overshoot with reperfusion after reversible episodes of ischemia, which could provide increased ATP production from phosphocreatine reserves during the episode of sustained ischemia. If a phosphocreatine overshoot exists after repeated 5 min episodes of ischemia and reperfusion, it is operating under a circumstance in which adenine nucleotides have been partially degraded by the previous ischemic episode. This deficit in adenine nucleotide high-energy phosphates would tend to offset a phosphocreatine overshoot. Thus, for the phosphocreatine overshoot to be solely responsible for slowed ATP depletion, two criteria must be met. First, the overshoot must be substantial, i.e., at least equal to and probably greater than the deficit in adenine nucleotide high-energy phosphates. Second, if it is the sole cause, phosphocreatine levels should remain elevated for a longer period in preconditioned myocardium than in "virgin" myocardium; in the latter, phosphocreatine is known to be depleted rapidly after the onset of ischemia.¹⁹

The protective effect of preconditioning also could result from a reduced accumulation of ischemic cata-

bolites. Intermittent ischemia results in degradation of larger molecules such as glycogen and adenine nucleotides, and their breakdown products, lactate, H^+ , NH_3 , inorganic phosphate, etc., are then washed out upon reperfusion. Preconditioned animals could thereby start the 40 min occlusion with a smaller pool of macromolecular precursors, and this would limit catabolite accumulation during the occlusion. Alternatively, a reduced energy demand might drive anaerobic glycolysis to a lesser extent, and thereby limit accumulation of glycolytic intermediates.

One ischemic catabolite that has been of considerable interest is the purine base hypoxanthine, a product of adenine nucleotide degradation. McCord²⁰ has proposed that the enzyme xanthine oxidase, which is converted from the dehydrogenase form during ischemia,²¹ contributes to myocardial cell death by generating superoxide ($\cdot O_2^-$) anions when hypoxanthine is oxidized to xanthine in the presence of molecular oxygen. Thus, in the setting of ischemia and reperfusion when hypoxanthine is produced and abundant oxygen is provided by the presence of reactive hyperemia, it is conceivable that some cell death could result from oxygen-derived free radicals. If this were true, then part of the protective effect of preconditioning

could be due to a decrease in the adenine nucleotide content of the myocardium before the sustained occlusion, which would limit hypoxanthine accumulation and superoxide production. However, when the xanthine oxidase inhibitor allopurinol was tested in our 40 min preparation of occlusion-reperfusion,²² there was no limitation of infarct size. This suggests that oxygen-derived free radicals, generated by the xanthine oxidase pathway, do not contribute to cell death in our preparation. Therefore, it is unlikely that reduced hypoxanthine accumulation contributes to the protective effect of preconditioning.

Neely and Grotyohann²³ tested the relationship between catabolite accumulation and functional recovery in isolated rat hearts after episodes of ischemia and reperfusion. They depleted glycogen levels by anoxic perfusion, and then subjected the hearts to total ischemia. At the end of the ischemic episode, less lactate accumulated in the hearts that were anoxically preperfused. Functional recovery was improved compared with that in hearts that were only made ischemic. This functional improvement was abolished when lactate was added to the preperfusion medium. They concluded that accumulation of glycolytic intermediates, and not ATP depletion, was involved in preventing functional recovery but made no observations on cellular viability.

There are many other mechanisms whereby preconditioning could protect ischemic myocardium. A reduction in catabolite accumulation could limit the osmotic load that occurs during ischemia. This, in turn, would limit the amount of cell swelling during ischemia and reperfusion, and possibly prevent rupture of damaged membranes.²⁴ Another possibility is that preconditioning could limit accumulation of chemotactic factors that attract neutrophils to ischemic/reperfused tissue. Several studies have implicated neutrophils as mediators of ischemic injury.^{25, 26} Repeated episodes of ischemia could conceivably alter basal sympathetic tone, e.g., by depleting catecholamines from adrenergic nerve terminals. However, Janes et al.²⁷ recently reported that 12 coronary occlusions of 5 min duration each did not diminish the increase in contractility (measured by development of intramyocardial pressure) from stellate ganglion stimulation. Although basal sympathetic tone was not measured, their data do indicate that postischemic stunned myocardium still was capable of responding to sympathetic nerve stimulation. Moreover, the failure of β -blockade with propranolol to limit infarct size in either the 40 min or 3 hr preparations²⁸ suggests that preconditioning is not due to loss of β -sympathetic tone. Finally, it is conceivable

that preconditioning could activate synthesis of an enzyme or activate enzyme systems that better enable the cell to withstand ischemia. However, at present no data are available to support this hypothesis.

Failure to limit infarct size after 3 hr of ischemia. Despite the dramatic protective effect observed after 40 min of ischemia, preconditioning failed to limit infarct size after 3 hr of sustained ischemia. It is useful to view this in two contexts:

(1) The previously protected subendocardium failed to survive the 3 hr occlusion. Thus, it appears that preconditioning can only delay cell death; the additional 140 min of ischemia overwhelmed the protective effect in the subendocardial region.

(2) Preconditioning failed to protect the mid and subepicardial myocardium from its normal time course of cell death. The failure to delay cell death in the mid and subepicardial myocardium must result from a difference in the effect of preconditioning on these layers, or intrinsic differences in the nature of ischemic injury in the outer vs the inner layers of the heart. It seems likely that the outer layers, by virtue of having greater collateral flow and a lower metabolic rate,²⁹ and therefore less severe ischemia, would not be as well preconditioned as the inner layer by the four 5 min occlusions. If this is so, it might be possible to protect the mid and subepicardial myocardium by use of longer occlusions in the preconditioning period. Also, if reduced catabolite accumulation plays a role in protecting the subendocardial region, one might expect that the mid and subepicardial myocardium would not be as dramatically protected, since greater collateral flow to these regions naturally limits their catabolite accumulation.

The results of this study parallel the findings from this laboratory when the calcium-channel blocker verapamil was evaluated for its cardioprotective effects.³⁰ In that study, pretreatment with verapamil was associated with a marked limitation of infarct size when reperfusion was established after 40 min of ischemia, but continuous treatment initiated 15 min after occlusion failed to limit infarct size when reperfusion was established after 3 hr of ischemia. We do not know whether there is a common mechanism of protection between verapamil and preconditioning. However, the parallel results do suggest that it may be more difficult to delay cell death in the mid and subepicardial myocardium, where ischemia is less severe and cell death is already delayed. Nevertheless, the fact that reperfusion after 3 hr of ischemia does limit infarct size by about 10%² indicates that the wavefront of cell death is still progressing at 3 hr. Thus, it is theoretically possi-

ble to delay cell death in regions of less severe ischemia, it simply may be difficult to improve upon the effects of residual collateral perfusion.

Clinical relevance. Results from controlled animal experiments must be viewed with appropriate caution when extrapolating to the clinical situation. However, in man, myocardial infarction often is preceded by multiple episodes of angina pectoris. It is possible, therefore, that patients who experience repeated episodes of angina may similarly precondition their myocardium, and in so doing, alter the time course of cell death after the onset of a sustained coronary occlusion. If this is true, then the onset and early progression of cell death may be slower in many patients than the results of animal studies have suggested may be the case. A slower progression of cell death implies a longer window of time in which it might be possible to salvage myocardium via reperfusion, e.g., with thrombolytic therapy or coronary angioplasty.

We are grateful for the expert technical assistance of Jean A. Wakefield for animal surgery, blood flow measurements and assistance with infarct sizing, and to Betty Goodfellow for preparation of histologic slides.

References

1. Reimer KA, Murry CE, Yamasawa I, Hill ML, Jennings RB: Four brief periods of myocardial ischemia cause no cumulative ATP loss or necrosis. *Am J Physiol* (in press)
2. Reimer KA, Jennings RB: The "wavefront phenomenon" of myocardial ischemic cell death. II. Transmural progression of necrosis within the framework of ischemic bed size (myocardium at risk) and collateral flow. *Lab Invest* **40**: 633, 1979
3. Jennings RB, Hawkins HK, Lowe JE, Hill ML, Klotman S, Reimer KA: Relation between high energy phosphate and lethal injury in myocardial ischemia in the dog. *Am J Pathol* **92**: 187, 1978
4. Reimer KA, Jennings RB, Cobb FR, Murdock RH, Greenfield JC Jr, Becker LC, Bulkley BH, Hutchins GM, Schwartz RP Jr, Bailey KR, Passamani ER: Animal models for protecting ischemic myocardium: results of the NHLBI cooperative study. Comparison of unconscious and conscious dog models. *Circ Res* **56**: 651, 1985
5. Reimer KA, Jennings RB: The changing anatomic reference base of evolving myocardial infarction. Underestimation of myocardial collateral blood flow and overestimation of experimental anatomic infarct size due to tissue edema, hemorrhage and acute inflammation. *Circulation* **60**: 866, 1979
6. Jennings RB, Schaper J, Hill ML, Steenbergen C, Reimer KA: Effect of reperfusion late in the phase of reversible ischemic injury. Changes in cell volume, electrolytes, metabolites, and ultrastructure. *Circ Res* **56**: 262, 1985
7. Gillmore RF Jr, Zipes DP: Different electrophysiological responses of canine endocardium and epicardium to combined hyperkalemia, hypoxia, and acidosis. *Circ Res* **46**: 814, 1980
8. Swain JL, Sabina RL, Hines JJ, Greenfield JC Jr, Holmes EW: Repetitive episodes of brief ischaemia (12 min) do not produce a cumulative depletion of high energy phosphate compounds. *Cardiovasc Res* **18**: 264, 1984
9. Lange R, Ingwall JS, Hale SL, Alker KJ, Kloner RA: Effects of recurrent ischemia on myocardial high energy phosphate content in canine hearts. *Bas Res Cardiol* **79**: 469, 1984
10. Basuk WL, Reimer KA, Jennings RB: Effect of repetitive brief episodes of ischemia on cell volume, electrolytes and ultrastructure. *J Am Coll Cardiol* **8**(suppl A): 33A, 1986
11. Lange R, Ware J, Kloner RA: Absence of a cumulative deterioration of regional function during three repeated 5 or 15 minute coronary occlusions. *Circulation* **69**: 400, 1984
12. Geft IL, Fishbein MC, Ninomiya K, Hashida J, Chau E, Yano J, Y-Rit J, Genov T, Shell W, Ganz W: Intermittent brief periods of ischemia have a cumulative effect and may cause myocardial necrosis. *Circulation* **66**: 1150, 1982
13. Selye H, Veilleux R, Grasso S: Protection, by coronary ligation, against isoproterenol-induced myocardial necrosis. *Proc Soc Exp Biol Med* **104**: 343, 1960
14. Dusek J, Rona G, Kahn DS: Myocardial resistance. A study of its development against toxic doses of isoproterenol. *Arch Pathol* **89**: 79, 1970
15. Schaper W: Residual perfusion of acutely ischemic heart muscle. In Schaper W, editor: *The pathophysiology of myocardial perfusion*. New York, 1979, Elsevier/North Holland Biomedical Press, p 345
16. Braunwald E, Kloner RA: The stunned myocardium: prolonged, postischemic ventricular dysfunction. *Circulation* **66**: 1146, 1982
17. Heyndrickx GR, Millard RW, McRitchie KJ, Maroko PR, Vatner SF: Regional myocardial functional and electrophysiological alterations after brief coronary artery occlusion in conscious dogs. *J Clin Invest* **56**: 978, 1975
18. Ichihara K, Abiko Y: Rebound recovery of myocardial creatine phosphate with reperfusion after ischemia. *Am Heart J* **108**: 1594, 1984
19. Allison TB, Ramey CA, Holsinger JW Jr.: Transmural gradients of left ventricular tissue metabolites after circumflex artery ligation in dogs. *J Mol Cell Cardiol* **9**: 837, 1977
20. McCord JM: Oxygen-derived free radicals in postischemic tissue injury. *N Engl J Med* **312**: 159, 1985
21. Chambers DE, Parks DA, Patterson G, Roy R, McCord JM, Yoshida S, Parmley LF, Downey JM: Xanthine oxidase as a source of free radical damage in myocardial ischemia. *J Mol Cell Cardiol* **17**: 145, 1985
22. Reimer KA, Jennings RB: Failure of the xanthine oxidase inhibitor allopurinol to limit infarct size after ischemia and reperfusion in dogs. *Circulation* **71**: 1069, 1985
23. Neely JR, Grotyohann LW: Role of glycolytic products in damage to ischemic myocardium. Dissociation of adenosine triphosphate levels and recovery of function of reperfused ischemic hearts. *Circ Res* **55**: 816, 1984
24. Steenbergen C, Hill ML, Jennings RB: Volume regulation and plasma membrane injury in aerobic, anaerobic and ischemic myocardium in vitro. Effects of osmotic cell swelling on plasma membrane integrity. *Circ Res* **57**: 864, 1985
25. Engler RL, Schmid-Schönbein GW, Pavelec RS: Leukocyte capillary plugging in myocardial ischemia and reperfusion in the dog. *Am J Pathol* **111**: 98, 1983
26. Romson JL, Hook BG, Kunkel SL, Abrams GD, Schork MA, Lucchesia BR: Reduction of the extent of ischemic myocardial injury by neutrophil depletion in the dog. *Circulation* **67**: 1016, 1983
27. Janes RD, Johnstone DE, Klassen GA, Armour JA: The function of cardiac sympathetic efferent nerves within a zone of repeated ischemia. *Circulation* **72**(suppl III): III-63, 1985 (abst)
28. Jennings RB, Reimer KA: Effect of beta-adrenergic blockage on acute myocardial ischemic injury. In Gross F, editor: *Modulation of sympathetic tone in the treatment of cardiovascular diseases*. Bern, 1979, Hans Huber Publishers, p 103
29. Murry CE, Reimer KA, Hill ML, Yamasawa I, Jennings RB: Collateral blood flow and transmural location: Independent determinants of ATP in ischemic canine myocardium. *Fed Proc* **44**: 823, 1985 (abst)
30. Reimer KA, Jennings RB: Verapamil in two reperfusion models of myocardial infarction. Temporary protection of severely ischemic myocardium without limitation of ultimate infarct size. *Lab Invest* **51**: 655, 1984