ACCELERATED RECOVERY OF POSTISCHEMIC STUNNED MYOCARDIUM AFTER INDUCED EXPRESSION OF MYOCARDIAL HEAT-SHOCK PROTEIN (HSP70)

In vitro studies suggest that interventions targeted at myocardial gene regulation of endogenous cytoprotective elements, such as heat-shock protein, may attenuate myocardial ischemic injury. We tested the hypothesis that heat shock–induced expression of myocardial heat-shock protein before ischemia accelerates functional recovery of postischemic stunned myocardium in the intact circulation. Sixteen dogs underwent partial femoral arteriovenous bypass and core temperature was raised to 42° C for 15 minutes in eight dogs (heat-shocked) and maintained at 37° C in eight dogs (nonheat-shocked). After 24 hours dogs were studied to measure myocardial segment length in the circumflex artery region with ultrasonic dimension transducers, left ventricular pressure with a micromanometer, and circumflex coronary flow with an ultrasonic probe. Regional contractile function was quantified by the area beneath the linear preload recruitable stroke work relationship at baseline and at intervals during reperfusion after a 15-minute circumflex artery occlusion followed by 3 hours of reperfusion. Baseline and peak reperfusion hyperemic circumflex flows were 37 ± 9 ml/min and 154 ± 33 ml/min, respectively, in heat-shocked dogs ($p < 0.001$) and 46 ± 24 ml/min and 171 ± 57 ml/min, respectively, in nonheat-shocked dogs ($p < 0.001$), with no differences between groups (p = not significant) at any time during reperfusion. Heart rate and left ventricular peak pressure, end-diastolic pressure, and first derivative of left ventricular pressure were similar (all p = not significant) in heat-shocked and nonheat-shocked dogs during ischemia and reperfusion. Before ischemia, preload recruitable stroke work relationship did not differ (p = not significant) in heat-shocked and nonheat-shocked dogs. Ischemia reduced preload recruitable stroke work relationship to 32% ± 8% control ($p < 0.001$) in heat-shocked dogs and to 19% ± 15% control in nonheat-shocked dogs ($p < 0.001$) at 15 minutes of reperfusion, indicating a similar (p = not significant) initial degree of injury. During 3 hours of reperfusion, preload recruitable stroke work relationship returned to 80% ± 38% control in heat-shocked dogs but to only 33% ± 13% control in nonheat-shocked dogs ($p < 0.0001$). Myocardial expression of heat-shock protein, quantified by optical densitometry of Western blots using an antibody specific for HSP70, was greater in heat-shocked than in nonheat-shocked dogs (108 ± 27 versus 71 ± 14 densitometry units, $p < 0.005$). Exact causal mechanisms remain to be defined, but these data indicate (1) hyperthermic bypass triggers induction of myocardial heat-shock protein and (2) elevated myocardial heat-shock protein is associated with accelerated recovery of stunned myocardium. Promotion of endogenous molecular cytoprotective systems represents a novel and potentially useful strategy for myocardial protection. (J THORAC CARDIOVASC SURG 1995;109:753-64)

Barbara L. Robinson, MD, Terumasa Morita, MD, David O. Toft, PhD, and James J. Morris, MD, *Rochester, Minn.*

From the Division of Thoracic and Cardiovascular Surgery and the Department of Biochemistry and Molecular Biology, Mayo Clinic and Foundation, Rochester, Minn.

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Address for reprints: James J. Morris, MD, Division of Thoracic and Cardiovascular Surgery, Mayo Clinic and Foundation, 200 1st St. SW, Rochester, MN 55905.

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Despite restoration of coronary blood flow and preservation of myocardial viability after brief periods of myocardial ischemia, prolonged abnormalities of myocardial contractile performance occur. Reoxygenation and reperfusion of previously ischemic myocardium has both detrimental and beneficial effects on myocellular function.[1, 2] After ischemia, the ensuing inflammatory response[2-6] and generation of cytotoxic products, in particular oxygen-derived free radicals,[2, 7, 8] are believed to be

important mediators of myocardial injury associated with reperfusion.

Experimental data suggest that interventions designed to inhibit formation and release of cytotoxic metabolites may attenuate reperfusion injury[5, 9-12] and accelerate recovery of impaired contractile function of postischemic stunned myocardium.[1, 8, 13, 14] Interestingly, recent experimental work also suggests that interventions targeted at promoting myocardial gene expression for an endogenous myocardial cytoprotective element, heat-shock proteins (HSPs), may also effectively attenuate myocardial reperfusion injury.[15-27]

HSPs, a family of proteins differentiated by molecular weight (e.g., HSP60, HSP70, HSP90), are constitutively expressed in virtually all organisms by a wide variety of cells. HSPs are known to function as molecular chaperons in normal cellular processes and are involved in facilitating protein folding, the assembly of macromolecular protein complexes, and protein trafficking among intracellular compartments.[28-30] HSPs also act to ensure cell membrane stability and to facilitate renaturation of denatured proteins and degradation of irreversibly damaged proteins.[31, 32]

Exposure of cells to mild environmental or metabolic stress induces increased HSP synthesis. The induction of HSPs conveys a protection to the cell against subsequent stress of greater severity. As an example, induction of HSP by prior exposure of cells to a mild sublethal heat shock results in the development of an acquired thermotolerance whereby cells are capable of surviving subsequent exposure to an otherwise lethal heat stress.[33, 34] Messenger ribonucleic acid (RNA) coding for HSP and HSP, in particular the HSP70 family, are induced in myocardial cells in response to a wide variety of cellular stresses including heat,[35] pressure or volume overload,[36] myocardial stretch,[37] hypoxia,[38] and ischemia.[39]

The induction of HSPs by one type of environmental or metabolic insult conveys a degree of cellular protection or cross-tolerance to subsequent insults of a different type, suggesting the HSP response may represent a generalized cellular defense mechanism. This possibility has stimulated research in the area of regulation of HSP synthesis as an endogenous myocardial protective mechanism against oxidative stress resulting from ischemia and reperfusion injury. In cell culture[25] and in isolated buffer-perfused hearts pretreated with heat shock,[15, 16, 22, 26] increased HSP expression results in cellular resistance to metabolic stress resulting from ischemia and reduction in myocardial infarct size. Heat shock–mediated myocardial protection in the intact circulation has not been as well characterized,[19, 21, 23, 24] however. In addition, the effect of prior HSP induction on the functional recovery of viable but stunned postischemic reperfused myocardium is not known.

For these reasons, we undertook a study to test the hypothesis that induction of the heat-shock response and expression of myocardial HSP70 before ischemia may have an important effect on attenuating myocardial stunning in the intact circulation. Such a finding would suggest that regulation of myocardial gene expression represents a novel and potentially useful strategy of myocardial protection.

Methods

Experimental protocol

Heat-shock delivery. Sixteen adult mongrel dogs (mean weight 27 ± 3 kg) were anesthetized with methohexital sodium (Brevitol, 12.5 mg/kg), intubated, and their lungs ventilated with oxygen and halothane 1.5%. Under sterile conditions, the right femoral artery and vein were exposed via a 3 cm skin incision. Heparin (100 U/kg) was administered and the femoral artery and vein were each cannulated (14F cannula, C.R. Bard, Inc., Billerica, Mass.). Partial arteriovenous extracorporeal bypass at 0.5 L/min was initiated with a circuit consisting of a centrifugal pump (Bio-Pump, Medtronic Bio-Medicus, Eden Prairie, Minn.) and an in-line heat exchanger (Avecor, Plymyth, Minn.) and primed with 250 ml of Plasma-Lyte solution. Dogs were selected in random order to undergo either hyperthermic (heat-shocked) or normothermic (nonheat-shocked) extracorporeal bypass. In eight dogs (heat-shocked) core body temperature was elevated to 42° C for 15 minutes. Induction of systemic hyperthermia was aided by use of a warming blanket and ventilation with warmed (38° C) gas. Temperature of blood exiting the in-line heat exchanger was monitored and did not exceed 42° C. After delivery of heat shock, core body temperature was reduced to 37° C. The other eight dogs (nonheat-shocked) were subjected to an identical protocol except that core temperature was kept at 37° C during extracorporeal bypass of an equivalent, matched duration. In all dogs arterial blood gases and arterial pressure were monitored during partial arteriovenous bypass with maintenance of arterial oxygen tension greater than 70 mm Hg and mean arterial pressure greater than 70 mm Hg by adjusting bypass flow. After termination of bypass, cannulas were removed, vessels repaired, the groin incision closed, and protamine (1 mg/kg) administered. Dogs recovered from anesthesia and were cared for after the operation. They received intramuscular torbugesic 0.5 mg for pain and intramuscular bicillin 1.25 million units.

Myocardial ischemia and reperfusion. The next day, dogs were returned to the laboratory, anesthetized with pentobarbital (30 mg/kg intravenously), intubated, and mechan-

ically ventilated with oxygen. Through a left thoracotomy, dogs were instrumented with hydraulic occluders around the superior and inferior venae cavae and around the proximal left circumflex coronary artery. A micromanometer (model PC-350, Millar Instruments, Inc., Houston, Tex.) was passed into the left ventricular apex via a 0.5 cm incision in the apex to record instantaneous left ventricular pressure, an ultrasonic flow probe (inner diameter 2 mm, Transonic Systems Inc., Ithaca, N.Y.) was placed around the circumflex artery distal to the occluder to measure circumflex coronary blood flow, and a pair of miniature subepicardial pulse-transit ultrasonic dimension transducers (1.5 mm outer diameter, Physiological Monitoring Systems Group, Durham, N.C.) were positioned approximately 1 cm apart in the distribution of the circumflex artery and connected to a sonomicrometer (Davis Associates, Durham, N.C.) to record instantaneous myocardial segment dimension. The sinus node was crushed and the right atrium was paced at a constant rate. Exactly 24 hours after the initiation of partial extracorporeal bypass, the left circumflex artery was occluded by inflation of the hydraulic occluder after administration of lidocaine (1 mg/kg intravenously). Circumflex occlusion was maintained for 15 minutes, the occluder was then released, and the heart was reperfused for 3 hours. Zero flow during circumflex occlusion and restoration of flow after occluder release were confirmed by flow probe measurement. At the conclusion of 3 hours' reperfusion, dogs were killed by deep barbiturate anesthesia and the hearts were excised.

All animals received humane care in compliance with the "Principles of Laboratory Animal Care" formulated by the National Society for Medical Research and the "Guide for the Care and Use of Laboratory Animals" prepared by the Institute of Laboratory Animal Resources and published by the National Institutes of Health (NIH publication No. 86-23, revised 1985). The procedures and handling of animals were reviewed and approved by the Institutional Animal Care and Use Committee of the Mayo Foundation.

Data acquisition and analysis

Myocardial contractile function. Left ventricular pressure, myocardial segment dimension, and coronary flow data were acquired at baseline before circumflex artery occlusion, during occlusion, and during 3 hours of reperfusion. At each data acquisition, static data were recorded and data were also recorded over a range of left ventricular end-diastolic volumes produced by transient (5 to 10 second) vena caval occlusion for 10 to 25 cardiac cycles. Physiologic data were filtered with a 50 Hz low-pass analog filter and digitized at an eight-channel sweep speed of 200 Hz by an analog to digital converter (model 5025MF, ADAC, Woburn, Mass.). The analog to digital conversion time per channel was 30 msec, creating a phase delay between channels of less than 4.5 degrees. After data were collected and stored on a hard disk by a personal computer (Reason Technology Inc., Minneapolis, Minn.), data analysis was accomplished on a microcomputer (DEC, Vaxstation 3100, Maynard, Mass.) with interactive software (Davis Associates, Durham, N.C.) and software developed in our laboratory. The first derivative of left ventricular pressure was determined from a running five-point polyorthogonal transformation of the digitized ventricular pressure waveform. Cardiac cycles were automatically defined on the basis of dP/dt criteria. Regional left ventricular systolic contractile function was quantified by means of the linear and load-independent preload recruitable stroke work (SW) relationship, an analysis of ventricular myocardial segment ability to generate SW as a function of end-diastolic length (EDL). This analysis is a highly sensitive, useful parameter to assess myocardial dysfunction after acute ischemic injury, as previously described in detail.[40-42] In brief, regional left ventricular SW values were calculated from instantaneous pressure (P) and dimension (L) data for each cardiac cycle as follows:

$$SW = \int P \cdot dL \qquad (1)$$

Data from each vena caval occlusion were fitted to the equation:

$$SW = M_w (EDL - L_w) \qquad (2)$$

relating SW to EDL where M_w is the slope and L_w is the x-axis intercept. Preload recruitable work area (PRWA) was defined as the area under the SW versus EDL regression line and was calculated as:

$$PRWA = M_w/2 (L_{w\,max} - L_w)^2 \qquad (3)$$

where $L_{w\,max}$ was the maximal x-intercept value obtained for a given myocardial segment over the entire experiment. Changes in ventricular contractile function are reflected as changes in M_w and L_w and by changes in PRWA, which reflects changes in both slope, M_w, and x-intercept, L_w (Fig. 1). For each dog, M_w, L_w, and PRWA were determined from equations 2 and 3 for each vena caval occlusion. Mean values were calculated for three to five vena caval occlusions performed at baseline before ischemia produced circumflex artery occlusion and after ischemia at 15, 30, 60, 90, 120, 150, and 180 minutes of reperfusion. Standard hemodynamic data (heart rate, left ventricular peak pressure, end-diastolic pressure, first derivative of left ventricular pressure, and circumflex artery flow) were also acquired.

Myocardial HSP determination. From excised hearts, transmural myocardial tissue samples from the distribution of the left circumflex artery were obtained, rapidly dissected free of epicardial fat and visible coronary vessels, and immediately placed in a −70° C freezer until subsequent analysis. The extent of cellular expression of HSP was quantified by optical densitometry of Western blots using a primary antibody specific for HSP70 employing conventional techniques previously described in detail.[43, 44] In brief, tissue samples were weighed and soluble tissue homogenates were prepared from approximately 0.5 gm of tissue diluted in eight equivalent volumes of buffer containing Tris 50 mmol/L, ethylenediaminetetraacetic acid, 1 mmol/L, thioglycerol 10 mmol/L, and phenylmethylsulfonyl fluoride 1 mmol/L at pH 7.50. Equivalent volumes of cytosol (10 μl) were analyzed, which contained 50 to 60 μg of total protein. Resultant comparisons were based on tissue wet weight. For Western blotting, sodium dodecyl sulfate–polyacrylamide gel electrophoresis gels equilibrated in Tris-glycine transfer

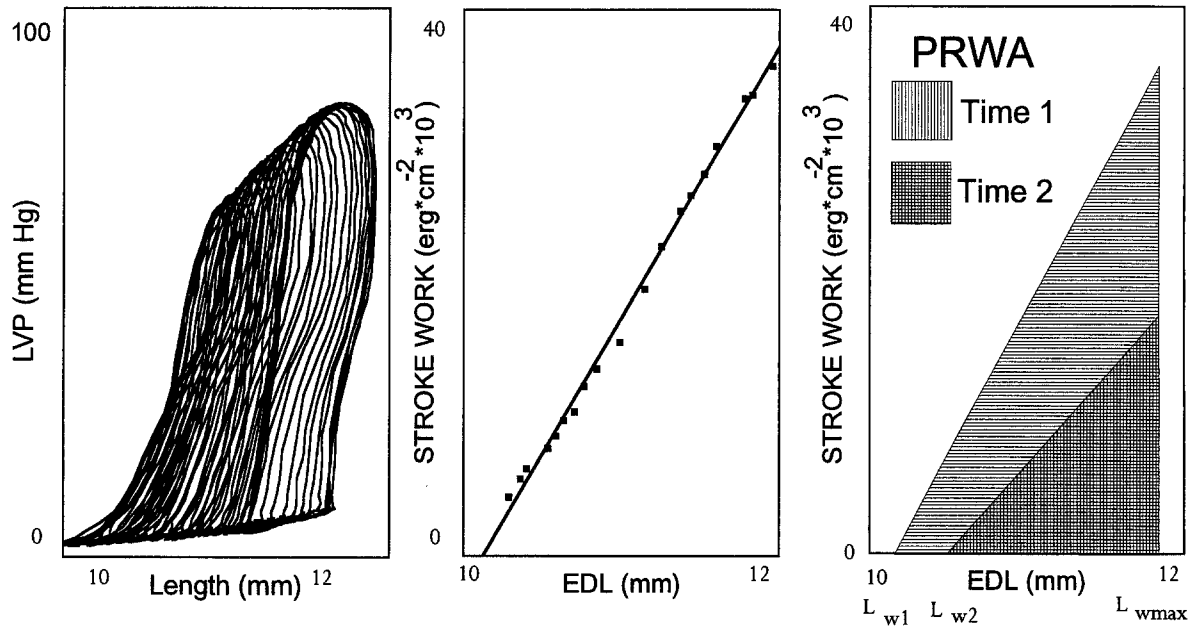

Fig. 1. *Left panel,* Characteristic plot of instantaneous left ventricular transmural pressure *(LVP)* and myocardial segment dimension in the region of the circumflex artery for cardiac cycles over a range of end-diastolic volumes produced by transient vena caval occlusion. *Center panel,* Expressing left ventricular regional SW as a function of end diastolic length *(EDL)* for each cardiac cycle (expressed by equations 1 and 2 in text) yields a highly linear relationship precisely quantifiable by a slope, M_w, and x-axis intercept, L_w. *Right panel,* Computation of preload recruitable work area *(PRWA)* as area under segmental SW versus EDL relationship (expressed by equation 3 in text) for two different SW versus EDL relationships in same dog at different times. L_{w1} and L_{w2} are relationship x-intercepts for individual study times and L_{wmax} is maximal value of L_w for entire experiment.

buffer (Tris 0.5 mol/L, glycine 3.84 mol/L, 0.01% sodium dodecyl sulfate, and 20% methanol, pH 8.30) were electrophoretically transferred to Immobilon-P membranes (IPVH 15150, Millipore, Bedford, Mass.) after a two-stage protocol in an electrophoresis unit (TE Series Transphor, Hoefer Scientific Instruments, San Francisco, Calif.). After transfer, the membranes were placed in Western buffer (Tris 20 mmol/L, NaCl 150 mmol/L, 0.5% Tween 20 polysorbate, and 1% powdered milk, pH 7.40) to blot at room temperature for 1 hour. After this, the Western buffer was replaced with 25 ml of Western buffer containing the primary antibody to the inducible form of HSP70 (SPA-810, 10 μg/ml, Stressgen, Victoria, B.C.) and incubated for 1.5 hours at room temperature. After the primary antibody incubation, the membrane was washed in 30 ml Western buffer, then placed in Western buffer containing the second antibody, goat antimouse immunoglobin G alkaline phosphatase (Southern Biotech, Pittsburgh, Pa.), diluted 1:500, and incubated for 30 minutes. The membranes were then washed and stained to the desired intensity with 25 ml of NBT-BCIP (0.033% Nitroblue tetrazolium, 0.033% 5-bromo-4-chloro-3-indolyl phosphate, 0.31% dimethylformamide). The proportions of isolated proteins were then quantified by densitometry (CS9000U Scanner, Shimadzu, Kyoto, Japan). Band intensities on Western blots run simultaneously were measured directly from blot reflectance.

Western blotting assay for HSP70 was shown to be linear within a range of 0 to 1.0 μg as determined by analysis of HSP70 purified from chicken liver and quantitated with bovine serum albumin as a standard. Because total HSP70 comprises 1% of total protein, of which the inducible form is no more than 50%, tissue sample amounts tested were in the linear range.

All data are presented as mean ± standard deviation except as otherwise noted. Statistical analyses were performed by means of paired and unpaired *t* tests and one- and two-way analysis of variance for repeated measures with the Newman-Keuls test to localize differences as appropriate. Statistical significance was accepted at $p < 0.05$.

Results

Heat-shock delivery. The mean duration of partial arteriovenous bypass required to elevate core temperature to 42° C in heat-shocked dogs was 30 ± 7 minutes. Mean time required to reduce temperature to 37° C after delivery of heat shock was 15 ± 4 minutes. For all dogs the mean total duration of

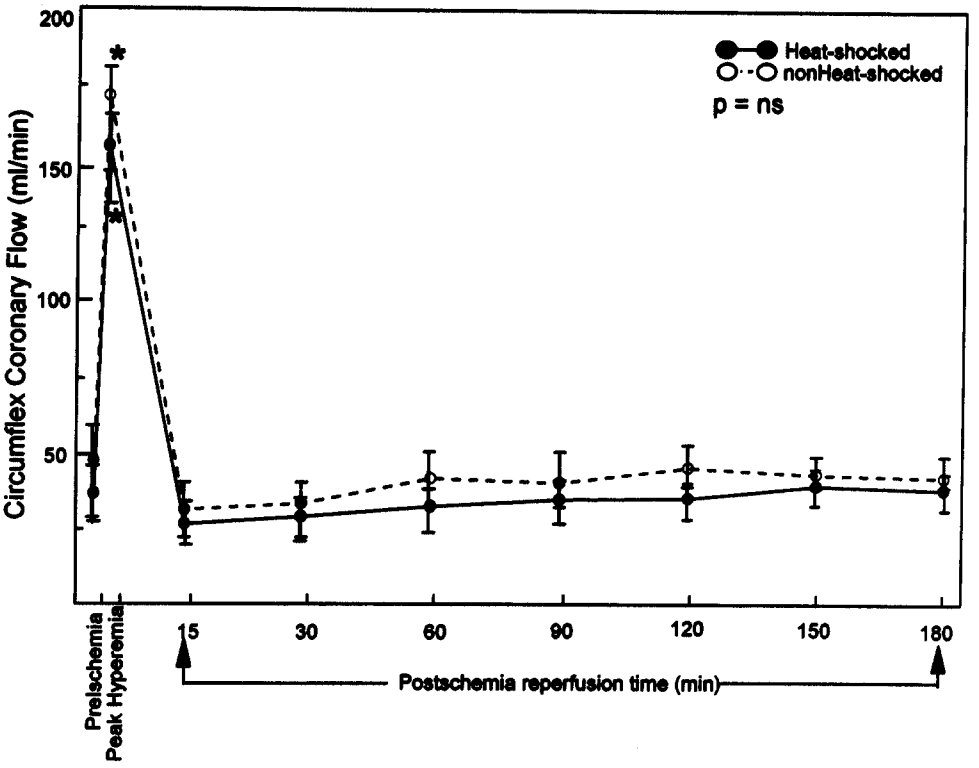

Fig. 2. Effects of circumflex artery occlusion and reperfusion on circumflex coronary blood flow in heat-shocked and nonheat-shocked dogs. Values are mean ± standard error of the mean. (*$p < 0.05$ versus preischemic value determined by one-way analysis of variance for repeated measures. Difference between heat-shocked and nonheat-shocked dogs tested by two-way analysis of variance for repeated measures.) *ns,* Not significant.

partial bypass was 64 ± 14 minutes and there was no difference (p = NS*) in duration of bypass in heat-shocked and nonheat-shocked dogs.

Circumflex coronary artery blood flow. At baseline before circumflex artery occlusion, mean circumflex coronary blood flow was 37 ± 9 ml/min in heat-shocked dogs and 46 ± 24 ml/min in nonheat-shocked dogs (p = NS). After release of the circumflex artery occlusion, peak hyperemic reperfusion flow was 154 ± 33 ml/min in heat-shocked dogs and 171 ± 57 ml/min in nonheat-shocked dogs (p = NS). Circumflex coronary blood flows did not differ (all p = NS) at any time during reperfusion in heat-shocked and nonheat-shocked dogs (Fig. 2).

Hemodynamics before and during ischemia and reperfusion. During partial arteriovenous bypass for heat-shocked and nonheat-shocked dogs, mean values for heart rate were 153 ± 29 beats/min and 116 ± 28 beats/min ($p < 0.01$); for mean arterial pressure, 79 ± 9 mm Hg and 87 ± 6 mm Hg ($p < 0.05$); and for bypass flow rate, 0.86 ± 0.03 L/min and 0.75 ± 0.01 L/min ($p < 0.01$), respectively.

As summarized in Fig. 3, the effects of circumflex artery occlusion and reperfusion on heart rate, left ventricular peak pressure, left ventricular end-diastolic pressure, and left ventricular dP/dt were similar (all p = NS) in heat-shocked and nonheat-shocked dogs before and during myocardial ischemia and reperfusion, indicating no differences in global hemodynamics in the two groups.

Regional myocardial contractile function. At baseline before circumflex artery occlusion, myocardial function did not differ in heat-shocked and nonheat-shocked dogs. The slope, M_w, and x-intercept, L_w, of the preload recruitable SW relationship were similar (both p = NS) in heat-shocked and nonheat-shocked groups (Table I).

In both heat-shocked and nonheat-shocked dogs, 15 minutes of circumflex artery occlusion resulted in significant depression of myocardial contractile

*NS = Not significant.

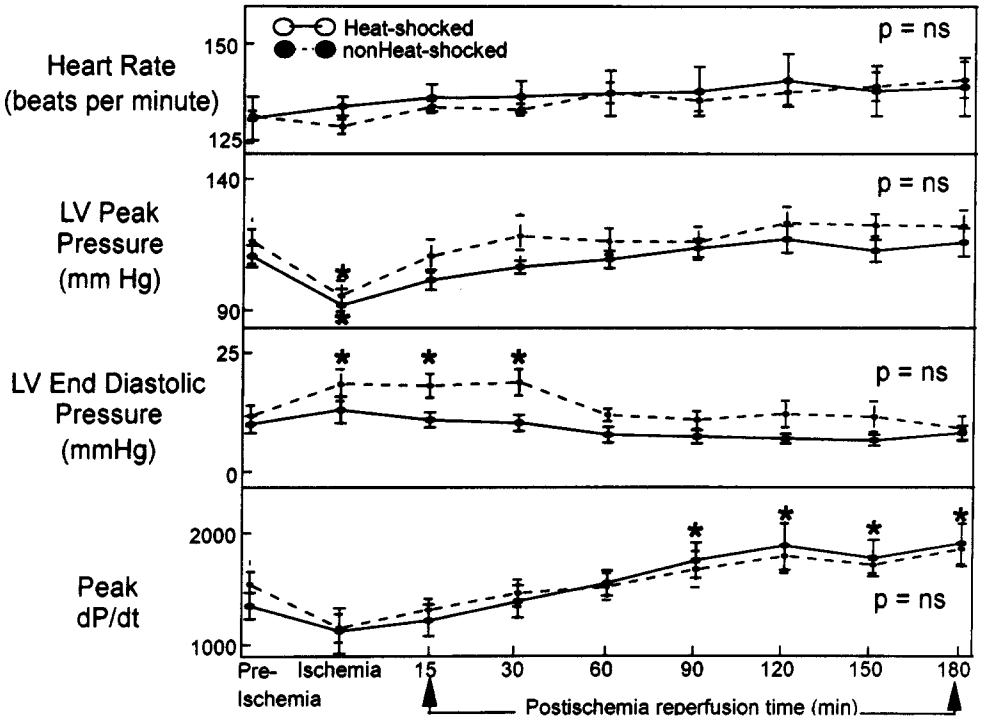

Fig. 3. Effects of circumflex artery occlusion and reperfusion on heart rate and left ventricular *(LV)* peak pressure, end-diastolic pressure, and peak first derivative of left ventricular pressure in heat-shocked and nonheat-shocked dogs. Values are mean ± standard error of the mean. (*$p < 0.05$ versus preischemic value determined by one-way analysis of variance for repeated measures. Difference between heat-shocked and nonheat-shocked dogs tested by two-way analysis of variance for repeated measures.)

Table I. *Effects of circumflex artery occlusion and reperfusion on myocardial contractile function quantified by slope, M_w, x-intercept, L_w, and area (PRWA) of the preload recruitable stroke work relationship*

		Reperfusion							
	Preischemia	*15 min*	*30 min*	*60 min*	*90 min*	*120 min*	*150 min*	*180 min*	*p Value*
M_w (erg · cm^{-3} · 10^3)									
Heat-shocked dogs	94 ± 26	74 ± 23*	83 ± 28	97 ± 39	114 ± 37	118 ± 49	135 ± 49	149 ± 58	p = NS
Nonheat-shocked dogs	93 ± 39	55 ± 21*	73 ± 40	105 ± 63	112 ± 55	137 ± 76	124 ± 51	129 ± 50	
L_w (mm)									
Heat-shocked dogs	10.7 ± 3.6	11.6 ± 3.5*	11.6 ± 3.5*	11.7 ± 3.6*	11.5 ± 3.5*	11.4 ± 3.4*	11.4 ± 3.4*	11.5 ± 3.5*	$p < 0.008$
Nonheat-shocked dogs	12.2 ± 3.7	13.5 ± 4.1*	13.7 ± 3.9*	13.9 ± 4.0*	13.8 ± 4.0*	13.7 ± 4.0*	13.7 ± 4.0*	13.6 ± 4.0*	
PRWA									
Heat-shocked dogs	100	32 ± 8*	32 ± 13*	38 ± 14*	57 ± 24*	69 ± 40*	76 ± 34	80 ± 38	$p < 0.0001$
Nonheat-shocked dogs	100	19 ± 15*	16 ± 13*	16 ± 15*	21 ± 12*	27 ± 13*	31 ± 15*	33 ± 13*	

Values are mean ± standard deviation. PRWA is expressed as percent of preischemic value.

*$p < 0.05$ versus preischemia determined by one-way analysis of variance for repeated measures. Differences between heat-shocked and nonheat-shocked dogs tested by two-way analysis of variance for repeated measures.

function, reflected as a reduction in M_w ($p < 0.01$) and rightward shift of L_w ($p < 0.01$) with a resultant marked reduction in PRWA (Table I). Circumflex artery occlusion reduced PRWA to 32% ± 8% of the preischemic control value in heat-shocked dogs ($p < 0.001$) and to 19% ± 15% of the preischemic control value in nonheat-shocked dogs ($p < 0.001$) at 15 minutes' reperfusion.

The decrease in M_w and rightward shift of L_w measured after ischemia at 15 minutes' reperfusion

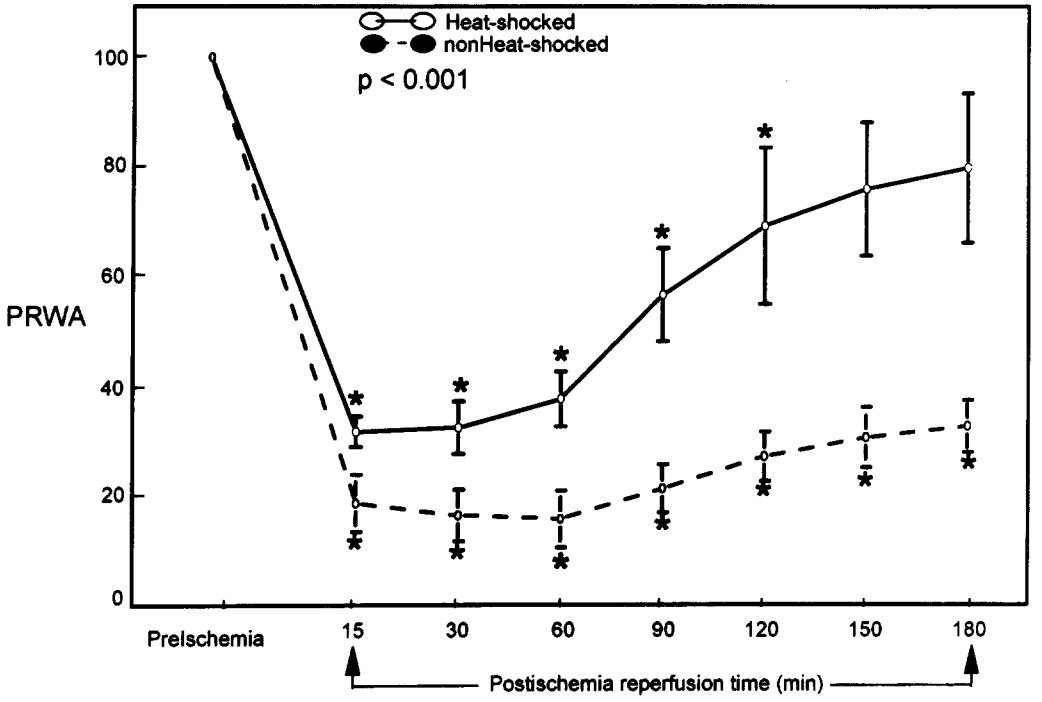

Fig. 4. Effects of circumflex artery occlusion and reperfusion on myocardial contractile function quantified by preload recruitable work area *(PRWA)* expressed as percent of preischemic control values in heat-shocked and nonheat-shocked dogs. Values are mean ± standard error of the mean. (*$p < 0.05$ versus preischemic value determined by one-way analysis of variance for repeated measures. Difference between heat-shocked and nonheat-shocked dogs tested by two-way analysis of variance for repeated measures.)

were similar (both p = NS) in heat-shocked and nonheat-shocked dogs. The decrease in PRWA measured at 15 minutes' reperfusion was also similar (p = NS) in heat-shocked and nonheat-shocked dogs, indicating a similar initial degree of ischemic injury measured during early reperfusion in the two groups of dogs (Table I).

During subsequent reperfusion, however, there was a markedly accelerated recovery of contractile function in the heat-shocked group compared with the nonheat-shocked group ($p < 0.001$) (Fig. 4). By 3 hours' reperfusion, PRWA returned to 80% ± 38% of preischemic control values in heat-shocked dogs but only to 33% ± 13% of preischemic control values in nonheat-shocked dogs. After 2 hours' reperfusion, PRWA in heat-shocked dogs did not differ significantly from the control preischemic value. In nonheat-shocked dogs, PRWA remained significantly depressed below preischemic values even at 3 hours' reperfusion. Recovery of contractile function in the heat-shocked dogs was attributable to recovery of M_w and a leftward shift of L_w back toward preischemic control values (Table I), whereas prolonged impairment of contractile function in nonheat-shocked dogs was principally attributable to a persistent rightward shift of L_w.

Myocardial HSP70. Induction of myocardial HSP70 expression was increased in heat-shocked dogs compared with nonheat-shocked dogs (108 ± 27 versus 71 ± 14 densitometry units, $p < 0.005$) (Figs. 5 and 6).

Discussion

These data demonstrate that in the intact canine model of regional myocardial ischemia and reperfusion, the heat-shock response with subsequent increased expression of myocardial HSP70 conferred a significant protection to myocardium subjected to a 15-minute period of coronary artery occlusion and 3 hours of reperfusion. Induction of myocardial HSP by a period of hyperthermic partial extracorporeal bypass 24 hours before ischemia was associated with markedly improved contractile function of postischemic myocardium. Increased expression of

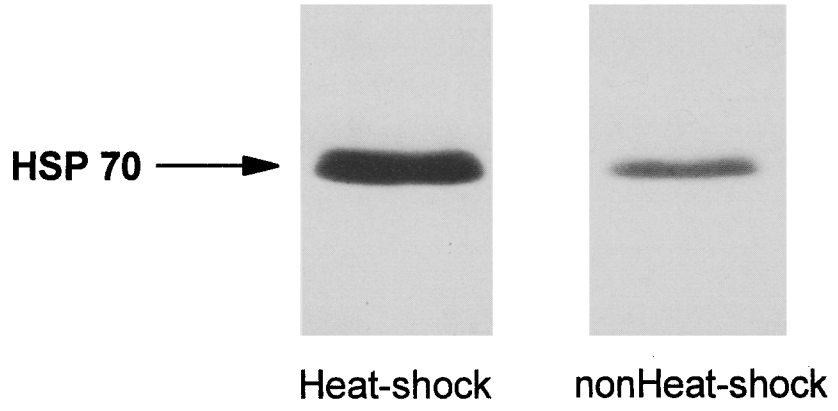

Fig. 5. Western blot depicting levels of heat-shock protein *(HSP70)* in myocardial samples obtained from the circumflex region of the left ventricle in representative heat-shocked and nonheat-shocked dogs. The protein band representing HSP70 is indicated *(arrow)*. There was greater induction of HSP70 in the heat-shocked dog.

myocardial HSP70 was not associated with a measurable difference in baseline, preischemic myocardial contractile function. Induction of myocardial HSP70 also did not significantly alter the immediate marked reduction in myocardial contractile function after transient coronary occlusion measured at 15 minutes' reperfusion, suggesting no attenuation of myocardial injury occurring during the ischemic period. During subsequent reperfusion, however, there was accelerated recovery of contractile function associated with elevated myocardial HSP70 levels in heat-shocked dogs compared with prolonged contractile dysfunction associated with lower myocardial HSP70 levels observed in nonheat-shocked dogs. This difference in myocardial functional recovery was not related to differences in coronary blood flow during reperfusion in the two groups of dogs or to differences in global hemodynamics during ischemia and reperfusion. These observations suggest that heat-shock induction of myocardial HSP70 expression before ischemia accelerated recovery of postischemic stunned myocardium by attenuation of myocardial reperfusion injury.

These results demonstrating the cardioprotective effects of heat shock–induced myocardial HSP70 expression in the intact circulation are consistent with findings of several investigations in different experimental models. Heat shock–induced HSP-mediated cross-tolerance to myocardial ischemia and reperfusion has been demonstrated in the isolated buffer-perfused rat and rabbit heart. Whole-body heat-shocked hearts showed enhanced postischemic recovery of left ventricular developed pressure, reduced creatine kinase release, reduced accumulation and release of oxidized glutathione, a marker of oxidative stress, and better preservation of both high-energy phosphate stores and mitochondrial respiratory control index, as well as a reduction in myocardial infarct size[15, 16, 22, 26] in response to a subsequent in vitro ischemic insult.

The effects of whole-body heat shock on inducing a cross-tolerance to myocardial ischemia and reperfusion injury in vivo has not previously been as well established, and results of recent investigations are somewhat incongruent.[19, 21, 23, 24] The disparate results of prior in vivo studies may be largely attributable to differences in experimental models, however. In the rat and rabbit heart, whole-body heat shock was shown to be protective, reducing infarct size resulting from subsequent 30-minute[24] and 35-minute in vivo ischemic periods.[19, 27] However, heat shock did not protect against a subsequent 45-minute in vivo ischemic period.[21] Heat shock confers in vitro myocardial protection for as long as 96 hours after the heat-shock event,[45] but in vivo heat shock–mediated protection appears more limited in duration. Reduction in infarct size is observed at 24 hours[19, 24] but not at 40 hours after heat-shock delivery.[24] Increased HSP messenger RNA may be detected as early as 1 hour and increased HSP70 as early as 2 hours after the shock event,[17] but the minimum time required for heat shock to convey in vivo myocardial protection has not been defined. At present, in vivo heat shock–mediated myocardial protection appears to be transient and the degree of protection is limited with more severe degrees of ischemic injury. The effect of

HSP induction on myocardial stunning has not previously been well characterized.

Cognizant of these factors, we designed the present study using a 24-hour interval between the heat-shock event and the ischemic period. We used a brief period of hyperthermic extracorporeal bypass for elevation of core temperature to 42° C for 15 minutes, a method with potential clinical application, rather than the method of transcutaneous whole-body heating previously used in smaller animal models. This intensity of hyperthermia has been shown to induce HSP expression whereas lower temperatures (40° to 41° C) do not.[27] Our method of heat shock resulted in a 44% increase in myocardial HSP70 measured approximately 27 hours later. We also chose to use an ischemic period limited to 15 minutes, a duration of ischemia known to cause no myocardial necrosis but to result in prolonged systolic contractile dysfunction that persists for as long as 24 hours of reperfusion.[42] The intent of this study was not to assess reduction of myocardial infarct size, a marker of severe and irreversible ischemic injury. Rather, our intent was to assess functional recovery of stunned myocardium, which represents a reversible ischemic and reperfusion injury. Transient coronary occlusion of 15 minutes' duration followed by reperfusion in the intact dog is a widely used and well-characterized[40, 42] experimental model of myocardial stunning. Precise quantification of the regional contractile dysfunction and recovery in this model using the load-independent preload recruitable work relationship has also previously been described.[40, 42] The use of such a practical, load-insensitive, and responsive functional parameter is crucial for detailed analysis of myocardial response to ischemia and reperfusion.

This study clearly demonstrated an association between accelerated recovery of postischemic stunned myocardium and heat-shock induction of myocardial HSP70. However, important questions remain to be addressed to completely define the mechanism and role of HSP in mediating myocardial protection in the intact circulation. The exact molecular basis of the apparent cytoprotective effects of HSP70 in postischemic myocardium has not yet been defined and is not apparent from this investigation. Increasing evidence indicates oxygen-derived free radicals directly contribute to the pathogenesis of prolonged contractile dysfunction after transient regional or global myocardial ischemia in the absence of myocardial cell death.[1, 2, 5, 6, 8-14] Free radicals generated by ischemia and reperfusion events are thought to damage cellular and subcellular compartment membranes, enzyme systems, and nuclear, nucleolar, and ribosomal proteins resulting in myocellular dysfunction. The cardioprotective mechanism of HSP70 may be similar to the known functions of HSPs as molecular chaperons in normal cells.[28-32] HSP70 may stabilize and facilitate repair of damaged proteins, membranes, and other macromolecular complexes and assist in oligomerization and translocation of newly synthesized proteins. The heat-shock response is also known to result in other cellular alterations including increased intracellular levels of catalase, adenosine triphosphate, calcium, and pH,[15, 45-47] which may have additional cardioprotective effects.

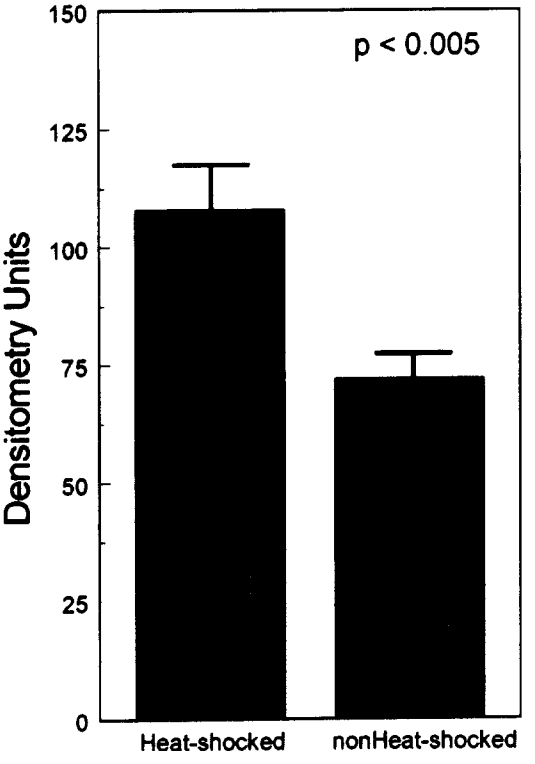

Fig. 6. Densitometric quantification of myocardial HSP70 in myocardial samples obtained from the circumflex region of the left ventricle in heat-shocked and nonheat-shocked groups of dogs. Values are mean ± standard error of the mean. (Difference between heat-shocked and nonheat-shocked dogs tested by unpaired *t* test.)

A cause and effect relationship between HSP70 induction and accelerated recovery of stunned myocardium is also not established by the present study. The delivery of whole-body heat shock likely resulted in other effects in addition to induction of myocardial HSP70 expression. These other effects may

have accounted for the observed accelerated postischemic myocardial functional recovery attributed to myocardial HSP70 induction. Noncardiac cells such as neutrophils and coronary vascular endothelial cells, important mediators of reperfusion injury,[1,2] were also heat shocked, which may have induced alterations in the coronary vascular and inflammatory responses to ischemia and reperfusion.

However, other work has more directly established a cytoprotective function of HSP during ischemia and metabolic stress. Monoclonal antibodies against HSP70 induced in response to heat shock have been shown to abolish the acquired cellular tolerance to thermal stress.[48] There is also an observed direct correlation between the amount of myocardial HSP induced and the extent of myocardial protection assessed by reduction in myocardial infarct size after coronary artery occlusion and reperfusion in the in vivo rat model.[27] Direct evidence of the cytoprotective function of HSP70 is provided by the observation that forced expression of a human HSP70 transgene in mammalian cell culture results in increased HSP70 and confers cellular resistance to metabolic stresses similar to those induced by ischemia.[25]

In summary, this study demonstrated that a brief period of hyperthermic extracorporeal bypass induced expression of myocardial HSP70. Induced expression of HSP70 was associated with accelerated functional recovery of stunned myocardium. Further research is necessary to define more clearly the exact role and mechanism of HSP-mediated myocardial protection in the intact circulation. However, these findings indicate that interventions targeted at myocardial gene regulation of endogenous molecular cytoprotective elements, such as shock proteins, may markedly attenuate ischemic and reperfusion injury. This suggests the intriguing possibility that pharmacologic or genetic approaches to promote expression of shock proteins may represent a novel and potentially useful strategy for molecular myocardial protection and treatment of cardiac disease.

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Discussion

Dr. David M. Follette *(Sacramento, Calif.).* Dr. Robinson is the first to perform an in vivo study. A fair amount of work has been accumulated on HSP, but these are all in vitro studies and not in a normal physiologic situation. It is also important that she has introduced to us the concept of the potential for gene therapy in cardiac surgery. This is a very important topic in tumor biology and other areas, and I was very pleased to have seen a novel application with respect to what we do.

Dr. Robinson focused on ischemia rather than necrosis. Many of the prior studies that have been done have concentrated on prevention of necrotic areas, but her study simply focused on the ability of an area that is ischemic to recover its function.

I have several questions for Dr. Robinson. Because in the study the only significant data points are related to the segmental wall motion, could you amplify on the measurement of the PRWA, especially knowing that in the dog the coronary anatomy is such that it can be difficult to exactly quantify a regional area of ischemia?

Dr. Robinson. Regional preload recruitable work is a parameter that has been extensively characterized and validated as a load-independent descriptor of myocardial contractile performance. In all cases we were certain to measure regional contractile performance in myocardial areas immediately adjacent to epicardial coronary arteries of either the ischemic or nonischemic regions.

Dr. Follette. One of the mechanisms that has been proposed and that you mentioned for the effect of HSP is the protective effects on oxygen free radicals. Have you had an opportunity to measure oxidized glutathione or any other free radicals to shed some light on this hypothesis?

Dr. Robinson. That is part of my thesis. In the future we plan to characterize more completely free radical production, neutrophil function, and coronary vascular endothelial cell function in heat shock–associated myocardial protection. In terms of the mechanism by which HSPs seem to function by stabilizing cell membranes and by facilitating renaturation of damaged proteins, perhaps HSPs also act to attenuate free radical–mediated damage during reperfusion.

Dr. Follette. We will look forward to your thesis not only with respect to the free radicals but also to the endothelial releasing factor and other changes.

Other forms of stress can lead to the release of HSP, such as hypoxia, ischemia, pressure and volume overload, and certain pharmacologic agents. Have you tested other ways to induce the HSPs that are not as severe as pretreatment with severe hyperthermia?

Dr. Robinson. Both pressure and volume overload, ischemia, myocardial stretch, different pharmacologic agents such as amphetamines and cadmium induce expression of HSPs. Heating is a means to produce rapidly a large amount of HSPs and has previously been used in other experimental models. Pharmacologic or genetic means of producing chronic up-regulation of inducible HSP expression may represent an important therapeutic option in the future and warrant further investigation.

Dr. Follette. A lot of attention is focused on ischemic preconditioning. I would like to thank you for introducing us to the concept that gene manipulations may play an additional role in ischemic preconditioning and protection of the myocardium.

Dr. William Brenner *(Hackensack, N.J.).* I am not a veterinarian but I do own dogs, and I am aware that their temperature is higher than 37° C normally. Perhaps it is more appropriate that you call your control dogs cold-shocked dogs rather than nonheat-shocked dogs.

Also, I think this may have exaggerated the differences in the outcome, but I do not know if that is the fact. Correct me if I am wrong.

Dr. Robinson. The amount of HSP in dogs subjected to normothermic bypass was the same as in briefly anesthetized dogs not subjected to bypass of any time.

Dr. Brenner. Clinically, because most cardiac surgery is coronary revascularization, the heat stress to induce HSP would exaggerate ischemia by increasing oxygen demand. I am concerned and wonder about its clinical application.