Hypothermia preserves function and signaling for mitochondrial biogenesis during subsequent ischemia

XUE-HAN NING, CHENG-SU XU, YING C. SONG, YUN XIAO, YING-JIA HU, FLAVIAN MARK LUPINETTI, AND MICHAEL A. PORTMAN
Cardiology Division, Department of Pediatrics and Cardiovascular Surgery Division, Department of Surgery, University of Washington, Seattle 98195; and Children's Hospital and Regional Medical Center, Seattle, Washington 98105

Ning, Xue-Han, Cheng-Su Xu, Ying C. Song, Yun Xiao, Ying-Jia Hu, Flavian Mark Lupinetti, and Michael A. Portman. Hypothermia preserves function and signaling for mitochondrial biogenesis during subsequent ischemia. Am. J. Physiol. 274 (Heart Circ. Physiol. 43): H786–H793, 1998.—Hypothermia is known to protect myocardium during ischemia, but its role in induction of a protective stress response before ischemia has not been evaluated. As cold incites stress responses in other tissues, including heat shock protein induction and signaling mitochondrial biogenesis, we postulated that hypothermia in perfused hearts would produce similar phenomena while reducing injury during subsequent ischemia. Studies were performed in isolated perfused rabbit hearts (n = 77); a control group (C) and a hypothermic group (H) subjected to decreasing infusate temperature from 37 to 31°C over 20 min. Subsequent ischemia during cardioplegic arrest at 34°C for 120 min was followed by reperfusion. At 15 min of reperfusion, recovery of left ventricular developed pressure (LVDP), maximum first derivative of left ventricular pressure (LV dP/dtmax), LV –dP/dtmax, and the product of heart rate and LVDP was significantly increased in H (P < 0.01) compared with C hearts. Ischemic contracture started later in H (97.5 ± 3.6 min) than in C (67.3 ± 3.3 min) hearts. Myocardial ATP preservation and repletion during ischemia and reperfusion were higher in H than in C hearts. mRNA levels of the nuclear-encoded mitochondrial proteins adenine nucleotide translocase isoform 1 (ANT1) and β-F1-adenosine triphosphatase (β-F1-ATPase) normalized to 28S RNA decreased in C hearts but were preserved in H hearts after reperfusion. Inducible heat shock protein (HSP70–1) mRNA was elevated nearly 4-fold after ischemia in C hearts and 12-fold in H hearts. These data indicate that hypothermia preserves myocardial function and ATP stores during subsequent ischemia and reperfusion. Signaling for mitochondrial biogenesis indexed by ANT1 and β-F1-ATPase mRNA levels is also preserved during a marked increase in HSP70–1 mRNA.

Materials and Methods
Preparation of Isolated Heart
Rabbits (male or female, 2.2–2.7 kg body wt) were anesthetized with pentobarbital sodium (45 mg/kg iv) and heparinized (700 U/kg iv). The heart was rapidly excised and immersed momentarily in ice-cold physiological salt solution (PSS), pH 7.4, containing (in mmol/l) 118.0 NaCl, 4.0 KCl, 22.3 NaHCO3, 11.1 glucose, 0.66 KH2PO4, 1.23 MgCl2, and 2.38 CaCl2. The aorta was cannulated in the Langendorff mode, and the heart was perfused with PSS that had been equilibrated with 95% O2-5% CO2 at 37°C and passed twice through filters with 3.0-µm pore size. Perfusion pressure was maintained at 90 mmHg. An incision was made in the left atrium, and a fluid-filled latex balloon was passed through the mitral orifice and placed in the left ventricle. The balloon was connected to a pressure transducer for continuous measurement of left ventricular pressure (LVP) and its first derivative to heat shock or ischemia have been a major focus of investigation with respect to enhancement of tissue resistance to subsequent ischemia (14, 21, 33, 35, 40). Furthermore, hypothermia either singly or accompanied by cardioplegia is regularly employed in myocardial protection during heart surgery. The operative mechanism is a putative reduction in myocardial high-energy phosphate utilization during ischemia (15). Hypothermic induction of a stress response, when applied before ischemia, has not been investigated in the heart.

In this study, we propose that even a mild, relatively brief exposure to hypothermia can improve resistance to a subsequent prolonged ischemic insult and that this response is associated with an alteration in signaling for mitochondrial biogenesis. As hypothermia can produce alterations in myocardial performance and energy utilization even during subsequent rewarming, cardioplegia was used during ischemia to negate this effect. Studies were performed in a perfused heart preparation, a model that has been used frequently to characterize other stress-related phenomena, including heat shock (33, 35, 40). Cardiac function and ATP preservation were measured to demonstrate that improved ischemic resistance occurred in this model. Additionally, Northern blot analyses of expression of an inducible heat shock protein (HSP70–1) gene (10, 16, 23), as well as genes regulating major constitutive mitochondrial membrane proteins [adenine nucleotide translocase isoform 1 (ANT1) and β-subunit F1-adenosinetriphosphatase (β-F1-ATPase)] (11, 36, 44, 47) were performed to index signaling for mitochondrial biogenesis and cold adaptation.

Cold-induced stress is a phenomenon associated with an increase in inducible heat shock protein expression in various tissues (31, 32). Particularly in brown adipose tissue, cold-induced stress or hypothermia also induces mitochondrial biogenesis (20, 29, 30). At the transcriptional level this is characterized by coordinated increases in expression of nuclear- and mitochondrial-encoded genes regulating mitochondrial membrane proteins (28). Although this signaling has been well characterized in brown adipose fat, it remains relatively unexplored in other mammalian tissues. This is surprising because stress responses in the heart are relatively unexplored in other mammalian tissues.

Copyright © 1998 The American Physiological Society
derivative with respect to time (LV dP/dt). The causal vena cava, the left and right cranial vena cavae, and the ayzygos vein were ligated. The pulmonary artery was cannulated to enable collection of coronary flow, which was measured with a flowmeter (T201, Transonic Systems, Ithaca, NY).

The analog signals were continuously recorded on a pressurized ink-chart recorder (Gould, Cleveland, OH) and an on-line computer (Macintosh, Biopac Analog Signal Acquisition System). To characterize cardiac function, left ventricular developed pressure (LVDP) is defined as peak systolic pressure (PSP) minus end-diastolic pressure (EDP). The product of heart rate and LVDP [pressure-rate product (PRP), mmHg/min] was calculated to provide an estimate of myocardial work. Myocardial O2 consumption (MVO2) was calculated as CF \times [(P_{O2} - P_{O2}) \times (c_{O2}/760)], where CF is coronary flow (ml\cdot min\cdot g wet tissue\(^{-1}\)), (P_{O2} - P_{O2}) is the difference in the partial pressure of O2 (mmHg) between perfusate and coronary effluent, and c_{O2} is the Bunsen solubility coefficient of O2 in perfusate at 37°C (22.7 µl O2 \cdot atm\(^{-1}\) \cdot ml perfusate\(^{-1}\)) (37, 38). O2 extraction was calculated as MVO2 divided by the O2 content in the perfusate. Wet weight of the heart was determined at the conclusion of each experiment after trimming the great vessels and fat and blot drying the heart with nine-layer cotton gauge. Procedures followed were in accordance with institutional and National Institutes of Health guidelines.

### Lactate, pH, and CO2 Measurements

The first 1.5 ml of coronary effluent were collected at ischemic flush time (see Experimental Protocols) and at reflow. Lactate concentration was measured with a GM7 Analyser (Analox micro-Stat, London). The concentrations of O2 and CO2 were measured with a Radiometer (ABL 3, Copenhagen, Denmark). The difference in CO2 content between the coronary outflow and inflow was calculated as (P_{CO2} - P_{CO2}) \times c_{CO2}/V_v, where P_{CO2} - P_{CO2} is the difference in the partial pressure of CO2 (mmHg) between coronary effluent and perfusate, c_{CO2} is the solubility coefficient of CO2 in perfusate at 37°C (0.53 ml CO2 \cdot atm\(^{-1}\) \cdot ml perfusate\(^{-1}\)), and V_m is molar volume of CO2 (22.4 ml CO2 \cdot atm\(^{-1}\) (38). An intramural pH electrode of a Khuri regional tissue pH monitor (Vascular Technology, Chelmsford, MA) was placed in the left ventricular wall between the branches of circumflex and posterior descending arteries, about midway between the base and apex of the heart (n = 6/group).

### ATP and Metabolites

To observe changes in tissue nucleotides (ATP, ADP, AMP, and IMP) and nucleosides (adenosine, inosine, hypoxanthine, and xanthine), we rapidly froze hearts in liquid N2 and then stored at −80°C. An aliquot (200 mg) of the frozen tissue was pulverized and homogenized, and total RNA was extracted with an RNA isolation kit (Ambion, Austin, TX). RNA samples were tested by UV absorption at 260 nm to determine the concentration. The quality and concentration of the RNA samples were further confirmed by electrophoresis on denatured 1% agarose gels.

Northern blot analysis. For Northern blot analysis, 15 µg of RNA were denatured and electrophoresed in a 1% formaldehyde agarose gel, transferred to a nitrocellulose transfer membrane (Micron Separations, Westboro, MA), and cross-linked to the membrane with short pulse UV cross linking. The prehybridizing and hybridizing solutions contained 50% formamide, 1× Denhardt’s solution, 6× sodium chloride-sodium phosphate-EDTA, and 1% sodium dodecyl sulfate (SDS). cDNA probes were labeled with [32P]dCTP by random primer extension (PRIME-IT II, Stratagene, La Jolla, CA) and added to the hybridizing solution to a specific activity. Hybridization was carried out at 42°C for 18 h. The blots were then washed several times with a final wash in 1× standard sodium citrate and 0.1% SDS at 65°C. The relative amount of mRNAs was determined using a PhosphorImager (model 400S, Molecular Dynamics, Sunnyvale, CA). The same size area at each band was taken to measure the intensity and the same size area at the closest upstream position of each band was taken as the background of the image, respectively. The blots were exposed on Kodak X-o-mat film (Eastman Kodak, Rochester, NY) at −70°C. RNA loading was normalized by comparison to that of 28S ribosomal RNA. Adenine nucleotide translocase isoform 1 (ANT1) mRNA levels were detected using a 1.4-kilobase (kb) cDNA fragment cloned from the human skeletal muscle [American Type Culture Collection (ATCC), Rockville, MD] (11, 36, 44, 47). β-F1-ATPase mRNA levels were detected using a 1.8-kb cDNA fragment cloned from human HeLa cell line (ATCC) (11, 36, 46, 47). HSP70–1 mRNA levels were detected using a 1.7-kb cDNA fragment cloned from human hippocampus (ATCC) (10, 16, 23, 35). To compare different mRNA levels in the same myocardial sample, we analyzed aliquots of 15 µg total RNA from the myocardium by means of sequentially reprobing the membranes with 28S, ANT1, β-F1-ATPase, and HSP70–1 cDNA probes.

### Experimental Protocols

After instrumentation was completed and calibrations were performed, left ventricular balloon volumes were varied over a range of values (H787HYPOTHERMIC SIGNALING OF MITOCHONDRIAL BIOGENESIS) to construct left ventricular function curves. In this manner, it is possible to define a specific balloon volume that is associated with a LVDP between 100 and 140 mmHg. This volume remained unchanged during both baseline and reperfusion conditions. The intraventricular balloon volumes were not adjusted to produce specific EDPs (rather, we defined a level of systolic pressure development), but EDPs at baseline >10 mmHg were not accepted (39). Data from hearts characterized by LVDPs < 100 mmHg...
Fig. 1. Myocardial temperature. Ischemia starts at 0 min and ends at 120 min, then reperfusion follows. Before ischemia, the temperature is stable at 37°C in control hearts (C) but the temperature decreases to about 31°C progressively from baseline of 37°C in hypothermia-exposed hearts (H; C vs. H, p < 0.05; n = 10/group). Application of cardioplegia transiently decreases the temperature. There is no significant difference in temperature between groups during ischemia and reperfusion.

Table 1. Hemodynamics in control and hypothermia-exposed hearts

<table>
<thead>
<tr>
<th></th>
<th>Baseline</th>
<th>Preischemia</th>
<th>Reperfusion</th>
</tr>
</thead>
<tbody>
<tr>
<td></td>
<td>C</td>
<td>H</td>
<td>C</td>
</tr>
<tr>
<td>EDP, mmHg</td>
<td>2.0 ± 0.22</td>
<td>1.3 ± 0.27</td>
<td>2.8 ± 1.4</td>
</tr>
<tr>
<td>PSP, mmHg</td>
<td>113.4 ± 2.0</td>
<td>117.9 ± 2.6</td>
<td>113.7 ± 4.3</td>
</tr>
<tr>
<td>LVDP, mmHg</td>
<td>11.4 ± 2.0</td>
<td>116.6 ± 2.5</td>
<td>110.9 ± 4.8</td>
</tr>
<tr>
<td>LV dP/dt max, mmHg/s</td>
<td>1.714 ± 78</td>
<td>1.538 ± 38</td>
<td>1.611 ± 186</td>
</tr>
<tr>
<td>LV – dP/dt max, mmHg/s</td>
<td>1.098 ± 53</td>
<td>967 ± 29</td>
<td>1.104 ± 130</td>
</tr>
<tr>
<td>HR, beats/min</td>
<td>207.6 ± 6.3</td>
<td>191.3 ± 7.1</td>
<td>190.7 ± 12.1</td>
</tr>
<tr>
<td>PRP, 10-5 mmHg/min</td>
<td>23.08 ± 0.6</td>
<td>22.12 ± 0.66</td>
<td>21.27 ± 1.84</td>
</tr>
<tr>
<td>CF, ml·min⁻¹·g⁻¹</td>
<td>6.55 ± 0.39</td>
<td>6.26 ± 0.54</td>
<td>6.23 ± 0.45</td>
</tr>
<tr>
<td>MVV₂O₂, µmol·min⁻¹·g⁻¹</td>
<td>4.01 ± 0.28</td>
<td>3.86 ± 0.29</td>
<td>4.02 ± 0.27</td>
</tr>
<tr>
<td>O₂ extraction, %</td>
<td>68.5 ± 2.47</td>
<td>71.3 ± 1.97</td>
<td>73.1 ± 2.62</td>
</tr>
</tbody>
</table>

Values are means ± SE; n = 25 control hearts (C) and n = 18 hypothermia-exposed hearts (H). The hemodynamic indexes were determined in isolated reperfused hearts at baseline, after 20-min perfusion at 37°C (C) or 31°C (H), and after 15 min of reperfusion as described in MATERIALS AND METHODS. EDP, end-diastolic pressure; PSP, peak systolic pressure; LVDP, left ventricular developed pressure; LV dP/dt max, maximum of first derivative of left ventricular pressure; HR, heart rate; PRP, pressure-rate product (HR × LVDP); CF, coronary flow; MVV₂O₂, myocardial oxygen consumption. *P < 0.05, †P = 0.08 vs. C group hearts.
no significant differences between the control and hypothermia groups in EDP, LVDP, LV dP/dt max, heart rate, PRP, CF, MV˙O2, and O2 extraction. Hemodynamic results are summarized in Table 1. Twenty minutes of hypothermia decreased LVDP, LV dP/dt max, heart rate, PRP, coronary flow, and MV˙O2, but the left ventricular EDP increased. No significant changes in these parameters occurred in the control group (Table 1) during 20 min of perfusion at 37°C.

Functional recovery during reperfusion. In Table 1, the data demonstrate that the preischemic hypothermia provided superior functional recovery compared with that observed in control hearts. The preischemic hypothermic hearts were characterized by higher LVDPs, higher LV dP/dt max values, and lower EDP values.

Ischemic contracture. As noted in MATERIALS AND METHODS, a specific balloon volume was adjusted and then maintained throughout the protocol, allowing comparisons of LVP under constant end-diastolic volume. After CP solution was injected, the LVP was always near 0 mmHg. The beginning of ischemic contracture was defined by an initial rise of >2 mmHg in LVP. Ischemic contracture started significantly later in preischemic hypothermic hearts (97.5 ± 3.6 min) than in control hearts (67.3 ± 3.3 min).

Energy Metabolism

A decrease in CO2 production was noted in hearts during hypothermia corresponding to the decrease in O2 consumption. Together these changes indicate that a decrease in aerobic metabolism was induced by hypothermia. Despite temperature elevation, metabolic downregulation persisted through early ischemia as illustrated in Fig. 2 by significantly lower levels of both CO2 and lactate production in the hearts previously exposed to hypothermia.

Table 2 summarizes data relevant to ATP and its principal metabolites. Myocardial ATP, ADP, AMP, adenosine, total nondiffusible nucleotide (TNN), and total diffusible nucleoside (TDN) concentrations were not affected by exposure to hypothermia. Although ATP was depleted substantially regardless of preischemic temperature at end ischemia, levels were significantly higher in the hearts exposed to hypothermia. AMP levels were similarly increased in both groups. Adeno-

Table 2. ATP and metabolites

<table>
<thead>
<tr>
<th></th>
<th>Bc (n = 4)</th>
<th>Bb (n = 4)</th>
<th>Cc (n = 6)</th>
<th>Hc (n = 4)</th>
<th>Cc (n = 6)</th>
<th>Hc (n = 6)</th>
</tr>
</thead>
<tbody>
<tr>
<td>ATP</td>
<td>20.45 ± 0.57</td>
<td>17.95 ± 2.73</td>
<td>18.0 ± 0.39*</td>
<td>8.67 ± 1.45†</td>
<td>2.38 ± 0.51*</td>
<td>12.98 ± 1.11†</td>
</tr>
<tr>
<td>ADP</td>
<td>4.47 ± 0.48</td>
<td>3.71 ± 0.55</td>
<td>3.33 ± 0.24*</td>
<td>4.80 ± 0.77</td>
<td>2.16 ± 1.0*</td>
<td>4.21 ± 0.51†</td>
</tr>
<tr>
<td>AMP</td>
<td>0.40 ± 0.07</td>
<td>0.27 ± 0.03</td>
<td>1.53 ± 0.31*</td>
<td>0.89 ± 0.27‡</td>
<td>0.53 ± 0.11</td>
<td>0.34 ± 0.04</td>
</tr>
<tr>
<td>TNN</td>
<td>25.33 ± 0.79</td>
<td>21.94 ± 3.21</td>
<td>6.82 ± 0.43*</td>
<td>14.3 ± 2.01†</td>
<td>5.08 ± 0.52‡</td>
<td>17.55 ± 1.50†</td>
</tr>
<tr>
<td>Ado</td>
<td>0.17 ± 0.04</td>
<td>0.11 ± 0.04</td>
<td>7.29 ± 0.48*</td>
<td>2.02 ± 0.62†</td>
<td>0.18 ± 0.04</td>
<td>0.13 ± 0.03</td>
</tr>
<tr>
<td>TDN</td>
<td>0.62 ± 0.14</td>
<td>0.61 ± 0.06</td>
<td>7.99 ± 0.41*</td>
<td>4.72 ± 1.72†</td>
<td>1.75 ± 0.06*</td>
<td>0.85 ± 0.22</td>
</tr>
</tbody>
</table>

Values are means ± SE in units of µmol/g dry tissue; n = no. of hearts. Bc, time control at baseline; Bb, baseline value just before ischemia after 20 min of hypothermia; Cc, control group at 120 min of ischemia; Hc, preischemic hypothermic group at 120 min of ischemia; Cc, control group at 15 min of reperfusion; Hc, preischemic hypothermic group at 15 min of reperfusion; Ado, adenosine; TNN, total nondiffusible nucleotides; TDN, total diffusible nucleosides. *P < 0.05 vs. baseline for control or exposed group, respectively. †P < 0.05, ‡P = 0.06, H vs. C for same condition.

Fig. 2. Effects of preischemic hypothermia on accumulation of lactate, CO2, and myocardial pH. Differences in CO2 content (dCO2) or in lactate concentration (dLactate) between coronary effluent and inflow perfusates (n = 23 in C group, n = 18 in H group), as well as myocardial pH (n = 6/group), were determined as described in MATERIALS AND METHODS.
sine and TDN concentrations were elevated in both groups but to a lesser extent in the hypothermic group (P < 0.05). At 15 min of reperfusion, AMP returned to levels similar to baseline in both groups. Levels of ATP, ADP, and TNN were significantly higher and TDN was lower in hearts exposed to hypothermia.

β-F1-ATPase, ANT1, and HSP70–1 mRNAs

Hybridization of the mRNA probes for β-F1-ATPase (11), ANT1 (36, 44), and HSP70–1 (16) is similar to that observed in human (10, 36, 44), rat (11, 35), mouse (16), or rabbit tissues (14) and is illustrated in Fig. 3. Comparison of hearts in situ and hearts perfused for 50 min, regardless of temperature, resulted in no significant differences in transcript levels. Steady-state mRNA levels for three genes (ANT1, β-F1-ATPase, and HSP70–1) normalized to 28S ribosomal RNA intensity are shown in Fig. 4 for tissue obtained during the protocol. Across individual membranes, there were no significant changes in 28S band intensities (Fig. 3). Data are shown for control hearts at baseline, hearts subjected to 20 min of hypothermia, control hearts after the ischemic protocol and 45 min of reperfusion, and hypothermic exposed hearts after the ischemic protocol and 45 min of reperfusion.

Hypothermia did not alter the pattern of expression for any of these genes (Fig. 4) within 20 min of exposure. Ischemia followed by reperfusion did affect expression of the three genes. Postischemic steady-state levels of β-F1-ATPase and ANT1 mRNA were markedly diminished in controls (Fig. 4A). Hypothermic exposure ameliorated this subsequent postischemic decrease in steady-state levels of mRNA for the two mitochondrial proteins (Fig. 4A). HSP70–1 mRNA steady-state levels were elevated as a result of ischemia and reperfusion (Fig. 4B). However, the increase in HSP70–1 mRNA was substantially higher after reperfusion in the group that had been exposed to prior hypothermia.

**DISCUSSION**

Cardioplegic arrest in this perfused heart model removes discrepancies in myocardial performance between the experimental groups during early ischemia. Thus differences in high-energy phosphate depletion should not be due to discrepancies in contractile energy cost during ischemia. Furthermore, repeated application of cold cardioplegia and rewarming at 34°C produced comparable temperature responses between the groups (Fig. 1), negating another possible source of discrepancy in ATP utilization. Hypothermia results in alterations in contractile state and rates of energy expenditure in the intact heart. A reduction in O2 consumption during preischemic hypothermia in this

---

**Fig. 3.** A representative Northern blot. Each lane was loaded with 15 µg total RNA from ventricular myocardium and probed specifically for 28S, adenine nucleotide translocase isomor 1 (ANT1), β-subunit of F1-adenosinetriphosphatase (β-F1-ATPase), and inducible heat shock protein (HSP70–1). Samples were taken from hearts in situ (I), hearts at control baseline (Bc) and after 20 min of hypothermia (Bh), and hearts after 2 h of ischemia and 45 min of reperfusion in C and H groups (see text).

**Fig. 4.** Steady-state mRNA levels. All transcript levels are relative to 28S band intensity and normalized to Bc lane.

A: RNA levels for nuclear-encoded genes for ANT1 and β-F1-ATPase. Hypothermic exposure yields no differences in these levels (Bc vs. Bh; n = 6/group). Ischemia and reperfusion result in decreased levels in C hearts (n = 9) but no significant change in H hearts (n = 5). B: mRNA levels for HSP70–1. Exposure to hypothermia does not alter these levels. Ischemia and reperfusion yield a substantial increase in these levels in C hearts and an even greater increase in H hearts.
study exemplifies a decrease in ATP synthesis and utilization. The reductions in LVDP and LV dP/dt max indicate that there is a concomitant decrease in contractile state during mild hypothermia. To our knowledge, studies evaluating contractile function and the specific effects of mild hypothermia in the isolated perfused rabbit heart have not been previously performed and are thus not available for comparison. Although the reduction in O2 consumption during hypothermia is probably related principally to the decrease in contractile performance, an energy-sparing effect may also contribute to the decrease in ATP depletion during subsequent ischemia. Monroe et al. (34) demonstrated that under conditions of constant peak systolic pressure, lowering temperature from 38 to 32°C resulted in a greater area under the pressure-volume curve with no significant change in O2 consumption. This implies that at lower temperature more cardiac work can be achieved for the same energy cost and that ATP utilization is more efficient. Accordingly, ATP depletion and accumulation of its degradation products are greater in the control hearts during ischemia. These are indications that a smaller imbalance between ATP production and utilization occurs in the cold-stressed group. Net CO2 and lactate accumulation during ischemia reflect, respectively, aerobic and anaerobic ATP production. These values support, although do not prove, the notion that the greater ATP imbalance in the control group is due to greater high-energy phosphate utilization and not to decreased ATP production. A reduction in ATP utilization initiated during hypothermia might continue or perhaps may influence high-energy phosphate utilization during the subsequent ischemic period.

Restoration of ATP levels after reperfusion is higher in the hypothermia-exposed hearts. Improved ATP repletion after reperfusion also occurs consistently after other forms of preischemic stress exposure. However, modes of stress are inconsistent with respect to ATP preservation during the ischemic episode. For example, ischemic preconditioning produces either no effect or a reduction in ATP preservation, depending on the model under study (22, 46). This implies that the mechanisms that control ATP preservation and restoration may vary according to the mode of stress applied. Decreased purine loss or increased salvage during reperfusion in hypothermically exposed hearts probably contributes to improved ATP restoration because ATP degradation was decreased during ischemia. Maintenance of mitochondrial membrane function may also enhance ATP restoration after ischemia (22, 46).

The mechanisms responsible for decreased ATP utilization and their contribution to preservation of contractile function in the hypothermic hearts after ischemia remain highly speculative. Disturbances in excitation-contraction coupling and contractile apparatus, generated by free radical formation and Ca2+ overload, prevail as proposed causes of myocardial damage during ischemia and reperfusion (5). Profound hypothermia at temperatures <15°C and of several hours duration exacerbates cellular Ca2+ overload and induces peroxide and free radical formation (4, 25, 43). However, the effects of mild or short periods of hypothermia on these processes have not been examined. Conceivably, brief, relatively mild decreases in temperature can induce rapid enzyme changes consistent with cold adaptation, which would improve Ca2+ and free radical handling during a subsequent ischemic episode. Circumstantial evidence supporting this notion is provided in studies of tissue from cold-adapted or hibernating species (8, 12). Cold-adapted tissue demonstrates high rates of cardiac sarcoplasmic reticulum Ca2+ uptake (27), which presumably accounts for low cytosolic Ca2+ levels noted in hibernating animals. Reduction in cytosolic Ca2+ would effectively reduce activation of a variety of ATPases as well as catalytic proteases (1, 3, 6). Similarly, exposure to low environmental temperatures induces increases in antioxidant enzyme levels in a variety of tissues. Thus cold-adapted tissues display mechanisms that combat deleterious effects of oxidative stress present during extreme cold and/or ischemia.

Changes in heat shock protein and mitochondrial membrane protein gene expression demonstrate that an adaptive process has occurred. The isolated perfused heart has frequently been used as a model for characterization of heat shock protein gene expression, although questions concerning the appropriateness of this model in such investigations have been raised. Knowlton et al. (21) demonstrated that even a single ventricular stretch or hampering of systolic shortening resulted in a rapid increase in HSP70 expression in erythrocyte-perfused rabbit heart. However, this finding could not be reproduced by Myrmel et al. (35), who found no change in HSP70 expression in rat hearts after isovolumic perfusion at 65 mmHg for 30 min. Delcayre et al. (9) did find that augmentation of coronary perfusion pressure in beating or KCl-arrested isolated hearts perfused for 2 h produced increases in protein synthesis as well as HSP68 mRNAs. The discrepancies between these studies may be related to level and duration of perfusion pressure. In the present protocol, perfusion was performed with constant aortic pressure and isovolume, thus minimizing alterations in systolic shortening and diastolic stretch. Accordingly, this procedure resulted in no detectable increase in expression of HSP 70–1 over 50 min in either normothermic or hypothermic hearts before ischemia.

This is the first report of a hypothermia-induced alteration in heat shock protein gene expression after ischemia and reperfusion in hearts. A primary objective was to determine if an alteration in steady-state transcript levels could be induced by hypothermia-induced stress before ischemia. Cold stress is known to influence induction of heat shock proteins in various tissues (13, 19, 31). The response is highly variable and can extend from induction to suppression according to tissue and/or temperature (13, 19, 31). Furthermore, the heat shock protein response to cold occurs in some tissues only on recovery to normal temperature (26). Studies of cold-induced alterations in expression of the HSP70 family of proteins and their RNAs after ischemia have been reported in brain only (24, 42). These
studies demonstrate the extreme temperature variability of the heat shock response to cold. Although deep hypothermia (15°C) represses ischemic induction of HSP72 mRNA relative to ischemia at 23°C in pig brain, even lower expression occurs at 29°C (42). The mechanism of these temperature-dependent responses to cold stress in brain has not been elucidated (42).

Mitochondrial biogenesis can be initiated by cold-induced stress in brown adipose tissues (20, 31, 32). In several studies this has been characterized by increases in steady-state mRNAs for the uncoupling protein, a specific component of the mitochondrial membrane in brown adipose tissue. Other studies (28) have demonstrated coordinated gene expression for the adenine nucleotide translocator and the β-subunit for F1-ATPase with the uncoupling protein. This is consistent with reports that imply that the β-F1-ATPase subunit in particular can be used as a reporter gene for mitochondrial biogenesis (2, 17, 18, 24, 26, 41). The cold-induced stress response in brown adipose tissue, including HSP70 and uncoupling protein induction, can be specifically blocked through α-adrenergic receptor antagonism, implying that signaling is mediated by norepinephrine (30). These responses, including activation and regulation of mitochondrial membrane protein genes by cold-induced stress, have not been studied in other tissues. The coordinate expression of HSP70 with the uncoupling protein in brown adipose tissue compelled us to investigate whether alterations in steady-state levels of transcripts from genes regulating these important constitutive mitochondrial proteins could be induced by hypothermia followed by ischemia in the heart. A decrease in transcript levels for the β-F1-ATPase subunit gene has previously been documented by Heads et al. (14) after prolonged ischemic preconditioning, ischemia, and reperfusion protocol in rabbit myocardium (14). In this study a decrease in steady-state mRNA levels for both genes controlling these constitutive mitochondrial membrane proteins after reperfusion was detected only in the normothermic group. This finding implies that hypothermia directly or indirectly induced either an increase in transcription or a stabilization of these mRNAs.

In summary, these data suggest that exposure to a brief period of mild hypothermia improves resistance to injury during a subsequent period of prolonged ischemia with cardioplegic arrest. This response is associated with maintenance of steady-state mRNA levels for the adenine nucleotide translocator and the β-F1-ATPase subunit, as well as an elevation in expression of HSP70–1. These results imply that hypothermia induces an adaptive response, which is apparent in the postischemic period. These signals are associated with mitochondrial biogenesis in other tissues and are usually followed by an increase in mitochondrial protein synthesis consistent with cold adaptation. Because of the brief time course of events in this study, it is unlikely that increased protein synthesis occurred rapidly enough to effect the preservation of function and ATP associated with the elevated signal. However, reduction in injury may contribute to preservation of signaling for mitochondrial biogenesis. Mitochondrial dysfunction and damage have been documented in various models of myocardial O2 deprivation and repletion, and recovery is related to content of proteins participating in oxidative phosphorylation (15). Factors regulating synthesis of these proteins are complex and involve coordination of both nuclear and mitochondrial genes (45). Intuitively, maintenance of signaling for mitochondrial biogenesis and protein synthesis seems necessary for recovery of respiratory function after injury. As relatively little is known concerning the role of mitochondrial biogenesis in myocardial recovery, the implications of the hypothermia-induced preservation in signaling have not been elucidated but remain an area for future investigations.

This study was supported in part by National Heart, Lung, and Blood Institute Grant HL-47805-6. Address for reprint requests: X.-H. Ning, Dept. of Pediatrics, Box 356320, Univ. of Washington, 1959 NE Pacific St., Seattle, WA 98195. Received 7 July 1997; accepted in final form 20 October 1997.

REFERENCES


Received 7 July 1997; accepted in final form 20 October 1997.


