HYPOTHERMIA INCREASES THE THRESHOLD FOR ISCHEMIC PRECONDITIONING

Kentaro Dote, MD^a
Roger A. Wolff, MS^b
Donna M. Van Winkle, PhD^{b,c,d}

Objectives: Both hypothermia and ischemic preconditioning are known to provide tolerance to myocardial ischemia and reperfusion. The aim of this study was to determine whether hypothermia during the ischemic preconditioning period attenuates the protective effect of ischemic preconditioning. **Methods:** Experiments were performed in buffer-perfused isolated rabbit hearts. All hearts underwent 45 minutes of regional ischemia, followed by 2 hours of reperfusion. Ischemic preconditioning was elicited by either one or four periods of 5 minutes of regional ischemia. Hypothermia (25° C) was induced beginning either 20 or 50 minutes before the 45-minute period of regional ischemia; normothermia (38° C) was restored 10 minutes before the 45-minute period of regional ischemia. Except for the hypothermic periods noted, hearts were maintained at 38° C. **Results:** Normothermic ischemic preconditioning with either one or four cycles of 5 minutes of coronary occlusion resulted in a profound reduction of infarct size (58% reduction with one cycle, $p < 0.05$; 95% reduction with four cycles, $p < 0.01$). Hypothermic ischemic preconditioning with one cycle of 5-minute coronary occlusion resulted in no reduction of infarct size but hypothermic ischemic preconditioning with four cycles of 5-minute coronary occlusions resulted in a 94% reduction of infarct size ($p < 0.01$). Myocardial glycogen and lactate levels were maintained near control levels during hypothermic ischemia. **Conclusions:** From these data we conclude that hypothermia during the preconditioning period increases the threshold for eliciting the infarct limitation of ischemic preconditioning. (J Thorac Cardiovasc Surg 1998;116:319-26)

Clinically, organ preservation during ischemia relies heavily on hypothermia. Moderate to profound hypothermia prolongs organ survival during global ischemia,¹ and induced hypothermia is common for procedures requiring cardiopulmonary bypass.² Cardiac hypothermia decreases myocardial oxygen consumption, slows the rate of ischemic

adenosine triphosphate depletion, and reduces infarct size.^{1,2}

Experimentally, ischemic preconditioning (i.e., one or more transient periods of sublethal ischemia) also provides profound tolerance to subsequent ischemic episodes.³ Ischemic preconditioning can be elicited either by total coronary occlusion^{3,4} or by low-flow ischemia⁵; however, in both cases marked ischemia must be present for at least 5 minutes to elicit the phenomenon.^{4,5} To date, all animal species tested have displayed the preconditioning phenomenon, and there is evidence that preconditioning can be elicited in human myocardium.⁶ Yet there has been relatively little investigation of ischemic preconditioning in a hypothermic environment or in hypothermic tissues. Cave and Hearse⁷ demonstrated that the preconditioning-induced preservation of postischemic contractile function was achieved with a normothermic preconditioning stimulus and a hypothermic long ischemic period. However, the effect of hypothermia during the preconditioning ischemia is unknown. We postulated that

From the Department of Anesthesiology, Ehime University, Japan^a; Research Service^b and Anesthesiology Service,^c Veterans Affairs Medical Center, Portland, Ore.; and Department of Anesthesiology, Oregon Health Sciences University, Portland, Ore.^d

Supported by a grant from the Department of Anesthesiology, Oregon Health Sciences University, Portland, Ore.

Received for publication Nov. 14, 1997; revisions requested Jan. 29, 1998; revisions received March 6, 1998; accepted for publication March 6, 1998.

Address for reprints: D. M. Van Winkle, PhD, Anesthesiology Service (199), VA Medical Center, 3710 SW US Veterans Hospital Rd., Portland, OR 97201.

12/1/90188

Fig. 1. Time line depicting the sequence of experimental manipulations. *Black bars* indicate periods of hypothermia, *stippled areas* indicate periods of myocardial ischemia, and *empty bars* indicate periods of normal perfusion.

hypothermia may reduce myocardial energy demand such that a typical preconditioning stimulus is insufficient to generate the depth of ischemia required to elicit the preconditioning phenomenon.

The primary goal of this study was to clarify the effect of temperature during the preconditioning period on infarct size. Additionally, the metabolic effects of hypothermia during preconditioning were evaluated by measurement of myocardial glycogen and lactate levels. Experiments were performed in buffer-perfused isolated rabbit hearts undergoing regional ischemia-reperfusion. Rabbits were chosen for this study because their lack of coronary collaterals simplifies infarct size analysis; isolated hearts were used to facilitate rapid and precise changes in temperature.

Methods

Animals used in this study were allowed access to food and water ad libitum until induction of anesthesia. All animals received humane treatment in compliance with both the "Guide for the Care and Use of Laboratory Animals" published by the National Institutes of Health (NIH Publication No. 86-23, revised 1985) and local institutional policy.

Experimental preparation. Male New Zealand White rabbits (2.5 to 3.0 kg) were anesthetized with 30 mg/kg sodium pentobarbital plus 10 mg supplements of intravenous pentobarbital as needed. After tracheostomy, all

rabbits received mechanical ventilation (MDI ventilator, Mobile, Ala.). A left thoracotomy was performed in the fourth intercostal space and the pericardium was opened to expose the heart. A 4-0 silk ligature was placed around the proximal segment of a major left coronary artery to form a snare.

The hearts were then rapidly excised, placed in saline solution for transport, and mounted on a nonrecirculating Langendorff apparatus. Excision, mounting, and restoration of perfusion were performed in less than 60 seconds. Hearts were perfused with a modified Krebs-Henseleit buffer at 100 cm H₂O. Buffer composition was as follows (in millimoles per liter, pH 7.4): NaCl 118, NaHCO₃ 24.8, dextrose 10.0, KCl 4.7, CaCl₂ 2.5, KH₂PO₄ 1.19, and MgSO₄ 1.19. The buffer was filtered with a 0.45 mm prefilter and a 5 μ m in-line filter and bubbled with 95% oxygen and 5% carbon dioxide. Perfusate temperature was maintained at approximately 38°C except during the hypothermic periods (described later). A temperature probe was introduced into the right ventricular lumen for continuous temperature measurement. A fluid-filled latex balloon was placed inside the left ventricle (LV) and attached to a pressure transducer for measurement of LV pressure. Hearts were atrially paced at 270 beats/min (model 58800, Grass Instruments, Quincy, Mass.). Total coronary arterial perfusate flow was measured by timed collection of effluent. Hearts were allowed to stabilize 15 minutes before the experimental protocol was begun.

Infarct size studies. Fifty-four hearts were assigned to seven groups: normothermic control (CON), normothermic one-cycle preconditioning (IP1), normothermic four-cycle preconditioning (IP4), 10-minute hypothermic con-

Fig. 2. Infarct size data expressed as a percentage of the area at risk. A single cycle of normothermic preconditioning ischemia resulted in infarct limitation, but a single cycle of hypothermic preconditioning ischemia had no effect on infarct size. Profound cardioprotection was conferred by four-cycle preconditioning, regardless of the absence or presence of hypothermia during preconditioning. Data are plotted as mean $\pm$ the standard error of the mean. Asterisks indicate statistically significant values compared with the relevant control group (CON vs IP1, H₁₀ vs H₁₀ + IP1, CON vs IP4, H₄₀ vs H₄₀ + IP4). See text for description of the groups.

Fig. 3. Comparison of glycogen (solid bars, left y-axis) and lactate (empty bars, right y-axis) levels in myocardium subjected to either normothermia or hypothermia and to either no ischemia or a 5-minute preconditioning ischemic period. Data are expressed as mean $\pm$ the standard error of the mean. Single asterisks indicate statistically significant values compared with CON group. Double asterisks indicate statistically significant values compared with normothermic preconditioning. See text for description of the groups.

trol (H₁₀), 10-minute hypothermia with one-cycle preconditioning (H₁₀ + IP1), 40-minute hypothermic control (H₄₀), and 40-minute hypothermia with four-cycle preconditioning (H₄₀ + IP4). A time line depicting the experimental manipulations is presented in Fig. 1.

Induction of ischemia. Regional ischemia was elicited by tightening the left coronary snare and was confirmed by a decrease in total flow and a decrease in LV developed pressure (LVDP). Reperfusion was accomplished by release of the snare and was verified by hyperemia and partial resolution of the contractile dysfunction. All hearts underwent 45 minutes of regional ischemia and 2 hours of reperfusion. Ischemic preconditioning was elicited by either one cycle of 5 minutes of regional ischemia beginning 15 minutes before the 45-minute coronary occlusion (IP1) or by four cycles of 5 minutes of regional ischemia and 5 minutes of reperfusion beginning 45 minutes before the 45-minute coronary occlusion (IP4).

Induction of hypothermia. Hypothermia (25° C) was induced beginning either 20 or 50 minutes before the 45-minute period of regional ischemia; normothermia (38° C) was restored 10 minutes before the 45-minute coronary occlusion. Hypothermia was accomplished by exchanging the warmed perfusate reservoir for a cooled perfusate reservoir and by switching from a 38° C heated water circulator perfusing the water-jacketed glassware and tubing to a 25° C water circulator (model 8005, Fisher Scientific, Pittsburgh, Pa.). Normothermia was accomplished by reexchanging the perfusate reservoirs and circulators. The transition from normothermia to hypothermia took less than 2 minutes to complete, and the transition back to normothermia from hypothermic conditions lasted from 3 to 5 minutes. Except for the hypothermic periods already noted, hearts were maintained at 38° C.

Measurement of infarct size. At the conclusion of the experiment, the coronary artery was reoccluded, and

Table I. LV pressures

	Baseline	Preconditioning								Norm. perf.
		Norm. perf.	IP	Norm. perf.	IP	Norm. perf.	IP	Norm. perf.	IP	
LVDP (mm Hg)										
CON	98 ± 3							99 ± 3	97 ± 3	92 ± 3
IP1	94 ± 4							91 ± 5	32 ± 3*†	71 ± 4*†
H ₁₀	93 ± 4							123 ± 11*	128 ± 9*†	78 ± 4
H ₁₀ + IP1	103 ± 5							119 ± 10	75 ± 9*‡§	82 ± 7
IP4	87 ± 3	86 ± 4	36 ± 2*	72 ± 1*	35 ± 4*	68 ± 2*	35 ± 4*	68 ± 2*†	36 ± 4*†	69 ± 2*†
H ₄₀	99 ± 4	99 ± 11	115 ± 12	110 ± 6	112 ± 8	111 ± 7	113 ± 6	110 ± 8	110 ± 8	92 ± 9
H ₄₀ + IP4	85 ± 8	96 ± 7	75 ± 11¶	131 ± 21	78 ± 8¶	140 ± 25	60 ± 13¶	111 ± 24	54 ± 12¶	49 ± 7¶
LVEDP (mm Hg)										
CON	5 ± 1							5 ± 0	5 ± 0	5 ± 1
IP1	5 ± 0							4 ± 1	4 ± 0	3 ± 0*
H ₁₀	4 ± 0							18 ± 3*†	20 ± 4*†	3 ± 1
H ₁₀ + IP1	5 ± 0							21 ± 4*‡	15 ± 3‡	3 ± 1
IP4	5 ± 1	4 ± 0	4 ± 1	4 ± 1	3 ± 1	3 ± 1*	3 ± 0	3 ± 1	3 ± 1	2 ± 0*†
H ₄₀	6 ± 1	29 ± 6*	25 ± 6*	27 ± 5*	25 ± 6*	25 ± 6*	27 ± 8*	24 ± 6*†	22 ± 4*†	3 ± 0
H ₄₀ + IP4	6 ± 1	23 ± 5*	9 ± 4	24 ± 9*	12 ± 7	15 ± 6	5 ± 2	17 ± 6*	5 ± 1¶	3 ± 1

Data within boxes reflect periods of hypothermia. See text for description of groups. Norm. Perf., Normal perfusion; IP, ischemic preconditioning.

* $p < 0.05$ vs baseline.

† $p < 0.05$ vs CON.

‡ $p < 0.05$ vs. IP1.

§ $p < 0.05$ vs H₁₀.

|| $p < 0.05$ vs H₄₀ + IP4.

¶ $p < 0.05$ vs H₄₀.

fluorescent particles (zinc cadmium sulfide, Duke Scientific Corp., Palo Alto, Calif.) were infused into the aortic root. The particles fluoresce bright yellow under ultraviolet light thereby delineating the risk area (previously ischemic tissue) as a negative image. The heart was removed from the Langendorff apparatus, weighed, and frozen. The heart was then cut into transverse slices approximately 2 mm thick and incubated in triphenyl tetrazolium chloride (1% [wt/vol] in phosphate buffer at 37°C) for 20 minutes. Triphenyl tetrazolium chloride produces a brick-red formazan pigment in viable myocardium; necrotic myocardium does not stain and appears whitish-tan. Risk and infarct areas were then traced. Risk and infarct areas (in square centimeters) were determined by computer-assisted planimetry, and the volume (in cubic centimeters) of infarcted myocardium at risk was calculated from the planimetered areas and the slice thickness (2 mm). All tissue processing, tracing, and planimetry steps were performed in a blinded fashion. Hearts demonstrating infarction outside of the risk area, or with a risk area size of less than 0.4 cm³, were excluded from analysis.

Tissue glycogen and lactate studies. To evaluate the metabolic effects of the hypothermia during the preconditioning ischemic period, myocardial tissue glycogen and lactate levels were determined in 12 separate hearts. These hearts were randomly assigned to one of four treatments: normothermia ($n = 3$), normothermia with one cycle of 5 minutes of global ischemia ($n = 3$), hypothermia ($n = 3$), and hypothermia with one cycle of 5 minutes of global ischemia ($n = 3$). Hypothermia was

instituted 5 minutes before induction of ischemia. At the end of the ischemic period, or at 10 minutes of perfusion in the absence of ischemia, the hearts were rapidly frozen in liquid nitrogen.

Myocardial tissue glycogen levels were determined by the enzymatic method of Keppler and Decker.⁸ Myocardial tissue lactate levels were determined by an adaptation of the methods of Fleischer⁹ and Gutmann and Wahlefeld.¹⁰ Determinations were performed in triplicate.

Data analysis. Temperature, heart rate, LV pressure, and the electrocardiogram were recorded continuously on a strip chart recorder (model RS3400, Gould Instruments, Valley View, Ohio). Data were analyzed with a statistical software package (Crunch version 4.07, Crunch Software, Oakland, Calif.). Differences within groups were assessed with one-way analysis of variance with the Dunnett post hoc test for multiple comparisons. Differences between groups in hemodynamics and infarct size as a percentage of the risk area were analyzed with analysis of variance with the Tukey post-hoc test. Differences between groups in infarct volume were assessed with analysis of covariance, with risk volume as the covariate. Data are expressed as means plus or minus the standard error of the mean.

Results

Fifty-four hearts were entered into the infarct size study; in 51 hearts the experimental protocol was completed and the data were analyzed (CON = 9,

Occlusion		Reperfusion		
Start	End	Start	Middle	End
31 ± 6*	55 ± 3*	66 ± 2*	71 ± 2*	61 ± 4*
29 ± 3*	54 ± 4*	71 ± 4*	77 ± 4*	68 ± 6*
36 ± 3*	59 ± 3*	70 ± 3*	76 ± 3	67 ± 4*
42 ± 5*	56 ± 5*	63 ± 6*	64 ± 6*	60 ± 6*
33 ± 2*	46 ± 3*	61 ± 2*	63 ± 1*	58 ± 2*
46 ± 12*	58 ± 9*	71 ± 9	68 ± 8	62 ± 11*
26 ± 7*	48 ± 9	55 ± 10	59 ± 12	58 ± 11
5 ± 1	9 ± 1	19 ± 3*	14 ± 2*	12 ± 2*
4 ± 1	6 ± 1	14 ± 1*	9 ± 1*	7 ± 1*
3 ± 1	7 ± 1	18 ± 3*	13 ± 2	14 ± 3*
9 ± 5	12 ± 5	21 ± 5*	17 ± 6	17 ± 6
3 ± 0	4 ± 1	7 ± 1	4 ± 1†	4 ± 1
4 ± 1	7 ± 1	18 ± 3	12 ± 2	11 ± 4
3 ± 1	6 ± 1	10 ± 3	7 ± 1	6 ± 2

IP1 = 9, IP4 = 4, H₁₀ = 9, H₁₀ + IP1 = 10, H₄₀ = 6, and H₄₀ + IP4 = 4). One heart was excluded because of infarction outside of the risk area, one heart was excluded because of a small risk area size (<0.4 cm³), and one heart was excluded because of a prolonged transfer and mounting time (>60 seconds). There were no significant differences between groups in exclusions ($p = 0.86$, χ^2). Twelve hearts were entered into the myocardial glycogen and lactate analyses; in all 12 the experimental protocol was completed and the data were contributed to the data set.

Ventricular function and coronary flow. Ventricular function and coronary perfusate flow were not significantly different between groups at baseline (before hypothermia and preconditioning ischemia).

LV end-diastolic pressure (LVEDP) and LVDP data are shown in Table I. Both the preconditioning ischemia and 45-minute ischemia resulted in a decrease in LVDP (except group H₄₀ + IP4, likely because of variability in LVDP in the preconditioning reperfusion intervals). Reperfusion resulted in a slight recovery in LVDP followed by maintenance of this value over time; however, LVDP was still significantly depressed compared with baseline values except in the H₄₀ + IP4 group.

The 45-minute period of ischemia, but not preconditioning ischemia, resulted in a modest nonsignificant gradual increase in LVEDP in all groups compared with baseline. Reperfusion resulted in an

abrupt rise in LVEDP in all groups compared with baseline (statistically significant in groups CON, IP1, H₁₀, and H₁₀ + IP1); this effect was less marked in hearts that underwent four cycles of preconditioning (IP4 and H₄₀ + IP4 groups). Hypothermia itself resulted in an increase in both LVDP (significant in group H₁₀) and LVEDP (significant in all hypothermic groups) during the hypothermic period.

Coronary perfusate flow data are shown in Table II. Preconditioning ischemia resulted in an approximately 40% decrease in coronary perfusate flow; in the repetitive preconditioning groups (IP4 and H₄₀ + IP4) the decrease in flow during each cycle of preconditioning ischemia was also approximately 40%, although there was a trend for the preischemic flow values to decline with each preconditioning cycle (statistically significant only for the fourth preconditioning cycle). Hypothermia itself had no effect on nonischemic flow as compared with the baseline value.

During the period of hypothermia, hearts did not follow the electrical pacemaker and heart rate fell from the paced rate of 270 beats/min to 60 to 80 beats/min ($p = 0.0001$). Thus heart rate was significantly different from baseline in all hypothermic groups during the hypothermic period. However, heart rate was comparable in the paired comparison groups (H₁₀ vs H₁₀ + IP1 and H₄₀ vs H₄₀ + IP4) at all times. Immediately on restoration of normothermia, hearts returned to the paced rate of 270 beats/min and at the time of the 45-minute occlusion there were no differences in heart rate between the groups.

Infarct size data. Heart weights and risk volumes are shown in Table III. Infarct size data are depicted in Fig. 2. There were no differences in biventricular weight, risk volume, or the risk/biventricular weight ratio between groups.

Normothermic ischemic preconditioning with either one or four cycles of 5-minute coronary occlusion resulted in a profound reduction of infarct size, from 45.6% ± 9.1% in CON to 19.1% ± 4.7% in IP1 and 2.5% ± 0.6% in IP4 hearts ($p < 0.05$ and $p < 0.01$, respectively). The antecedent hypothermia alone did not alter infarct size (CON 45.6% ± 9.1% vs H₁₀ 41.0% ± 7.0% and H₄₀ 57.0% ± 8.1%; $p = 0.37$). Hypothermic ischemic preconditioning with one cycle of 5-minute coronary occlusion resulted in no reduction of infarct size (H₁₀ 41.0% ± 7.0% vs H₁₀ + IP1 43.1% ± 7.8%; $p = 0.33$), but hypothermic ischemic preconditioning with four cycles of 5-minute coronary occlusion resulted in a profound

Table II. Total coronary flow (in milliliters per minute)

	Preconditioning									
	Baseline	Norm. perf.	IP	Norm. perf.	IP	Norm. perf.	IP	Norm. perf.	IP	Norm. perf.
CON	54 ± 1							56 ± 2	55 ± 2	55 ± 3
IP1	55 ± 3							54 ± 3	30 ± 2*†	48 ± 3*
H ₁₀	54 ± 3							50 ± 4	47 ± 3	41 ± 2*†
H ₁₀ + IP1	54 ± 2							52 ± 4	31 ± 2*‡	45 ± 4*
IP4	59 ± 4	57 ± 3	36 ± 2*	56 ± 5	34 ± 3*	50 ± 4*	32 ± 3*	49 ± 5*§	32 ± 3*†§	46 ± 3*
H ₄₀	63 ± 3	66 ± 3	62 ± 2	59 ± 4	52 ± 4	49 ± 4*	47 ± 4*	44 ± 4*†	42 ± 4*†	52 ± 5*
H ₄₀ + IP4	50 ± 2	54 ± 4	34 ± 3*	51 ± 7	29 ± 3*	42 ± 7	23 ± 3*	33 ± 4*	19 ± 1*	34 ± 2*

Data within boxes reflect periods of hypothermia. See text for description of groups. Norm. Perf., Normal perfusion; IP, ischemic preconditioning.

* $p < 0.05$ vs baseline.

† $p < 0.05$ vs CON.

‡ $p < 0.05$ vs. H₁₀.

§ $p < 0.05$ vs H₄₀ + IP4.

|| $p < 0.05$ vs H₄₀.

reduction of infarct size, from $57.0\% \pm 8.1\%$ in H₄₀ to $3.3\% \pm 1.0\%$ in H₄₀ + IP4 ($p < 0.01$). This degree of cardioprotection was comparable to that achieved with four cycles of normothermic preconditioning.

Tissue glycogen and lactate data. As shown in Fig. 3, normothermic 5-minute global ischemia resulted in a profound increase of tissue lactate content (1.9 ± 0.16 $\mu\text{mol/gm}$ wet weight in normothermia vs 13.1 ± 1.1 $\mu\text{mol/gm}$ wet weight in normothermia plus ischemia; $p < 0.001$). In contrast, hypothermic 5-minute global ischemia resulted in no increase of tissue lactate content as compared with that in nonischemic hypothermic hearts (2.9 ± 0.13 $\mu\text{mol/gm}$ wet weight vs 1.7 ± 0.22 $\mu\text{mol/gm}$ wet weight, respectively; $p = 0.26$). Hypothermia alone did not alter myocardial tissue lactate content (1.7 ± 0.22 $\mu\text{mol/gm}$ wet weight hypothermia vs 1.9 ± 0.16 $\mu\text{mol/gm}$ wet weight normothermia; $p = 0.35$).

Normothermic ischemic preconditioning of 5 minutes of global ischemia resulted in a 67% reduction of tissue glycogen content as compared with that of nonischemic normothermic hearts (1.6 ± 0.28 mg/gm wet weight vs 4.8 ± 0.15 mg/gm wet weight, respectively; $p < 0.001$). Hypothermic ischemic preconditioning with 5 minutes of global ischemia resulted in no reduction of tissue glycogen content (5.0 ± 0.43 mg/gm wet weight hypothermia vs 4.6 ± 0.37 mg/gm wet weight hypothermia plus ischemia; $p = 0.43$). Hypothermia alone did not alter myocardial tissue glycogen content (5.0 ± 0.43 mg/gm wet weight hypothermia vs 4.8 ± 0.15 mg/gm wet weight normothermia; $p = 0.44$).

Discussion

The present data show that preischemic hypothermia (25°C) alone does not affect infarct size, but hypothermia during preconditioning ischemia increases the threshold of ischemia necessary to elicit cardioprotection. Thus, whereas a single 5-minute period of normothermic coronary occlusion was sufficient to induce preconditioning, multiple 5-minute periods of hypothermic coronary occlusions were required to induce preconditioning. Additionally, the maintenance of tissue glycogen and lactate levels near control levels during the hypothermic preconditioning coronary occlusion suggests that despite a cessation of arterial perfusion the tissue was not profoundly ischemic.

Normothermic preconditioning. Although ischemic preconditioning was originally observed after repetitive coronary occlusions in the dog,³ subsequent canine studies have shown that a single occlusion of 5 minutes confers cardioprotection comparable to that elicited with multiple preconditioning periods.^{11,12} Similarly, in in situ rabbit hearts a single 5-minute occlusion has been shown to be equipotent with two 5-minute coronary occlusions in preconditioning the myocardium.⁴ However, two 2-minute periods of coronary occlusion were insufficient to elicit preconditioning. In isolated rabbit hearts previous investigators have successfully elicited preconditioning with a single 5-minute coronary occlusion.¹³⁻¹⁵ In the present study we compared one and four 5-minute occlusions and found that both paradigms produced infarct limitation, although the four 5-minute coronary occlusions produced a more robust cardioprotective

Occlusion		Reperfusion		
Start	End	Start	Middle	End
29 ± 2*	31 ± 2*	52 ± 2	44 ± 3*	38 ± 2*
30 ± 2*	33 ± 2*	55 ± 3	45 ± 3*	41 ± 3*
26 ± 1*	32 ± 1*	52 ± 1	44 ± 1*	41 ± 1*
29 ± 2*	31 ± 2*	49 ± 2	41 ± 1*	38 ± 2*
32 ± 4*	31 ± 4*	57 ± 3	40 ± 4*	37 ± 2*
33 ± 5*	36 ± 6*	57 ± 4	47 ± 4*	44 ± 4*
24 ± 2*	27 ± 2*	52 ± 2	43 ± 3	39 ± 3*

effect. Therefore, in normothermic rabbit hearts, the threshold duration of coronary occlusion necessary to elicit preconditioning is approximately 5 minutes and there appears to be a temporal duration “dose-response” in eliciting the preconditioning effect.

Ischemia during hypothermia. Moderate to profound hypothermia prolongs organ survival during global ischemia.¹⁶ Previous studies have demonstrated a reduction of infarct size with hypothermia (25° C),^{17, 18} and recently it was reported that mild hypothermia (34° C) limits myocardial infarct size.² The preservation of postischemic ventricular function conferred by normothermic preconditioning is additive to the protection resulting from hypothermia during sustained ischemia.⁷ The converse situation—hypothermic preconditioning ischemia and normothermic sustained ischemia—has not previously been studied. The present study demonstrates that, rather than augmenting the protective effect of preconditioning ischemia, hypothermia instead increases the threshold of ischemia necessary to elicit cardioprotection.

Myocardial metabolism and oxygen consumption are decreased during hypothermia,^{16, 19} and the oxygen consumption of arrested dog hearts cooled to a temperature of 15° C is about 70% of that of normothermic arrested hearts.²⁰ Hypothermia is associated with a significant decrease in the rate of high-energy phosphate depletion and lactate production that occurs in ischemic tissue.²¹ Similarly, hypothermic arrest of dog hearts cooled to 17° C results in a threefold slower decline in adenosine triphosphate levels compared with results with normothermic potassium citrate arrest.^{19, 22} Hearts arrested during hypothermia (17° C) use one fourth less glycogen and produce one third less lactate than hearts arrested at normothermia.^{19, 22} These studies

Table III. Heart weights and ratios

	Biventricular weight (gm)	Risk (cm ³)	Risk/biventricular weight ratio (%)
CON	6.7 ± 0.3	0.90 ± 0.06	13.6 ± 0.9
IP1	6.7 ± 0.2	0.81 ± 0.06	11.9 ± 0.8
H ₁₀	7.2 ± 0.2	0.85 ± 0.09	11.8 ± 1.1
H ₁₀ + IP1	6.4 ± 0.2	0.82 ± 0.07	12.9 ± 1.0
IP4	6.8 ± 0.3	0.76 ± 0.04	11.1 ± 0.4
H ₄₀	7.0 ± 0.4	0.88 ± 0.14	12.8 ± 1.6
H ₄₀ + IP4	6.9 ± 0.1	1.01 ± 0.13	14.5 ± 1.6

See text for description of groups.

suggest that both the basal and working metabolic demands of hypothermic hearts are less than those of normothermic hearts.

How does hypothermia increase the threshold for ischemic preconditioning? Although there are many definitions of ischemia, one common definition characterizes ischemia as a condition in which oxygen and substrate delivery to the myocardium is reduced to an extent that results in a shift from aerobic to anaerobic metabolism.²³ Given this definition, the hypothermic isolated hearts in the current study were not markedly ischemic inasmuch as glycogen stores were not depleted and lactate was not produced. These data suggest that despite a cessation of perfusion, the balance between oxygen/substrate supply and metabolic demand was not greatly perturbed, likely because demand was also drastically reduced. Thus, because profound ischemia did not exist during the single hypothermic preconditioning occlusion, there was no cellular signal to initiate the preconditioning process.

Summary and conclusions

In summary, this study demonstrates that although antecedent hypothermia does not alter myocardial infarct size, it does increase the amount of transient antecedent ischemia necessary to elicit ischemic preconditioning.

We gratefully acknowledge the assistance of Michiko Dote in the preparation of the manuscript.

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