Hypothermia preserves myocardial function and mitochondrial protein gene expression during hypoxia

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Hypothermia preserves myocardial function and mitochondrial protein gene expression during hypoxia. Am J Physiol Heart Circ Physiol 285: H212–H219, 2003. First published March 13, 2003; 10.1152/ajpheart.01149.2002.—Hypothermia before and/or during no-flow ischemia promotes cardiac functional recovery and maintains mRNA expression for stress proteins and mitochondrial membrane proteins (MMP) during reperfusion. Adaptation and protection may occur through cold-induced change in anaerobic metabolism. Accordingly, the principal objective of this study was to test the hypothesis that hypothermia preserves myocardial function during hypoxia and reoxygenation. Hypoxic conditions in these experiments were created by reducing O2 concentration in perfusate, thereby maintaining or elevating coronary flow (CF). Isolated Langendorff-perfused rabbit hearts were subjected to perfusate (PO2 = 38 mmHg) with glucose (11.5 mM) and perfusion pressure (90 mmHg). The control (C) group was at 37°C for 30 min before and 45 min during hypoxia, whereas the hypothermia (H) group was at 29.5°C for 30 min before and 45 min during hypoxia. Reoxygenation occurred at 37°C for 45 min for both groups. CF increased during hypoxia. The H group markedly improved functional recovery during reoxygenation, including left ventricular developed pressure (DP), the product of DP and heart rate, dP/dt max, and O2 consumption (MVO2) (P < 0.05 vs. control). MVO2 decreased during hypothermia. Lactate and CO2 gradients across the coronary bed were the same in C and H groups during hypoxia, implying similar anaerobic metabolic rates. Hypothermia preserved MMP βF1-ATPase mRNA levels but did not alter adenine nucleotide translocase-1 or heat shock protein-70 mRNA levels. In conclusion, hypothermia preserves cardiac function after hypoxia in the hypoxic high-CF model. Thus hypothermic protection does not occur exclusively through cold-induced alterations in anaerobic metabolism.

EVALUATION OF HYPOTHERMIC myocardial protection has occurred predominantly in experimental models employing reductions in coronary flow to reduce oxygen supply. Exposure to mild or moderate hypothermic insult promotes cross-tolerance to variable forms of subsequent injury, particularly those produced by ischemia and/or reperfusion (19, 21). A reduction in ATP utilization rate initiated by hypothermia persists during rewarming and plays a central role in preserving myocardial function after ischemia and reperfusion (20). Hypothermic ATP preservation may promote specific molecular responses observed during reperfusion in the isolated rabbit heart model, including stabilization of steady-state mRNA levels for nuclear-encoded mitochondrial membrane proteins, enhanced induction of the stress protein heat shock protein-70 (HSP70) (19, 21, 22) and promotion of signaling for antiapoptotic pathways (18).

Hypothermia under no-flow conditions also reduces anaerobic ATP synthesis, thereby minimizing accumulation of glycolytic end products and raising myocardial pH (21). The anaerobic metabolites are known to exacerbate myocardial injury during reperfusion and to putatively regulate signaling for heat shock responses (15). Thus the hypothermic adaptive effect may operate exclusively under low-flow conditions and result directly from reductions in accumulated anaerobic products. The possibility that hypothermia may induce myocardial protection and adaptation during hypoxia with high coronary flow has not been examined. Coronary vasodilator response to low oxygen provides metabolic conditions differing substantially from those prevalent during ischemia and reperfusion. Hypoxia and associated elevation in coronary flow can induce high rates of myocardial anaerobic metabolism without affecting intracellular pH or promoting accumulation of glycolytic end products (25). Consequently, the principal objective of this study was to test the hypothesis that hypothermia preserves myocardial function during hypoxia and reoxygenation with maintained coronary flow. Furthermore, we determined whether the hypothermic adaptive responses for gene expression of HSP70 and two constitutive mitochondrial proteins occurred under metabolic conditions created by these hypoxic conditions.

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MATERIALS AND METHODS

Preparation of isolated hearts. Rabbit heart isolation and perfusion from our laboratory have been previously reported in detail (18, 21). In brief, hearts from rabbits (male or female, 2.3–2.5 kg body wt) anesthetized with pentobarbital sodium were rapidly excised and immersed momentarily in ice-cold physiological salt solution (PSS) (pH 7.4) containing (in mM) 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 equilibrated with 95% O2-5% CO2 at 37°C. Perfusion pressure was maintained at 90 mmHg (18). 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 and its first derivative with respect to time (dP/dt). The pulmonary artery was cannulated to enable collection of coronary flow measured with a flow meter (model T201; Transonic Systems, Ithaca, NY).

Analogue signals were continuously recorded on an on-line computer (Macintosh) and an analog signal acquisition system (Biopac Systems; Santa Barbara, CA). To characterize cardiac function, developed pressure is defined as peak systolic pressure (PSP) minus end-diastolic pressure. The product of heart rate and developed pressure (mmHg/min) was calculated to provide an estimate of myocardial work. Myocardial oxygen consumption (MVO2) was calculated as MVO2 = CF × [(Pao2 – PVCO2) × (c/760)], where CF is coronary flow (ml/min g wet tissue–1), Pao2 – PVCO2 is the difference in the partial pressure of oxygen (Pao2, mmHg) between perfusate and coronary effluent, and c is the Bunsen solubility coefficient of O2 in perfusate (22). Oxygen extraction was calculated as O2 Ext = MVO2/O2 content in the perfusate. Wet weight of the heart was determined at the conclusion of each experiment. All procedures were in accordance with institutional and National Institutes of Health guidelines.

Lactate and CO2 measurements. The coronary inflow and effluent were collected, and the concentrations of O2 and CO2 were immediately measured with a blood gas analyzer (model ABL5; Radiometer, Copenhagen, Denmark). Difference in CO2 content (dCO2) between coronary outflow and inflow was calculated as dCO2 = (PVCO2 – Paco2) × c/Vm, where PVCO2 – Pco2 is the difference in the partial pressure of carbon dioxide (PVCO2, mmHg) between coronary effluent and perfusate, c is the solubility coefficient of CO2, and Vm is molar volume (22.4 ml·mM–1·l–1) (18). Lactate concentration was measured with a microstat analyzer (model GM7; Analox, London, UK).

RNA isolation. After removal of excess fat and connective tissues, the left ventricular wall was brieﬂy blotted on nine-layer gauze, frozen in liquid nitrogen, and then stored at −80°C. An aliquot (150 mg) of the frozen tissue was pulverized and homogenized; total RNA was extracted with an RNA isolation kit (Ambion; Austin, TX). RNA samples were tested by ultraviolet absorption at an analytical wavelength of 260 nm to determine the concentration. The quality and concentration of the RNA samples were further conﬁrmed by electrophoresis on denatured 1% agarose gels (22).

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 by a short-wave ultraviolet cross-linker. The prehybridizing and hybridizing solutions contained 50% formamide, 1× Denhardt’s solution, 6× sodium chloride-sodium phosphate-EDTA, and 1% SDS. βF1-ATPase mRNA levels were detected by using a 1.8-kb cDNA fragment cloned from human HeLa cell line [American Type Culture Collection (ATCC), Manassas, VA] (2, 6, 9, 30). Adenine nucleotide translocator isofrom 1 (ANT1) mRNA levels were detected by using a 1.4-kb cDNA fragment cloned from human hippocampus (ATCC). Complementary DNA (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 (3). The blots were then washed several times with a final wash in 1× SSC and 0.1% SDS at 65°C. The relative amount of mRNAs was evaluated by using a Phosphor Imager (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. The blots were exposed on Kodak Biomax film (Eastman Kodak; Rochester, NY) at −70°C. RNA loading was determined by comparison to that of GAPDH. To compare different mRNA levels in the same myocardial sample, aliquots of 15–μg total RNA from the myocardium were analyzed by means of sequentially reprobing the membranes with GAPDH, βF1-ATPase, ANT1, and HSPO70–1 cDNA probes (17).

Experimental protocols. Left ventricular balloon volumes were varied over a range of values to construct left ventricular function curves to define a specific balloon volume associated with a developed pressure 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 end-diastolic pressures; rather, we defined a level of systolic pressure development. End-diastolic pressures at baseline >8 mmHg were not accepted (20). Data from hearts characterized by developed pressures <100 mmHg or >140 mmHg were not used. Baseline data were obtained after a 30-min equilibration period. The same procedures were followed in each experiment. During the baseline period, data were obtained from the hearts maintained at 37°C by passing water at this temperature through the walls of the organ bath. Hypoxia was induced by infusing PSS bubbled with 95% N-5% CO2 mixture gas (PO2 = 38 mmHg) for 45 min. Reoxygenation was followed for 45 min at 37°C. The control group was at 37°C during the entire experiment period (n = 6). Hypothermia was induced by progressively decreasing PSS temperature to 29.5°C during a 20-min period and maintained (Fig. 1) for 10 min before hypoxia and 45 min during hypoxia (n = 8). Reoxygenation was followed for 45 min at 37°C. The pulmonary outflow temperature was monitored continuously with a thermal probe to adjust the infused temperature. Hemodynamic data were recorded for 45 min, followed by sample collection for Northern blot analysis. Myocardial samples were also taken from hearts in situ as a nonperfused normal control group (n = 5).

Myocardial temperatures were not measured routinely in each experiment to avoid potential problems associated with traumatic introduction of needle-mounted temperature probes. In parallel experiments, myocardial temperature was monitored with a Khuri regional tissue temperature monitor (Vascular Technology; Chelmsford, MA) (21) to determine changes in the myocardial temperature profile by using our standard experimental protocol. Results of those experi-
**Table 1. Hemodynamics**

<table>
<thead>
<tr>
<th></th>
<th>Baseline</th>
<th>Prehypoxia</th>
<th>15 min</th>
<th>30 min</th>
<th>45 min</th>
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<tr>
<td><strong>EDP, mmHg</strong></td>
<td></td>
<td></td>
<td></td>
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<td></td>
</tr>
<tr>
<td>C</td>
<td>1.3 ± 0.5</td>
<td>2.6 ± 0.6</td>
<td>44.7 ± 8.3†</td>
<td>40.5 ± 9.6†</td>
<td>34.0 ± 8.9†</td>
</tr>
<tr>
<td>H</td>
<td>1.9 ± 0.4</td>
<td>21.6 ± 3.4†</td>
<td>8.9 ± 2.3*</td>
<td>4.6 ± 1.3*</td>
<td>5.9 ± 1.4*</td>
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<td><strong>PSP, mmHg</strong></td>
<td></td>
<td></td>
<td></td>
<td></td>
<td></td>
</tr>
<tr>
<td>C</td>
<td>132.1 ± 3.0</td>
<td>129.2 ± 3.4</td>
<td>66.2 ± 8.6†</td>
<td>65.9 ± 12.6†</td>
<td>63.3 ± 10.5†</td>
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<tr>
<td>H</td>
<td>133.2 ± 1.8</td>
<td>122.4 ± 5.9</td>
<td>100.0 ± 2.6†</td>
<td>104.7 ± 2.5*</td>
<td>110.2 ± 2.1*</td>
</tr>
<tr>
<td><strong>dP/dt max, mmHg/s</strong></td>
<td></td>
<td></td>
<td></td>
<td></td>
<td></td>
</tr>
<tr>
<td>C</td>
<td>1,942 ± 75</td>
<td>1,934 ± 72</td>
<td>211 ± 45†</td>
<td>301 ± 70†</td>
<td>366 ± 78†</td>
</tr>
<tr>
<td>H</td>
<td>2,013 ± 62</td>
<td>661 ± 44†</td>
<td>1,232 ± 141†</td>
<td>1,541 ± 108*</td>
<td>1,639 ± 65*</td>
</tr>
<tr>
<td><strong>dP/dt min, mmHg/s</strong></td>
<td></td>
<td></td>
<td></td>
<td></td>
<td></td>
</tr>
<tr>
<td>C</td>
<td>1,591 ± 65</td>
<td>1,574 ± 67</td>
<td>311 ± 70†</td>
<td>398 ± 78†</td>
<td>431 ± 68†</td>
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<tr>
<td>H</td>
<td>1,681 ± 59</td>
<td>618 ± 49†</td>
<td>1,065 ± 114†</td>
<td>1,233 ± 91*†</td>
<td>1,273 ± 54*†</td>
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<td><strong>HR, beats/min</strong></td>
<td>191.7 ± 6.8</td>
<td>194.3 ± 7.2</td>
<td>172.0 ± 5.1</td>
<td>193.0 ± 19.4</td>
<td>189.8 ± 13.0</td>
</tr>
<tr>
<td>C</td>
<td>204.5 ± 5.6</td>
<td>91.1 ± 8.4†</td>
<td>179.4 ± 13.4</td>
<td>202.0 ± 5.6</td>
<td>209.8 ± 6.2</td>
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<tr>
<td><strong>PRP, 10^3 mmHg/min</strong></td>
<td>25.08 ± 1.11</td>
<td>24.59 ± 1.04</td>
<td>3.66 ± 0.74†</td>
<td>4.59 ± 1.08†</td>
<td>5.28 ± 1.06†</td>
</tr>
<tr>
<td>C</td>
<td>26.89 ± 0.96</td>
<td>9.19 ± 0.88†</td>
<td>16.43 ± 1.57†</td>
<td>20.21 ± 0.82†</td>
<td>21.84 ± 0.65*</td>
</tr>
<tr>
<td><strong>CF, ml·min⁻¹·g⁻¹</strong></td>
<td>11.9 ± 0.49</td>
<td>12.1 ± 0.37</td>
<td>11.9 ± 1.13</td>
<td>11.3 ± 1.10</td>
<td>10.5 ± 0.89</td>
</tr>
<tr>
<td>C</td>
<td>11.4 ± 0.59</td>
<td>7.5 ± 0.95†</td>
<td>12.3 ± 0.90</td>
<td>11.4 ± 0.67</td>
<td>11.5 ± 0.69</td>
</tr>
<tr>
<td><strong>MVO₂, µM·min⁻¹·g⁻¹</strong></td>
<td>4.96 ± 0.36</td>
<td>4.94 ± 0.38</td>
<td>1.49 ± 0.26†</td>
<td>1.34 ± 0.28†</td>
<td>1.02 ± 0.21†</td>
</tr>
<tr>
<td>H</td>
<td>5.67 ± 0.27</td>
<td>1.15 ± 0.13†</td>
<td>3.89 ± 0.54*</td>
<td>4.25 ± 0.26*</td>
<td>4.53 ± 0.37*</td>
</tr>
<tr>
<td><strong>O₂ Ext, %</strong></td>
<td>47.3 ± 2.7</td>
<td>46.0 ± 3.4</td>
<td>13.8 ± 1.3†</td>
<td>13.2 ± 1.8†</td>
<td>11.1 ± 2.2†</td>
</tr>
<tr>
<td>C</td>
<td>56.5 ± 1.7</td>
<td>18.8 ± 2.1†</td>
<td>35.8 ± 3.9†</td>
<td>42.6 ± 2.6†</td>
<td>44.7 ± 2.7†</td>
</tr>
</tbody>
</table>

Values are means ± SE; n = 6 rabbit hearts in the control group (C) and 8 rabbit hearts in the hypothermia-treated group (H). Hemodynamic index was determined in isolated infused hearts at baseline and at 15, 30, and 45 min of reoxygenation as described in MATERIALS AND METHODS. Reo, reoxygenation; CF, coronary flow; DP, developed pressure; ± dP/dt max, maximum of the positive or negative first derivative of left ventricular pressure; EDP, end-diastolic pressure; HR, heart rate; PRP, product of HR and DP; PSP, the peak left ventricular systolic pressure; MVO₂, myocardial oxygen consumption; O₂ Ext, oxygen extraction. *P < 0.05 compared with C. †P < 0.05 compared with baseline.
first derivative maximums of left ventricular pressure ($\pm dP/dt_{\text{max}}$), and the product of developed pressure and heart rate. The left ventricular end-diastolic pressure was distinctly higher in the control than that in hypothermia hearts (Fig. 2).

Coronary flow. Heart weight and the left ventricular volume were similar between groups (6.1 ± 0.3 g and 5.5 ± 0.2 g and 1.4 ± 0.03 ml for the hypothermia group, respectively). Figure 3 illustrates coronary flow response throughout the protocol. Coronary flow did not differ significantly between groups during baseline (11.9 ± 0.49 ml·g⁻¹·min⁻¹ for the control group and 11.4 ± 0.59 ml·g⁻¹·min⁻¹ for the hypothermia group). Hypothermia applied before hypoxia decreased coronary flow ($P < 0.05$). Hypoxia increased coronary flow initially ($P < 0.05$) although there was no significant difference between the groups ($P > 0.05$). No subsequent differences in coronary flow occurred throughout the remainder of the protocol.

$MVO_2$. $MVO_2$ shown in Table 1 did not differ among the groups at baseline. Hypothermia before hypoxia decreased $MVO_2$ significantly ($P < 0.05$). Hypoxia decreased $MVO_2$ dramatically, but there was no significant difference among the groups ($P > 0.05$) (Fig. 4). The decrease in $MVO_2$ relates to deficits in oxygen extraction not coronary flow. $MVO_2$ recovered during reoxygenation in the hypothermia hearts but not in the controls.

Lactate and $CO_2$ concentration and production. No significant d$CO_2$ or lactate gradients between perfusate and effluent occurred in either group during hypoxia (Fig. 5). $CO_2$ release reflects the substrate oxidation rate. As expected in this relatively high coronary flow model, the rate of $CO_2$ production parallels $MVO_2$ (Fig. 4). However, the lactate production rate increased significantly in both groups during hypoxia but did not vary significantly among the groups at any time in the protocol (Fig. 4).

$\beta F_1$-ATPase, ANT1, and HSP70–1 mRNAs. Hybridization of the mRNA probes for $\beta F_1$-ATPase (6) and ANT1 has been previously reported in this rabbit model (19, 21, 22). A representative Northern blot illustrating $\beta F_1$-ATPase and ANT1 is shown in Fig. 6A. Statistical comparisons of hearts in situ versus control and hypothermia after hypoxia are illustrated in Fig. 6B. Values are normalized to GAPDH intensity (18). The steady-state mRNA levels for $\beta F_1$-ATPase in the control group were significantly depressed compared with the normal group ($P < 0.05$). However, $\beta F_1$-ATPase mRNA in the hypothermia group was maintained at normal group levels and increased over the control group levels. In contrast, no significant differences in ANT1 occurred among the three groups. HSP70–1 was induced greater than twofold and to a similar degree in both groups after hypoxia (Fig. 7B).
**DISCUSSION**

Hypothermic preservation of myocardial function after hypoxic insult represents the principal and novel finding in this study. It has been previously documented that hypothermia induced resistance to ischemic injury in the rabbit heart model (20, 21). Hypothermic mechanisms providing protection have not been elucidated but may be related to alterations in anaerobic metabolism. A prevailing hypothesis appearing in the literature states: conditions enhancing anaerobic energy production, while facilitating removal of glycolytic by-products, promote preservation of myocardial function (1, 5, 27, 29). This hypothesis lacks consensus, because the relationships between anaerobic metabolism and postischemic myocardial function greatly depend on particular flow conditions in each model under study. The current experimental model provides low oxygen supply with relatively high coronary flow and deviates substantially from traditional no-flow or low-flow ischemic modes.

Glycolysis during no-flow ischemia generates lactate and proton accumulation, which eventually inhibits further ATP production through this metabolic pathway (27). Proton accumulation accentuates deleterious sodium and calcium entry into myocardial cells during reperfusion. However, recent studies dispute the detrimental role of glycolysis during no-flow ischemia and indicate that ischemic glycolysis preserves sarcolemmal selective permeability and integrity (1). Studies (5, 29) employing low-flow ischemic conditions demonstrate that maintenance of lactate production and lactate-H⁺ cotransporter flux reduce myocardial injury after reperfusion. Reduction in lactate transport accompanied by lactate accumulation exacerbates myocardial injury after reperfusion in some low-flow ischemia models (5).

Previous work in this laboratory showed that hypothermia applied to no-flow ischemia reduces lactate production and lactate-H⁺ cotransporter flux (18–21). Thus one may presume that hypothermia-induced alterations in anaerobic metabolism provide a protective mechanism against injury from no-flow ischemia. This contention can be challenged, because glucose provided in cardioplegia during no-flow normothermic or hypothermic conditions paradoxically enhances glycolysis and lactate accumulation while promoting improvements in contractile performance after reperfusion (19, 20).

In the current experiments, hypoxia is defined as a substantial reduction in perfusate oxygen concentra-
tion without coronary flow limitation. A clear distinction exists between our experimental hypoxic condition and those interventions creating oxygen deprivation by coronary flow reduction. The hypoxic, isolated, perfused heart and hypoxic hearts in situ respond initially to oxygen deprivation through vasomotor relaxation, which produces a substantial increase in coronary flow as noted in Fig. 3. Previous work (25, 26) in our laboratory demonstrated that coronary flow elevation prevents accumulation of specific intracellular ions during hypoxia. Although myocardial intracellular pH decreases rapidly at initial onset of ischemia (24), cellular H+ concentration is relatively stable during early hypoxia without coronary flow limitation, despite marked depletion in high-energy phosphate stores. Because mechanisms of injury may differ between ischemia and hypoxia, one cannot presume that actions of hypothermia are similar for these two conditions.

Hypothermia diminished contractile function in parallel to a decrease in metabolic demand as evidenced by the decrease in MVO2 and CO2 production in this model. Consistent with previous studies (13, 21) hypothermia also produced an increase in respiratory efficiency of cardiac work-assessed by-product of developed pressure and heart rate-to-MVO2 ratio. Thus the hypothermia-perfused rabbit heart requires less carbon substrate oxidation per unit contractile work than the normothermic counterpart. The previous studies also demonstrated that metabolic downregulation during hypothermia before ischemic insult is not accom-panied by the metabolic changes characteristic of ischemic preconditioning. Accordingly, the ATP depletion and increased lactate production observed during brief single or multiple ischemic preconditioning periods (7, 14) do not occur during hypothermia. Therefore, the mechanisms of protection in this study are likely somewhat different than those provided by ischemic preconditioning.

Although hypothermia induces changes in metabolic demand and respiratory efficiency under aerobic conditions, the current model does not provide evidence that similar energy sparing occurs throughout the hypoxic period. However, our previous studies indicate that basal metabolic requirements are decreased by hypothermia during ischemia-induced asystole in the perfused rabbit heart (20). Additionally, the greater coronary reserve in the hypothermic hearts provides a brief advantage in cardiac energy supply-to-demand ratio in early hypoxia although coronary flow and oxygen consumption are similar between the two groups by 5 min into hypoxia. During the majority of the hypoxic period, the hearts were asystolic, and therefore, both groups used similarly diminished contractile energy for contractile work. Meager, but equivalent oxygen consumption rates persisted in both groups during the hypoxic period due to low oxygen concentration in the perfusate. Anaerobic metabolism as determined by lactate production predominated and supplied the vast majority of ATP production during hypoxia. Additionally, both groups maintained the lactate gradient implying that high coronary flow prevented aerobic metabolism and lactate export.

![Fig. 6. A: Northern blot for βF₁-ATPase and adenine nucleotide translocator isoform (ANT₁).](image_url)

- Each lane was loaded with 15 μg total RNA from ventricular myocardium and probed specifically for GAPDH, βF₁-ATPase, and ANT₁. Samples were taken from hearts in situ (N) and hearts after 45 min of hypoxia followed by 45 min of reoxygenation in the C and H groups. B: steady-state mRNA levels. Transcript levels are relative to GAPDH band intensity and are normalized to N. *P < 0.05 vs. N.

![Fig. 7. A: Northern blot for HSP70-1.](image_url)

- Each lane was loaded with 15 μg total RNA from ventricular myocardium and probed specifically for GAPDH and HSP70-1. Samples were taken from hearts in situ (N) and hearts after 45 min of hypoxia followed by 45 min of reoxygenation in the C and H groups. B: steady-state mRNA levels for HSP70-1. Transcript levels are relative to GAPDH band intensity and normalized to N. *P < 0.05 vs. N.
accumulation of this anaerobic metabolite. Thus these two experimental groups exhibit nearly identical patterns of anaerobic energy metabolism during the vast majority of the hypoxic period, although the hypothermic group demonstrated far superior contractile function after reoxygenation. Accordingly, these results challenge the hypothesis that hypothermia induces protection from hypoxic injury solely through moderation of anaerobic metabolism and lactate accumulation.

Despite similar coronary flow rates and, therefore, comparable oxygen supply in the two groups after reoxygenation, MVo₂ appears markedly limited in the control group. This limitation arises from the inability of the heart to extract oxygen, implying that a deficit in mitochondrial respiration exists. Thus one might postulate that hypothermia preserves mitochondrial membrane potential and integrity. Studies examining effects of hypothermia on mitochondrial structure and integrity after ischemia are planned for the future.

Analysis of gene expression under metabolic conditions of hypothermia and hypoxia represented the secondary objective for this study. Enhanced gene expression for stress-related proteins and constitutive mitochondrial proteins after ischemic insult represents a principal characteristic of the hypothermic adaptation (18, 21). This response emulates changes in expression induced by cold stress in tissues from hibernating species exposed to low levels of mitochondrial respiration (10). Postulated regulators for the stress proteins include specific metabolites, such as ATP (4) or lactate (15). However, glucose augmentation of glycolytic energy production and lactate accumulation in the ischemic heart did not enhance HSP70–1 expression in our previous study (19) employing a no-flow ischemic rabbit heart model.

Hypothermia failed to affect HSP70–1 expression in this hypoxic model. These data demonstrate the heterogeneity of the HSP70–1 response enhanced by hypothermia with no-flow ischemia but not enhanced with hypoxia to the degree applied in these protocols. Conceivably, even the low aerobic ATP production level that occurred in the hypoxic hearts was adequate to suppress expression for this gene. However, the mechanisms through which hypothermia exaggerates induction of this gene during ischemia require further elucidation. This cold and heat-induced stress protein could be regulated by an alternative, not-yet-defined thermogenic process.

The major mitochondrial constitutive proteins, βF₁-ATPase and ANT, are nuclear encoded. They participate in ADP phosphorylation and adenylate transfer across the mitochondrial membrane and are thus intimately involved with oxidative phosphorylation. The βF₁-ATPase, in particular, is often used as a marker gene for mitochondrial biogenesis (8, 11, 23, 31). Hypothermia enhances expression for the genes that regulate these proteins after application of ischemic stress (19, 20). This adaptive response has been demonstrated in the company of metabolic alterations that include reductions in lactate and proton accumulation during a stress period accompanied by improved preservation of ATP levels during reperfusion. However, glucose augmentation, which also promotes ATP preservation in the ischemic stress model, does not enhance expression of these mRNAs (19). Thus the studies performed in the ischemic model cast doubt on whether metabolic regulation exists for signaling of these particular proteins.

The present study indicates that hypothermic enhancement of βF₁-ATPase mRNA levels occurs after hypoxia. The response is less extreme than noted after ischemia (21) and is not accompanied by statistical differences in ANT mRNA levels. However, ANT mRNA appears fairly resilient and is not diminished by hypoxia, possibly reflecting the limited severity of the hypoxic stress. Results obtained in the current model also suggest that the hypothermic enhancement of mRNA levels for these nuclear-encoded mitochondrial proteins is not associated with the anaerobic metabolic rate and/or lactate accumulation. Thus an alternative mode of regulation for signaling for these proteins through cold may be operative.

The relatively brief time course of the protocol likely precludes occurrence of mRNA-mediated changes in expression for these mitochondrial membrane proteins during the posthypoxic recovery period. Although, hypothermia appears to alter signaling for these proteins, their specific role in myocardial protection needs to be more clearly defined. Maintenance in levels of these two proteins have been linked to long-term compensatory adjustments in myocardial high-energy phosphate metabolism and contractile function after ischemic injury (12, 17). Therefore, triggers, such as hypothermia, which positively affect signaling for these proteins, require closer scrutiny. In summary, these studies demonstrate that hypothermia preserves contractile function after hypoxic insult. However, the superior cardiac function apparent in the hypothermic group during the reoxygenation period cannot be explained by alterations in anaerobic metabolite accumulation. Furthermore, the hypothermic response of mRNAs for HSP70–1 and mitochondrial constitutive genes varies according to the specific form of applied oxygen deprivation condition.

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REFERENCES


