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ORIGINAL CONTRIBUTION

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Mild Hypothermia reduces infarct size in the beating rabbit heart: A practical intervention for acute myocardial infarction?

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Abstract The present study describes a method for rapidly cooling the whole body via its blood pool and tests whether cooling instituted after ischemia has begun can still limit infarction. We also evaluated whether the cardiac protection seen with cooling could be added to that from ischemic preconditioning. Recently it was reported that lowering myocardial temperature by only several degrees greatly slows the extent of myocardial infarction in the beating heart experiencing regional ischemia. To further explore the potential of hypothermia for myocardial protection, rabbits underwent either a 30-, 45- or 60-min coronary artery occlusion and 3-h reperfusion. Blood from a carotid artery was allowed to circulate through a heat exchanger immersed in ice water and return to a jugular vein until the blood temperature in the left atrium reached the target temperature of 35 or 32 °C. Furthermore, to elucidate the mechanism of hypothermia's protection, we also examined its effect on isolated cardiomyocytes. Rewarming began upon reperfusion in all protocols. Cooling to 32 °C before a 30-min ischemia reduced infarct size from 37.3 ± 2.5 % (n = 6) of the risk zone in normothermic controls to 3.6 ± 0.3 % (n = 6). When cooling was begun 10 or 20 min after the onset of ischemia infarct size was still significantly smaller [8.1 ± 1.2 % and 22.8 ± 1.8 %, respectively (n = 6 in each group)]. Less but significant protection was also seen with cooling to 35 °C. Cooling caused only mild bradycardia and hypotension and no apparent arrhythmias. Forty-five min of regional ischemia caused 50.7 ± 3.3 % (n = 6) of risk zone to infarct in untreated hearts. Preconditioning with 5-min ischemia/10-min reperfusion reduced infarct size to 27.5 ± 2.5 % (n = 6). Cooling to 32 °C starting 20 min after the onset of ischemia protected the heart (28.7 ± 2.6 % infarction, n = 8), and this protection could be added to the effect from ischemic preconditioning (6.3 ± 2.3 % infarction, n = 6). In the myocyte model, hypothermia and ischemic preconditioning delayed the progressive increase in osmotic fragility that occurs during simulated ischemia in an additive way, but only hypothermia delayed the appearance of contracture suggesting that different mechanisms are involved. Hence blood pool cooling was easily induced and well tolerated and protected the beating heart against infarction even when hypothermia was started after the onset of coronary occlusion. We conclude that hypothermia might be a simple and useful therapy for patients presenting with acute myocardial infarction.

Key words Hypothermia – ischemic preconditioning – myocardial infarction – myocytes – rabbit

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Introduction

There is a need for a therapeutic intervention which could make the heart resistant to infarction from regional myocardial ischemia as occurs in the patient with sudden coronary occlusion. One such intervention, ischemic preconditioning, has stimulated a great deal of interest in this regard because of its anti-infarct effect. When the heart is preconditioned with a brief period of ischemia, it quickly adapts to become markedly protected against infarction from a subsequent period of ischemia. While many agents have been identified which will pharmacologically induce ischemic preconditioning such as adenosine (38) and bradykinin (14), these agents must all be administered prior to ischemia in order to realize protection against infarction. While the requirement for pretreatment has made these agents impractical in patients presenting with acute myocardial infarction, it is hoped that future studies will reveal interventions which can induce preconditioning's protection even when administered after the onset of ischemia (3, 42).

Hypothermia also prolongs an organ's survival during ischemia. Cardiac hypothermia decreases myocardial oxygen consumption (5) and slows the rate of ATP depletion during ischemia (22), and hypothermia has been established as a fundamental method for cardiac preservation during cardiac surgery (17). Recently several laboratories have reported that relatively small changes of temperature profoundly influence myocardial infarct size in dogs (35), pigs (8), and rabbits (7, 16). In those studies infarct size changed by 8–20 % of the risk zone for each change of one degree between 35–42 °C (7, 8). Most recently topical cooling of the ischemic zone in rabbits has been described as a possible infarct-sparing intervention for acute myocardial infarction (15, 16). Infarct size was limited when topical cooling was started either prior to the onset of ischemia (16) or after ischemia had begun (15). In the present study we propose cooling of the body's blood pool as an alternative method of cooling the infarcting heart. With blood pool cooling the hypothermic heart continues to beat and circulate blood quite adequately even at temperatures as low as 32 °C. A method is described for rapidly cooling the blood pool using a heat exchanger inserted between a peripheral artery and vein which should pose fewer technical challenges than attempting to topically cool the heart. We explored the effect of two different temperatures (35 and 32 °C) and the utility of starting hypothermia at various times after the onset of the coronary occlusion. We also tested whether protection from ischemic preconditioning could be combined with that from cooling to get even greater protection. Finally, we examined the effect of hypothermia on a model of simulated ischemia in isolated cardiomyocytes to gain insight into the mechanism of its protection.

Materials and methods

This study was conducted in accordance with The Guide for the Care and Use of Laboratory Animals (National Academy Press, Washington, DC, 1996).

In situ model

Surgical preparation

New Zealand White rabbits of either sex, weighing 1.8–2.4 kg, were anesthetized with intravenous sodium pentobarbital (30 mg/kg). Anesthesia was supplemented with intravenous boluses of pentobarbital as needed to maintain the surgical plane (approximately every 20 min). The neck was opened with a ventral midline incision and a tracheotomy was performed. The rabbits were ventilated with 100 % oxygen and a positive pressure respirator (MD Industries, Mobile, AL). The respiratory rate and tidal volume were adjusted to keep the arterial blood pH in the physiological range. A catheter was inserted into the femoral artery for monitoring of blood pressure. Other catheters were placed in the carotid artery and jugular vein for cooling the blood with a heat exchanger. A left thoracotomy was performed in the fourth intercostal space, and the pericardium was opened to expose the heart. A 2–0 silk suture on a curved taper needle was passed around a prominent branch of the left coronary artery, and the ends were pulled through a small vinyl tube to form a snare. The coronary branch was occluded by pulling the snare, which was then fixed by clamping the tube with a small hemostat. Temperature was measured in the left atrium with a Thermalert TH-5 (Physiotemp, Clifton, NJ) and in the rectum with a glass thermometer (Fisher Scientific, Pittsburgh, PA).

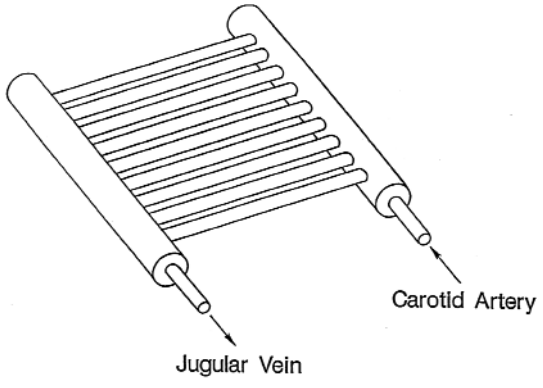

Fig. 1 A diagram of the heat exchanger used in these studies. Vinyl tubing was used to connect the exchanger to a carotid artery and a jugular vein of the rabbit. The actual exchanger is about 10 cm wide, has a volume of about 3 ml, and is made from brass and plastic tubing.

Rabbits were anticoagulated with 2000 units of heparin and a heat exchanger (Fig. 1) was installed between a carotid artery and a jugular vein. The heat exchanger had a hydraulic resistance to blood of 0.5 ml/min/mmHg and a volume of 3 ml. Circulation through the exchanger was controlled by a clamp on the carotid cannula. After the surgical preparation was completed the rabbit was allowed to stabilize for 10 min. Rabbits were then subjected to one of the protocols described below. Myocardial ischemia was induced by applying traction to the snare and was confirmed by appearance of regional cyanosis. Reperfusion was achieved by releasing the snare and was confirmed by visible hyperemia on the ventricular surface. After 3 h of reperfusion, an intravenous bolus of 2000 units of heparin was again administered and then a pentobarbital overdose. The heart was excised for postmortem analysis.

Measurement of infarct and risk zone

At the end of the experiment hearts were quickly removed from the chest and mounted on a Langendorff apparatus. The aortic root was perfused with saline at a pressure of 80 cm H_2O to wash out blood from the coronary arteries. Then the snared coronary artery was reoccluded, and 1–10 µm zinc cadmium sulfide fluorescent particles (Duke Scientific Corp., Palo Alto, CA) were infused into the perfusate to demarcate the risk zone as the tissue without fluorescence. The heart was weighed, frozen, and cut into 2-mm-thick slices. The slices were incubated in 1 % triphenyltetrazolium chloride (TTC) in pH 7.4 buffer for 15–20 min at 37 °C. The areas of infarct (TTC negative) and risk zone (non-fluorescent under ultraviolet light) were determined by planimetry. Infarct and risk zone volumes were then calculated by multiplying each area by the slice thickness and summing the products. Infarct size was expressed as a percentage of the risk zone.

Experimental Protocols

30-min ischemia studies Seven groups of open-chest rabbits experienced 30 min of regional ischemia followed by 3 h of reperfusion. In the control group the heat exchanger was immersed in warm water (38 °C) and the carotid artery clamp was removed 5 min prior to the 30-min ischemia and replaced 30 min after reperfusion. Temperature was maintained near 38 °C in these rabbits. The second (35/0) group of rabbits was cooled using the heat exchanger which was put into ice water (0 °C) starting 5 min prior to the 30-min period of ischemia. By adjusting the clamp on the carotid cannula the temperature of the blood in the left atrium was maintained at 35 °C until the end of ischemia. After initiation of reperfusion the heat exchanger was put into tepid water (38 °C) and the rabbit was allowed to rewarm. A heating pad was also used to aid rewarming. Body temperature could be restored to 38 °C in 30–60 min. The third (35/10) and fourth (35/20) groups of rabbits were cooled similarly, but cooling started 10 and 20 min after the onset of the coronary occlusion, respectively. Temperature in the left atrium was lowered to 35 °C by placing the heat exchanger in ice water for only the final 10 or 20 min of the ischemic period. After reperfusion rabbits were again rewarmed as in the 35/0 group. In the next three groups, 32/0, 32/10, and 32/20, cooling was started 5 min before or 10 or 20 min after the onset of the 30-min ischemia, respectively, and temperature in the left atrium was kept at 32 °C until the end of ischemia. At reperfusion rabbits were rewarmed as described above. Time for rewarming was usually slightly more than 1 h.

45-min ischemia studies Four groups of rabbits experienced 45 min of regional ischemia followed by 3 h of reperfusion. In control rabbits blood maintained near 38 °C was circulated through the heat exchanger placed in warm water starting 20 min after the onset of ischemia and continuing until reperfusion. The second group, PC, was ischemically preconditioned with 5 min of regional ischemia and 10 min of reperfusion prior to the 45-min ischemic period. Left atrial temperature was maintained near 38 °C as blood was allowed to circulate through the heat exchanger inserted into a warm water reservoir from 20 min after the onset until the end of ischemia. The third group of rabbits, Hypo, was cooled to 32 °C using the heat exchanger placed in ice water starting 20 min after coronary occlusion and continuing until the end of ischemia. Rabbits were rewarmed after reperfusion as already described. In the PC+Hypo group, rabbits were preconditioned as in the PC group and cooled to 32 °C starting 20 min after the onset of ischemia as in the Hypo group.

60-min ischemia studies One group of rabbits was subjected to 60 min of regional ischemia. In this group, blood was cooled to 32 °C starting 20 min after coronary occlusion and maintained at that temperature until after reperfusion. The purpose of this group was to accurately measure the progression of infarction during cooling by comparing infarct size to that in the 32/20 (30-min ischemia) and the Hypo (45-min ischemia) groups.

Isolated myocyte studies

Isolation of myocytes

Rabbits (2.4–3.0 kg) were anesthetized and intubated as described above. The hearts were quickly excised, mounted on a Langendorff apparatus and perfused for 5 min with modified Krebs-Henseleit (K-H) solution containing (mmol/L) NaCl 125, KCl 4.7, $MgCl_2$ 1.2, $NaHCO_3$ 25, KH_2PO_4 1.2, $CaCl_2$ 2,

and glucose 10. The perfusate was bubbled with 95 % O_2/5 % CO_2 and maintained at 37 °C. Hearts were then perfused with a calcium-free perfusate for 5 min, after which the perfusion solution was switched to one containing collagenase (0.8 mg/ml, type II, Worthington Biochemical Co., Freehold, NJ). After about 15 min of collagenase perfusion, hearts were removed from the apparatus, and atria were trimmed away. The ventricles were minced and cells dispersed with a large bore pipette, followed by filtration through nylon mesh. Cells were washed by a centrifugation and resuspension in modified K-H solution as specified above but containing (mmol/L) glutamine 0.68, taurine 60, creatine 20, 1 % BSA, 1 % BME amino acid, 1 % MEM non-essential amino acid, 1 % BME vitamin solution. Then calcium was added to a final concentration of 1.25 mmol/L and calcium tolerant cells were harvested by brief centrifugation. The supernatant was discarded. Preparations were considered satisfactory only if rod-shaped cells (not in contracture) accounted for more than 65 % of the cells at the beginning of each experiment.

Simulated ischemia model

At the end of each experimental protocol (see below), cells were resuspended in modified K-H buffer enriched with amino acids as detailed above, placed in a 1.8 ml microcentrifuge tube, and centrifuged into a pellet. Each cell pellet occupied a volume of about 0.5 mL and measured 0.8–1.0 cm in height. Excess supernatant was removed to leave a fluid layer above the pelleted cells of about one third the volume of the pellet. Mineral oil was layered on top to exclude gaseous exchange, and the cell pellet was incubated without agitation at either 37 or 32 °C for 180 min (see protocol). A 25 µL sample was taken from the center of the cell pellet with a pipette every 30 min and resuspended for 3–5 min at 30 °C in 200 µL of hypotonic (85 mOsM) buffer containing 3 mM amytal, a mitochondrial inhibitor to prevent cell rounding during reoxygenation. A 25 µL sample was then mixed on a microscope slide with an equal volume of 85 mOsM media (0.5 % glutaraldehyde in modified Tyrode solution with reduced NaCl containing 1 % trypan blue) for 3–5 min at room temperature. Both the morphology and the permeability of the cells to trypan blue were determined with microscopic examination at 100X magnification. Normal myocytes can tolerate the hypotonic medium and do not admit the stain. After a period of simulated ischemia, however, the cells become osmotically fragile and the number of stained cells increases at a predictable rate.

Experimental protocols

Each isolation provided enough cells for four experimental groups and two oxygenated control groups. Cells in the control (Control) and hypothermia (Hypo) groups were resuspended for 25 min at 37 °C in 3 ml of modified K-H buffer before ischemic pelleting. The two preconditioned groups, PC and PC+Hypo, were preconditioned by centrifuging cells into a pellet as described above for 10 min followed by resuspension in oxygenated buffer for 15 min prior to ischemic pelleting and incubation for 180 min. The Control and the PC tubes continued to be incubated at 37 °C for the rest of the study. Cells in the two hypothermic groups , Hypo and PC+Hypo, were incubated at 32 °C after pelleting. Cells in the two oxygenated control groups were not pelleted, but rather remained suspended in oxygenated glucose-containing media for the entire 180 min of the experiment. One was maintained at 37 °C and the other at 32 °C.

Statistics

All data are presented as mean ± SEM. One-way ANOVA was used to analyze infarct size and Scheffé's post-hoc test was used to test for differences between groups. Group size was normally 6 animals. The data in the 45 min hypothermia group, however, exibited a strong bimodal distribution after only 6 animals and it was felt that more animals should be studied to try to resolve the true mean. The same was true of the 32/20 group. ANOVA with replication was used to test for differences in hemodynamics in any given group. The difference was considered significant if the p value was less than 0.05. For the myocyte studies the areas under the % rods vs time and the % stained cells vs time plots were calculated. By reducing the temporal data to a single number analysis could be performed by one-way ANOVA. A replication model was used in which all myocytes from a single isolate were considered to constitute a replication. When a significant group difference was detected, a paired t-test with a Dunn-Šidàk correction was used to test for group differences. The difference was considered significant if the p value was less than 0.05.

Results

In situ model

30-min ischemia studies

We excluded two rabbits from analysis. The risk zone of one rabbit in the 35/0 group was below 0.5 cm^3 (44) and one rabbit in the 35/10 group experienced irreversible ventricular fibril-

Table 1 Hemodynamic parameters for 30-min ischemia studies

	Baseline	Ischemia 5 min	Ischemia 15 min	Ischemia 30 min	Reperfusion 60 min	Reperfusion 180 min
Heart rate (bpm)						
Control	259 ± 9	263 ± 8	260 ± 9	261 ± 9	263 ± 8	270 ± 8
35/0	257 ± 13	204 ± 7 **	207 ± 8 **	207 ± 10 **	258 ± 13	266 ± 14
35/10	260 ± 11	262 ± 11	214 ± 9 **	204 ± 7 **	263 ± 9	265 ± 10
35/20	256 ± 14	258 ± 14	254 ± 13	205 ± 6 **	263 ± 14	274 ± 11
32/0	261 ± 8	170 ± 7 **	172 ± 7 **	172 ± 8 **	246 ± 7 **	267 ± 10
32/10	259 ± 10	258 ± 10	191 ± 7 **	172 ± 5 **	248 ± 12	261 ± 13
32/20	261 ± 9	261 ± 11	262 ± 10	178 ± 6 **	264 ± 8	266 ± 8
Mean blood pressure (mmHg)						
Control	80 ± 2	78 ± 3	75 ± 3 *	73 ± 3 *	71 ± 3 *	68 ± 4 *
35/0	77 ± 5	63 ± 3 **	57 ± 3 *	57 ± 3 *	64 ± 5	64 ± 5
35/10	80 ± 3	77 ± 2	70 ± 2 **	67 ± 2 **	66 ± 2 **	59 ± 3 *
35/20	80 ± 2	76 ± 2 **	73 ± 2 *	66 ± 3 **	62 ± 4 **	64 ± 5 *
32/0	78 ± 2	67 ± 3 **	70 ± 2 **	70 ± 3 *	65 ± 3 **	66 ± 4 **
32/10	79 ± 4	77 ± 3	63 ± 3 **	65 ± 2 *	69 ± 2 **	66 ± 4 **
32/20	80 ± 1	78 ± 3	76 ± 2	65 ± 2 **	69 ± 2 **	63 ± 5 **
Rate-pressure product (X100)						
Control	261 ± 10	261 ± 13	245 ± 13	244 ± 15	237 ± 13	238 ± 21
35/0	264 ± 26	175 ± 12 **	164 ± 13 **	159 ± 16 **	224 ± 18	230 ± 24
35/10	269 ± 18	256 ± 13	195 ± 9 **	181 ± 7 **	223 ± 13 *	209 ± 12 *
35/20	264 ± 16	250 ± 15 *	238 ± 12 *	179 ± 8 **	216 ± 13 **	231 ± 12
32/0	259 ± 9	146 ± 4 **	151 ± 5 **	150 ± 5 **	208 ± 10 **	230 ± 21
32/10	260 ± 11	247 ± 10	157 ± 10 **	141 ± 7 **	216 ± 9 **	220 ± 15 *
32/20	264 ± 9	253 ± 14	249 ± 12	147 ± 8 **	230 ± 8 *	218 ± 14 *

Values are mean ± SEM; * $p < 0.05$ vs. Baseline, ** $p < 0.01$ vs. Baseline

lation. All other rabbits contributed data. Transient atrioventricular block was observed in one rabbit in the 35/0 group during ischemia and in another rabbit in the 35/10 group during reperfusion. One rabbit in the 35/20 group fibrillated during ischemia, while one each in the control, 35/10, and 32/0 groups fibrillated during reperfusion. All but the one in the 35/10 group converted to sinus rhythm either spontaneously or with mechanical stimulation within 2 min and finished the protocol.

Hemodynamic parameters Hemodynamic parameters in the seven groups are summarized in Table 1. Heart rate, mean blood pressure, and rate-pressure products were comparable in all groups under baseline conditions. Circulation of blood through the heat exchanger when immersed in 38 °C water did not change heart rate or mean blood pressure. Cooling significantly decreased heart rate and caused mean blood pressure to fall by approximately 10 mmHg. With rewarming heart rate returned to the baseline value and blood pressure rose to a level not different from that seen in the normothermic control groups at comparable time periods. There was a gradual decline in blood pressure in all groups over the 3 h of the study which is normal for this model. Temperatures in the left atrium and rectum in the seven groups are summarized in Table 2. Both temperatures were comparable in all groups under baseline conditions. When the rabbit was cooled, temperature of blood in the left atrium could be reduced as desired to either 35 or 32 °C within 2 min. However, rectal temperature declined much more slowly and it consistently remained above 35 °C in all of the experiments except in the 32/0 group. Rewarming occurred much more slowly requiring 30–60 min for temperature in the left atrium to return to baseline. Rectal temperature usually continued to fall for several minutes by an additional 0.1–0.2 °C after cooling was discontinued and then rose toward normal at a rate similar to that of blood in the left atrium.

Infarct size data Table 3 presents animal body weight, heart weight, and risk zone size data. There were no significant differences in these parameters among the groups. Table 3 also summarizes infarct size data for the groups while Fig. 2 shows the infarct size for each animal. Infarct size expressed as a percentage of risk zone was 37.3 ± 2.5 % in the Control group. Cooling the rabbit prior to and for the duration of the ischemic period to 35 °C (35/0 group) significantly reduced infarct size

Table 2 Temperature data for 30-min ischemia studies

	Baseline	Ischemia 5 min	15 min	30 min	Reperfusion 60 min	180 min
Temperature in the left atrium (°C)						
Control	38.4 ± 0.2	38.5 ± 0.2	38.4 ± 0.2	38.5 ± 0.2	38.6 ± 0.2 *	38.7 ± 0.2 *
35/0	38.5 ± 0.3	34.9 ± 0.0 **	34.9 ± 0.1 **	35.0 ± 0.0 **	38.2 ± 0.3 *	39.0 ± 0.3 *
35/10	38.6 ± 0.1	38.7 ± 0.2	35.0 ± 0.0 **	35.0 ± 0.0 **	38.5 ± 0.2	39.1 ± 0.2 *
35/20	38.5 ± 0.4	38.6 ± 0.4	38.6 ± 0.4	35.0 ± 0.0 **	38.4 ± 0.3	39.1 ± 0.3 *
32/0	38.4 ± 0.1	32.0 ± 0.0 **	32.0 ± 0.0 **	32.0 ± 0.1 **	37.7 ± 0.1 **	38.9 ± 0.2 *
32/10	38.3 ± 0.3	38.3 ± 0.3	32.0 ± 0.1 **	32.0 ± 0.0 **	38.0 ± 0.1	38.8 ± 0.2 *
32/20	38.2 ± 0.2	38.2 ± 0.2	38.2 ± 0.2	32.0 ± 0.0 **	38.2 ± 0.2	38.9 ± 0.3 **
Temperature in the rectum (°C)						
Control	38.5 ± 0.2	38.5 ± 0.2	38.5 ± 0.2	38.4 ± 0.2	38.6 ± 0.2	38.6 ± 0.2
35/0	38.7 ± 0.3	36.6 ± 0.2 **	36.1 ± 0.2 **	35.8 ± 0.2 **	38.3 ± 0.2	39.2 ± 0.3 *
35/10	38.8 ± 0.2	38.8 ± 0.2	37.8 ± 0.1 **	36.6 ± 0.1 **	38.5 ± 0.2	39.2 ± 0.2
35/20	38.7 ± 0.4	38.7 ± 0.4	38.7 ± 0.4	37.1 ± 0.3 **	38.4 ± 0.3 *	39.3 ± 0.3 *
32/0	38.5 ± 0.2	35.7 ± 0.3 **	34.7 ± 0.3 **	33.8 ± 0.2 **	37.0 ± 0.2 **	38.7 ± 0.3
32/10	38.4 ± 0.3	38.4 ± 0.3	37.0 ± 0.3 **	35.1 ± 0.3 **	37.3 ± 0.3 **	38.7 ± 0.3
32/20	38.2 ± 0.2	38.2 ± 0.2	38.2 ± 0.2	35.7 ± 0.3 **	37.8 ± 0.2 *	38.8 ± 0.3 **

Values are mean ± SEM; * $p < 0.05$ vs. Baseline, ** $p < 0.01$ vs. Baseline

to 11.1 ± 2.6 % ($p < 0.01$ vs. Control). When cooling to 35 °C was delayed until 10 min after the onset of ischemia (35/10 group), the heart was still protected (18.2 ± 2.5 % infarction, $p < 0.01$ vs. Control). However, when cooling was instituted 20 min after ischemia had begun (35/20 group), protection was no longer observed (33.9 ± 2.3 % infarction, p = NS vs. Control). When the temperature was lowered to 32 °C, additional protection was seen. Infarct size was only 3.6 ± 0.3 % in the 32/0 group and 8.1 ± 1.2 % in the 32/10 group ($p < 0.01$ vs. Control). Surprisingly, cooling the rabbit to 32 °C for only the last 10 min of the 30-min ischemic period (32/20 group) was also protective (22.8 ± 1.8 % infarction, $p < 0.01$ vs. Control).

45-min ischemia studies

We excluded one rabbit in the PC group because the risk zone was below 0.5 cm^3. One rabbit in each of the Control, PC, and PC+Hypo groups fibrillated during ischemia. Also one rabbit in the Control group fibrillated during reperfusion. All fibrillating hearts converted to sinus rhythm either spontaneously or with mechanical stimulation within 2 min.

Hemodynamic parameters Hemodynamic parameters in the four groups are summarized in Table 4. Heart rate, mean blood pressure, and rate-pressure products were comparable in all

Table 3 Infarct size data for 30-min ischemia studies

	N	Body weight (kg)	Heart weight (g)	Risk zone (cm^3)	Infarct size (cm^3)	Infarct (% of Risk)
Control	6	2.0 ± 0.0	6.2 ± 0.3	0.96 ± 0.12	0.37 ± 0.06	37.3 ± 2.5
35/0	6	2.1 ± 0.0	6.4 ± 0.3	0.92 ± 0.10	0.11 ± 0.02 **	11.1 ± 2.6 **
35/10	6	2.2 ± 0.1	6.5 ± 0.3	0.85 ± 0.08	0.17 ± 0.04 *	18.2 ± 2.5 **
35/20	6	2.2 ± 0.0	6.6 ± 0.2	0.88 ± 0.05	0.30 ± 0.03	33.9 ± 2.3
32/0	6	2.1 ± 0.1	6.8 ± 0.2	0.96 ± 0.06	0.03 ± 0.00 **	3.6 ± 0.3 **
32/10	6	2.3 ± 0.1	6.8 ± 0.1	0.94 ± 0.06	0.08 ± 0.01 **	8.1 ± 1.2 **
32/20	8	2.1 ± 0.1	6.8 ± 0.2	0.93 ± 0.07	0.21 ± 0.03	22.8 ± 1.8 **

Values are mean ± SEM; * $p < 0.05$ vs. Control, ** $p < 0.01$ vs. Control; Abbreviation: N = number of rabbits in each group

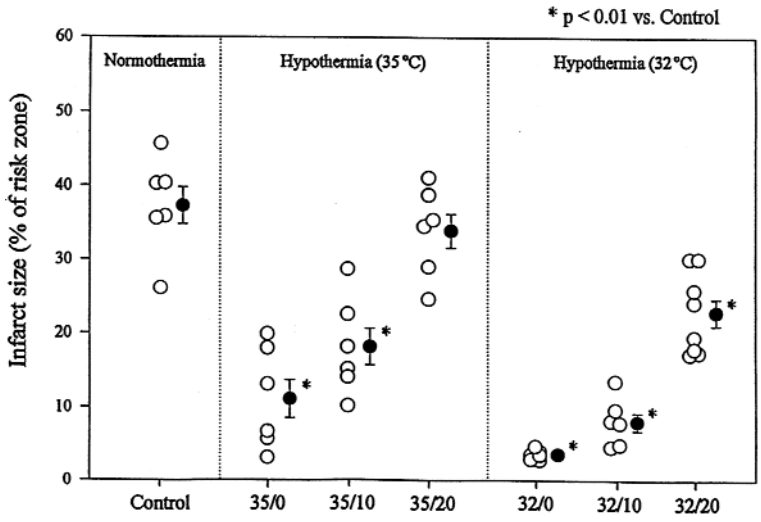

Fig. 2 Infarct size normalized as a percent of the risk zone is plotted for the groups experiencing 30 min of regional ischemia. Open and closed circles indicate individual experiments and group means, respectively. Note that the protection observed depends both on the temperature and the duration of cooling. Abbreviations: see text.

groups under baseline conditions. As in the 30-min ischemia studies, hypothermia significantly decreased both heart rate and mean blood pressure. After rewarming heart rate and blood pressure recovered.

Infarct size data As can be seen in Table 5, there were no significant differences in body weight, heart weight, or risk zone size among the groups. Figure 3 shows the normalized infarct sizes for the 4 groups. In the Control group 50.7 ± 3.3 % of the risk zone infarcted, whereas preconditioning with 5-min ischemia/10-min reperfusion (PC) significantly reduced infarct size to 27.5 ± 2.5 % ($p < 0.01$ vs. Control). Cooling to 32 °C begun 20 min after the onset of ischemia protected the heart to a similar degree (28.7 ± 2.6 % infarction, $p < 0.01$ vs. Control). The cardioprotective effect of hypothermia could be added to that from ischemic preconditioning when the two interventions were combined in the PC+Hypo group (6.3 ± 2.3 % infarction, $p < 0.01$ vs. Control, PC, and Hypo).

Table 4 Hemodynamic parameters for 45-min ischemia studies

	Baseline	Ischemia 5 min	Ischemia 30 min	Ischemia 45 min	Reperfusion 60 min	Reperfusion 180 min
Heart rate (bpm)						
Control	273 ± 8	277 ± 9	259 ± 9	265 ± 11	259 ± 10	245 ± 12 *
PC	262 ± 11	263 ± 12	266 ± 10	264 ± 10	243 ± 12	254 ± 16
Hypo	262 ± 11	264 ± 11	178 ± 7 **	175 ± 6 **	258 ± 12	261 ± 12
PC+Hypo	265 ± 10	267 ± 12	190 ± 5 **	181 ± 4 **	257 ± 12	268 ± 15
Mean blood pressure (mmHg)						
Control	81 ± 3	76 ± 3	72 ± 4	70 ± 4	65 ± 5 *	59 ± 4 **
PC	84 ± 2	80 ± 3 **	78 ± 2 **	76 ± 2 **	72 ± 1 **	65 ± 3 **
Hypo	81 ± 1	82 ± 3	69 ± 4 **	71 ± 3 **	76 ± 3	71 ± 3 *
PC+Hypo	82 ± 3	83 ± 2	75 ± 1 *	77 ± 2	77 ± 3	78 ± 3
Rate-pressure product (X100)						
Control	261 ± 12	249 ± 15	218 ± 14	221 ± 16 *	205 ± 16 *	178 ± 11 **
PC	275 ± 12	259 ± 11	258 ± 7	251 ± 10 *	219 ± 9 **	211 ± 14 *
Hypo	280 ± 11	278 ± 16	163 ± 10 **	161 ± 8 **	257 ± 19	244 ± 19 *
PC+Hypo	277 ± 16	277 ± 16	186 ± 6 **	181 ± 5 **	255 ± 15	269 ± 19

Values are mean ± SEM; * $p < 0.05$ vs. Baseline, ** $p < 0.01$ vs. Baseline; Abbreviations: PC = ischemic preconditioning, Hypo = hypothermia

Table 5 Infarct size data for 45-min ischemia studies

	N	Body weight (kg)	Heart weight (g)	Risk zone (cm^3)	Infarct size (cm^3)	Infarct (% of Risk)
Control	6	2.1 ± 0.1	6.4 ± 0.1	0.94 ± 0.11	0.49 ± 0.07	50.7 ± 3.3
PC	6	2.1 ± 0.1	6.7 ± 0.2	0.88 ± 0.08	0.25 ± 0.04	27.5 ± 2.5 *
Hypo	8	2.1 ± 0.0	6.7 ± 0.3	0.96 ± 0.12	0.29 ± 0.06	28.7 ± 2.6 *
PC+Hypo	6	2.0 ± 0.0	6.7 ± 0.3	0.88 ± 0.11	0.07 ± 0.03 *	6.3 ± 2.3 *†

Values are mean ± SEM; * p < 0.01 vs. Control, † p< 0.01 vs. PC and Hypo; Abbreviations: see Tables 2 and 4

60-min ischemia studies

Two of six rabbits fibrillated during ischemia, but converted to sinus rhythm within 1 min. Cooling reduced the heart rate and mean blood pressure by an amount similar to that seen in the other groups subjected to hypothermia. Infarct size in this hypothermic group was 28.6 ± 1.9 % of the risk zone (Fig. 4). While this group experienced 30 more minutes of ischemia than the 32/20 group, only an additional 5 % of the risk zone infarcted over this period. This difference was not significant. Thus, the rate of infarction during cooling to 32 °C was slowed to a negligible rate.

Isolated myocyte model

Figure 5 indicates that the rate of development of contracture (conversion of rod-shaped cells to square cells) during simulated ischemia at 37 °C was similar in control and preconditioned groups. Cooling the cells to 32 °C, however, greatly delayed the onset of contracture both in the presence and absence of preconditioning (p < 0.05 vs. Control). Figure 6 reveals that ischemically preconditioned cells were significantly protected against the increase in osmotic fragility that accompanies simulated ischemia (p < 0.01 vs. Control). Hypothermia during ischemic pelleting at 32 °C also delayed

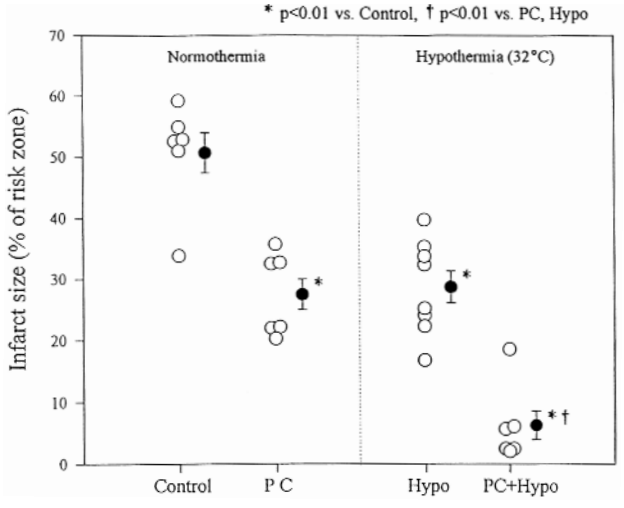

Fig. 3 Infarct size normalized as a percent of the risk zone is plotted for the groups experiencing 45 min of regional ischemia. Open and closed circles indicate individual experiments and group means, respectively. Either hypothermia starting 20 min after the onset of ischemia or ischemic preconditioning protected the hearts. If both interventions were combined additional protection was observed. Abbreviations: see text.

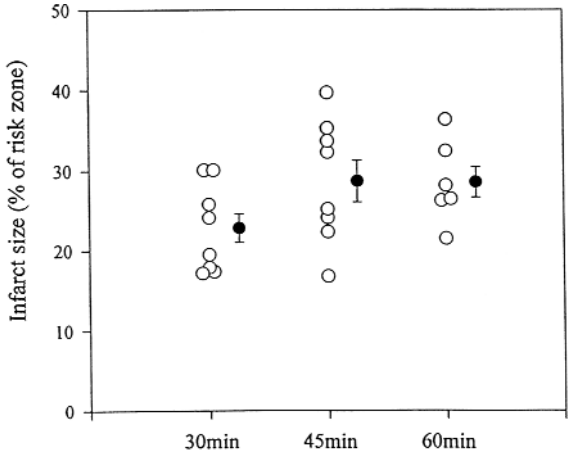

Fig. 4 Infarct size normalized as a percent of the risk zone is plotted. Open and closed circles indicate individual experiments and group means, respectively. When the heart was cooled to 32°C starting 20 min after ischemia, infarct sizes were the same regardless of whether the hearts experienced 30 (32/20 group), 45 (Hypo group) or 60 min of ischemia.

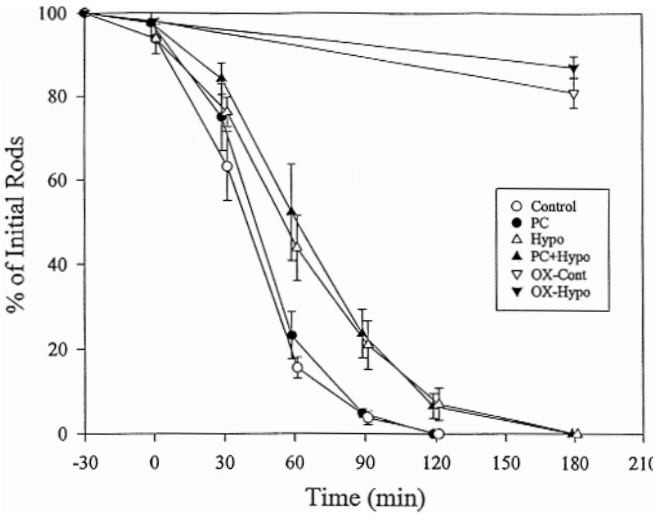

Fig. 5 A plot of the effect of ischemic preconditioning (PC) and hypothermia at 32 °C (Hypo) on the rates of development of rigor contracture (rod to square transition) in isolated cardiomyocytes from 5 hearts exposed to simulated ischemia. Control and PC groups underwent contracture at similar rates. However, the cooled groups, Hypo and PC+Hypo, experienced a delay of contracture development. Abbreviations: OX-Cont = oxygenated controls at 37 °C, OX-Hypo = oxygenated controls at 32 °C.

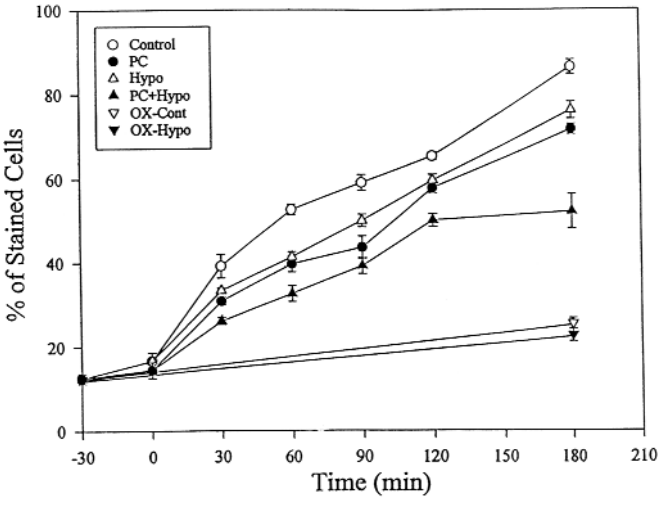

Fig. 6 A graph showing the effect of ischemic preconditioning (PC) and hypothermia (Hypo) on the development of osmotic fragility in cardiomyocytes from 5 hearts. The percentage of myocytes which admit trypan blue after a hypotonic shock is plotted against the duration of simulated ischemia. The appearance of osmotic fragility was delayed in the PC group. Hypothermia also delayed the appearance of fragility, and this effect could be added to that from PC. Abbreviations: see Fig. 5.

the onset of osmotic fragility ($p < 0.01$ vs. Control) to an extent similar to that of ischemic preconditioning. When ischemic preconditioning and hypothermia were combined, a further shift of the curves to the right was observed ($p < 0.01$ vs. Control, PC and Hypo). Cells in the oxygenated groups retained a rod shaped morphology and continued to exclude trypan blue for the duration of the experimental protocol.

Discussion

Several studies have reported that even a small decrease of temperature can profoundly protect the beating heart against infarction, and the amount of protection depended on the degree of hypothermia (7, 8, 16, 35). In the present study we describe a simple method for rapidly cooling the entire blood pool with a heat exchanger inserted between an artery and vein. We confirm the protective influence of temperature on infarct size and clearly show that the progression of infarction can be greatly slowed even when cooling is instituted after the onset of ischemia. When ischemic preconditioning and hypothermia are combined, additive protection is observed. This additive effect of hypothermia and preconditioning was also documented in a myocyte model suggesting that ischemic preconditioning and cooling protect the heart by fundamentally different mechanisms. Most importantly, cooling the blood to 32 °C has no adverse effect on the heart's ability to maintain a normal hemodynamic state, thus, precluding the need for any extracorporeal assistance. These data would indicate that the protection of cooling can be applied to the beating heart and could form the basis of a therapeutic intervention for patients with acute myocardial infarction.

The presented data suggest that cooling to 32 °C virtually arrests the infarction process in the rabbit heart. When the heart was cooled to 32 °C starting 20 min after ischemia, infarct sizes were the same regardless of whether the hearts experienced 30 (32/20 group), 45 (Hypo group) or 60 min of total ischemia (22.8 ± 1.8 %, 28.7 ± 2.6 % and 28.6 ± 1.9 % infarction, respectively; p = NS). The progression of infarction appears to have been nearly stopped by the hypothermia. Gho et al. (11) tested the effect of combining hypothermia and ischemic preconditioning in the rat. In their study hypothermia had no effect on infarct size against ischemic periods of one hour (11) but was protective when 30 min ischemic periods were employed (12). Cooling also magnified the efficacy of ischemic preconditioning using the one hour protocol. In the present study we found that myocardial temperature was an important determinant of the progression of infarction as has been reported by others (7, 8 16, 35).

An important observation is the significant decrease in infarction even when cooling was initiated after the onset of coronary occlusion. A delay in treatment is inevitable in the

clinical setting in which nearly all patients present to emergency rooms only after their coronary occlusion has produced symptoms. Hale et al. (15) also observed that cooling after the onset of coronary occlusion is protective. In their protocol topical cooling induced by placing a balloon filled with ice water on the ischemic epicardium was employed. However, adaptation of that approach to a clinical setting would present daunting technical problems. Furthermore, topical cooling would primarily cool the epicardium, whereas our method would primarily cool the endocardium, that region of the heart which is most susceptible to ischemia.

The mechanism by which temperature influences infarct size is probably a decrease in the rate of high energy phosphate utilization during hypothermia. Jones et al. (22) showed in the dog heart that decrements in temperature from 40 to 28 °C slowed both the rate of high energy phosphate utilization and production from anaerobic glycolysis. Ichihara et al. (21) reported that glucose utilization in both nonischemic and ischemic hearts was significantly reduced at 20 °C and accumulation of lactate was reduced from that seen at 37 °C, suggesting that the metabolic demand of hypothermic hearts is less than that of normothermic hearts. Evidence of an improved energy balance was seen in the present study in the myocyte model in which the rate at which contracture developed during simulated ischemia was reduced during hypothermia. In this model contracture occurs as individual cells become severely depleted of ATP (1, 18, 19, 26, 39). This would suggest that cooling acted to spare ATP which is in stark contrast to the case with ischemic preconditioning which itself had no apparent effect on the rate of contracture in the cardiomyocytes (Fig. 5). In the present study, hypothermia caused moderate bradycardia as has been previously reported in small animals (7, 11, 16). However, bradycardia by itself has not been shown to have any effect on infarct size in the rabbit model of ischemia-reperfusion (30). In addition, increase in heart rate by pacing did not change the relationship between infarct size and temperature in either the rabbit (7, 16) or rat (12). Therefore, the protective effect of hypothermia is unlikely to be related to induced bradycardia.

The mechanism by which preconditioning protects is currently unproven. Murry et al. (32) proposed that preconditioning reduces ATP utilization during ischemia. In their dog study ATP was rapidly depleted during the brief preconditioning ischemia, but then declined more slowly during prolonged ischemia in the preconditioned hearts. On the other hand Kolocassides et al. (25) found that preconditioned rat hearts became depleted of ATP sooner than non-preconditioned hearts resulting in earlier ischemic contracture in the preconditioned hearts. In the rabbit myocyte model preconditioning has no effect on the rate of development of individual cell contracture, and this observation has been interpreted to imply that the utilization of ATP was unaltered (2). But this has not been confirmed by direct ATP measurements.

Both preconditioning and hypothermia delayed the onset of cell osmotic fragility during ischemia. Osmotic fragility is thought to reflect a modification of the cytoskeleton that leads to membrane failure in the ischemic cardiomyocytes (10, 40). We have noted that virtually all interventions which delay the increase in osmotic fragility in the myocyte model also limit infarction in the whole heart (2). If preconditioning does act through a change in membrane structure, then the differing mechanisms could explain why it was possible to combine the two interventions and realize additional protection in both the myocyte and whole heart models.

Recently several studies have shown that inhibition of Na^+/H^+ exchange can protect the heart (6, 23). This protection is thought to result from prevention of calcium overload at reperfusion (24). Hypothermia inhibits Na^+/K^+ ATPase activity and activates the Na^+/H^+ exchanger (28) which should worsen sodium loading during ischemia. However, hypothermia also inhibits the Na^+/Ca^{++} exchanger by both a phase transition of membrane lipids (13) and by the temperature dependence of the exchanger protein (13). Thus, cooling may have protected by preventing calcium overload at reperfusion. However, the effect does not appear to be a reperfusion phenomenon since cooling just prior to reperfusion offered no protection as shown in our 35/20 group and in Hale's rabbits (15). Clearly the earlier we started cooling, the more effective it was.

The present results suggest that hypothermia could be used to protect the hearts of patients who are experiencing an acute myocardial infarction and that considerable benefit could be derived even when cooling is begun after the onset of ischemia. The data would indicate that body temperature in rabbits can be lowered by as much as 5 °C without compromising hemodynamics or triggering arrhythmias. We experimented with a number of strategies for cooling the rabbits but found that a heat exchanger connected between a large artery and vein was the only practical way to induce rapid cooling. We also tested a clinical heat exchanger used in a heart-lung machine in one anesthetized dog. The exchanger was connected between the femoral artery and vein, and we found that we could reduce the dog's systemic blood temperature as quickly as in the rabbits. No problems with arrhythmias or hypotension were encountered in the one dog studied.

In a clinical setting the earlier the cooling could be initiated, the more effective the protection from cooling would be. It should be appreciated that during coronary occlusion infarction progresses in the rabbit about twice as fast as in the dog heart (29, 31, 34) and more than 5 times faster than in the baboon heart (41). It is not known how rapidly infarction progresses in the human heart, but it must occur fairly slowly since salvageable tissue is thought to be present in the human heart up to 6 h after the onset of a coronary occlusion (37). If one can extrapolate our results to the clinical setting, cooling a patient to 32 °C even starting several hours after the onset of

symptoms might still be expected to significantly limit infarction. Obviously cooling could only be an effective clinical intervention if it could be initiated before most of the ischemic myocardium is irreversibly injured. Thus, the practicality of the method will depend on how rapidly the human heart actually does infarct.

A potential complication with blood pool cooling is that it may delay the action of a thrombolytic agent. However, in the study by Yenari et al. (43) clot lysis by tPA was slowed by approximately 0.5 % per degree of cooling. Thus, cooling to 32 °C would be expected to delay clot lysis by only 3 %. Of course, cooling should not affect the efficacy of a rescue angioplasty procedure. The requirement for an anticoagulant for the heat exchanger should also be acceptable in the setting of acute myocardial infarction.

We noted that during cooling rectal temperature lagged behind left atrial temperature, and we assume that the delay is related to the low arterial flow to the rectum and consequently a slower equilibration with the cooled blood. On the other hand, temperature of well perfused myocardium would be expected to equilibrate with that of chilled blood much more rapidly, although deeply ischemic segments in a large heart like that of a human could cool more slowly. Appropriate large animal studies could easily answer this question.

The present procedure was performed in anesthetized rabbits in which comfort and shivering were not issues. However, ample data suggest that whole blood pool cooling in man would be safe. In recent clinical trials involving patients with head trauma, cooling to 32–34 °C for 24–48 h resulted in improved neurologic recovery and reduced mortality (27, 36). No increase in the incidence of complications was seen. Furthermore, similar degrees of hypothermia have been induced in healthy volunteers by Hayward's group (9, 20). Shivering and a feeling of discomfort can be problems in hypothermia, but interestingly these symptoms can be suppressed with topical warming of the skin even when core temperature remains at 33–34 °C (9, 20) or with pharmacologic agents such as pethidine (Demerol) (33).

In summary, mild hypothermia protected the beating rabbit heart against infarction without compromising its ability to support the circulation. The degree of protection depended on the degree and duration of hypothermia and significant protection could be obtained even when cooling was instituted after ischemia had begun. Furthermore, the cardioprotective effect of hypothermia could be added to that of ischemic preconditioning. Thus, entire blood pool cooling could be a useful therapy for patients with acute myocardial infarction.

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