COLD CARDIOPLEGIC ARREST ENHANCES HEAT SHOCK PROTEIN 70 IN THE HEAT-SHOCKED RAT HEART

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Background: Myocardial content of the 70-kd heat shock protein has been found to correlate with improved cardiac recovery after ischemia, but the mechanisms and conditions that regulate its level, particularly under clinical conditions, are unclear. The aim of this study was to assess the effect of hypothermic cardioplegic arrest and reperfusion on the expression of 70-kd heat shock protein in a protocol mimicking conditions of preservation for cardiac transplantation.

Methods: Heat-shocked and control hearts were subjected to 4 hours of cardioplegic arrest and global ischemia at 4°C and then to 20 minutes of reperfusion. Hearts were freeze clamped at different time points—after 15 minutes of Langendorff perfusion, at the end of ischemia, and after 20 minutes of reperfusion, and analyzed for heat shock protein 70 content by Western blotting. Another set of hearts was subjected to 10 minutes of normothermic ischemia and 20 minutes of reperfusion followed by freeze clamping and analysis of heat shock protein 70 content as in cardioplegic arrest protocol. Cardiac function was measured by means of a left ventricular balloon at the end of reperfusion.

Results: Preischemic concentration of 70-kd heat shock protein was increased in heat-shocked hearts compared with control hearts. The content of 70-kd heat shock protein in heat-shocked hearts was further increased from 5.0 ± 2.4 ng/μg at the end of ischemia to 11.0 ± 4.9 ng/μg ($n = 8$, mean $\pm$ SD; $P < .05$) at 20 minutes of reperfusion after cold cardioplegic arrest. No further rise in 70-kd heat shock protein of the heat-shocked hearts was observed after normothermic ischemia. Maximal developed pressure was 120.8 ± 13.4 mm Hg in control hearts compared with 164.7 ± 22.5 mm Hg in heat-shocked hearts ($n = 5$, mean $\pm$ SD; $P = .037$) after cardioplegic arrest. By contrast, after normothermic ischemia, maximum developed pressure was 111.2 ± 10.9 mm Hg in control hearts compared with 139.2 ± 11.0 mm Hg in heat-shocked hearts ($n = 4$, mean $\pm$ SD; $P = .031$).

Conclusion: Hypothermic cardioplegic arrest but not short normothermic ischemia triggered a further increase in the level of 70-kd heat shock protein in heat-shocked rat hearts, which may enhance endogenous cardiac protection. (J Thorac Cardiovasc Surg 2001;121:1130-6)

The accumulation of 70-kd heat shock protein (Hsp70) enhances cardiac resistance to ischemia/reperfusion injury and improves postischemic recovery of cardiac function.¹ Synthesis of Hsp in cardiac cells can be induced by a variety of stimuli such as elevated tempera-

ture,¹ ischemia,² hypoxia,³ and depletion of adenosine triphosphate.⁴ The exact mechanism of this protective effect is not known, but it is thought that this is partly due to the ability of Hsp to act as molecular chaperones by binding to denaturing proteins to protect their native structure. In addition to increases in expression of Hsp, heat shock causes several other changes such as increased antioxidant capacity,⁵ improved preservation of high-energy phosphates,⁶ and activation of K⁺ channels,^{7,8} which could contribute to the protective effect. Despite the controversy surrounding the protective effects of Hsp70, the degree of postischemic recovery

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was found to be related to the level of Hsp contained within the heart.⁹ Therefore, any additional stimulus to increase the level of Hsp could be of clinical importance. Cardiac cooling as a strategy for myocardial protection is known to exert numerous effects on the cell, which can be detrimental or beneficial; however, the net result is cytoprotective. The effect of hypothermia on the heat shock response has not been fully investigated. In this present study, in which we used a protocol that mimics donor preservation for cardiac transplantation, we investigated how hypothermic cardioplegic arrest affects Hsp70 content in heat-shocked hearts.

Methods

Animals. Male Sprague-Dawley rats weighing between 300 and 330 g were used in all experiments. In all studies, 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, National Research Council, and published by the National Academy Press, revised 1996.

Induction of heat shock. Rats were anesthetized with an intraperitoneal injection of sodium pentobarbital (50 mg/kg), then placed on a temperature-controlled heating pad (IMS K-Temp control unit; Congleton, Cheshire, United Kingdom) set at 45°C until body temperature reached 42°C. Body temperature was monitored with a rectal temperature probe and maintained between 42°C and 42.5°C for 15 minutes as previously described.¹⁰ The animals were left to recover for 24 hours. Control animals did not undergo this procedure.

Experimental time course. The Langendorff-perfused isolated rat heart preparation used in this study has already been described in detail elsewhere.¹¹ In brief, all animals were put to death by intraperitoneal injection of sodium pentobarbital (50 mg/kg). The femoral vein was immediately exposed and heparin (200 IU) was injected. The heart was then excised and immediately placed in ice-cold (4°C) Krebs solution. The aorta was rapidly cannulated (within approximately 30 seconds) and Langendorff perfusion was initiated. The heart was perfused with Krebs-Henseleit bicarbonate buffer (pH 7.4) consisting of (in millimoles per liter) NaCl 118.5, NaHCO₃ 25.0, KCl 4.8, MgSO₄ 1.2, KH₂PO₄ 1.2, CaCl₂ 1.25, and glucose 11.0, which was continuously gassed with 95% oxygen and 5% carbon dioxide and maintained at 37°C. The Krebs buffer flowed from a reservoir 100 cm above the heart.

The heart was suspended in a water-jacketed chamber maintained at 37°C. After initial perfusion in the Langendorff mode, both heat-shocked (n = 5) and control hearts (n = 5) were subjected to protocol 1, including cardioplegic arrest with St Thomas' Hospital No. 1 cardioplegic fluid, 4 hours of global ischemia at 4°C, and reperfusion for 20 minutes, or to protocol 2, including 10 minutes of global normothermic ischemia (without cardioplegic arrest) at 37°C followed by 20 minutes of reperfusion (heat-shocked hearts, n = 4; control hearts, n = 4). Hearts were freeze clamped at different time points during both

protocols: after 15 minutes of Langendorff perfusion, at the end of ischemia, and after 20 minutes of reperfusion. Mechanical function was assessed with the use of a balloon catheter inserted into the left ventricle to determine systolic and end-diastolic pressure/volume relations after ischemia, as previously described.¹¹ The balloon was inflated with water in increments of 50 µL. Left ventricular peak systolic and end-diastolic pressures were recorded at each loading of the balloon and were used to calculate left ventricular developed pressure (peak systolic minus end-diastolic pressure). The diastolic stiffness coefficient was calculated as previously described¹² from the end-diastolic pressure/volume relation by logarithmic transformation of pressure at each balloon volume. Diastolic stiffness coefficient was the slope value of this relation. In another series of hearts, left ventricular biopsy specimens from control and heat stressed hearts were taken at identical time points: after 15 minutes of Langendorff perfusion, at the end of ischemia, after 20 minutes of reperfusion, to examine myocardial Hsp70 content by Western immunoblotting (heat shocked, n = 8, control, n = 8, cardioplegic arrest protocol; heat shocked, n = 4-5, normothermic ischemia protocol).

Assessment of Hsp expression. The induction of Hsp70 was assessed by sodium dodecyl sulfate (SDS), polyacrylamide gel electrophoresis, and Western immunoblotting as previously described.¹³ Whole heart homogenates were solubilized in 1% w/v SDS and assayed for total protein using the Bradford assay, denatured by heating at 100°C in Laemmli buffer, and separated on 10% SDS gels until the bromophenol blue tracking dye reached the end of the gel. Gels were equilibrated for 30 minutes in transfer buffer, and transfer of the proteins was performed for 1 hour at 500 mA. Western blots were blocked with 3% w/v nonfat dried milk (Marvel; Premier Beverages, Stafford, United Kingdom) in phosphate-buffered saline solution containing 0.05% w/v Tween-20 for 1 hour to block nonspecific binding sites. The blots were then probed with mouse antibodies specific to inducible Hsp70 (Bioquote Ltd, North Yorkshire, United Kingdom) diluted to a final concentration of 1:1000 for 1 hour. Blots were washed 3 times and incubated with secondary horseradish-peroxidase-conjugated rabbit anti-mouse antibody for 1 hour. The result was visualized with the use of an enhanced chemiluminescence (ECL) detection system (Amersham Pharmacia Biotech Inc, Piscataway, NJ). Hyperfilm myeloperoxidase was exposed to blots treated with ECL for 30 seconds and developed in an automatic film processor. After ECL exposure, antibodies were removed from blots by incubation in a solution of 2% w/v SDS, 6.25% v/v 1 mol/L Tris hydrochloride, pH 6.8, and 0.7% v/v 2-mercaptoethanol. Proteins were then visualized by staining with 0.01% amido black in a solution of methanol, water, and acetic acid (ratio of 45:45:10 v/v). Amido black-stained blots and ECL films were scanned with a Molecular Dynamics 300A laser densitometer (Molecular Dynamics, Inc, Sunnyvale, Calif), and Hsp70 levels were determined as a proportion of total protein loaded using the Quantity One software package (PDI, Huntington, NY).

Chemicals. St Thomas' Hospital cardioplegic solution No. 1, supplied as concentrate (David Bull Laboratories, Mulgrave,

Fig 1. A, Concentration of Hsp70 in heat-shocked and control hearts subjected to cardioplegic arrest, 4 hours of total global ischemia at 4°C, and 20 minutes of reperfusion at various time points of the experiment: end of a 15-minute period of initial Langendorff perfusion, end of ischemia, and 20 minutes of reperfusion. **B,** Concentration of Hsp70 in heat-shocked and control hearts subjected to 10 minutes of normothermic ischemia at 37°C and 20 minutes of reperfusion at various time points of the experiment, end of a 15-minute period of initial Langendorff perfusion, end of ischemia, and 20 minutes of reperfusion. Values represent the mean variation of sample size in groups $\pm$ SD ($n = 4-8$; * $P < .05$ vs control at the same time point; # $P < .05$ vs 15 minutes of Langendorff perfusion and end of ischemia).

Victoria, Australia), was diluted in Ringer solution (Travenol Laboratories, Thetford, Norfolk, England) and passed through a 0.2-mm filter (Pall Biomedical, Glen Cove, NY).

Statistical analysis. Experimental results were compared by a 1-way analysis of variance and where appropriate by a 2-way analysis of variance for repeated measures followed by the Student *t* test to indicate differences between groups. Values are presented as the mean $\pm$ SD.

Results

Cardiac Hsp70 content. The Hsp70 concentrations at different time points of cardioplegic arrest protocol 1, in both heat-shocked and control hearts, are shown in Fig 1, A. Initial Hsp70 content was higher in heat-shocked hearts than in control hearts. Heat-shocked

hearts subjected to 4 hours of cold cardioplegic arrest followed by 20 minutes of reperfusion had a 2-times greater concentration of Hsp70 than the heat-shocked hearts after 15 minutes of Langendorff perfusion ($P < .05$) or at the end of ischemia ($P < .05$).

The Hsp70 levels at different time points of the normothermic ischemia protocol 2, in which both heat-shocked and control hearts were subjected to 10 minutes of normothermic ischemia, are shown in Fig 1, B. The Hsp70 content was higher in all heat-shocked hearts than in controls at their respective time points. No further enhancement was observed on 20 minutes of reperfusion after 10 minutes of normothermic ischemia. The time course for the natural expression of Hsp70 during the experimental protocol is shown in

Fig 2. The time course of Hsp70 concentration changes after heat stress. Values represent the mean $\pm$ SD (n = 5 in all groups, except 29 hrs, n = 4; * P = .013 vs control; ** P < .001 vs control).

Fig 3. Diastolic stiffness coefficient for heat-shocked and control hearts subjected to cold cardioplegic arrest (n = 5) and ischemia for 4 hours or 10 minutes of normothermic ischemia (n = 4). Values represent the mean $\pm$ SD.

Table I. Mechanical function of heat-shocked and control hearts subjected to cardioplegic arrest for 4 hours at 4°C

Balloon volume (μL)	Systolic pressure (mm Hg)	Diastolic pressure (mm Hg)	LVDP (mm Hg)
<i>Control</i>			
50	55.6 $\pm$ 25.1	0.5 $\pm$ 0.6	61.0 $\pm$ 24.6
100	68.7 $\pm$ 25.5	2.1 $\pm$ 1.4	72.4 $\pm$ 24.1
150	82.4 $\pm$ 29.2	4.2 $\pm$ 2.1	85.5 $\pm$ 27.5
200	98.4 $\pm$ 30.7	6.8 $\pm$ 4.2	96.8 $\pm$ 27.1
250	112.8 $\pm$ 27.0	10.8 $\pm$ 7.8	107.6 $\pm$ 20.4
300	124.9 $\pm$ 22.7	17.2 $\pm$ 14.2	117.1 $\pm$ 12.9
<i>Heat shock</i>			
50	66.1 $\pm$ 8.7	2.2 $\pm$ 1.3	63.9 $\pm$ 9.4
100	92.7 $\pm$ 18.9	7.5 $\pm$ 3.3	85.2 $\pm$ 16.7
150	124.1 $\pm$ 31.1	11.9 $\pm$ 6.0	112.2 $\pm$ 25.6
200	146.4 $\pm$ 31.9	20.0 $\pm$ 12.9	126.4 $\pm$ 20.1
250	177.2 $\pm$ 40.1	34.0 $\pm$ 22.5	143.2 $\pm$ 18.6
300	191.1 $\pm$ 36.1	43.1 $\pm$ 27.1	148.0 $\pm$ 14.8

LVDP, Left ventricular developed pressure.

Table II. Mechanical function of heat-shocked and control hearts subjected to 10 minutes of normothermic ischemia at 37°C

Balloon volume (μL)	Systolic pressure (mm Hg)	Diastolic pressure (mm Hg)	LVDP (mm Hg)
<i>Control</i>			
50	37.7 $\pm$ 27.4	0.8 $\pm$ 0.7	36.9 $\pm$ 27.0
100	51.0 $\pm$ 36.7	1.9 $\pm$ 2.2	49.1 $\pm$ 34.9
150	63.1 $\pm$ 38.3	3.1 $\pm$ 1.1	59.9 $\pm$ 35.4
200	79.2 $\pm$ 37.1	5.0 $\pm$ 7.1	74.2 $\pm$ 31.5
250	99.8 $\pm$ 38.3	7.7 $\pm$ 11.7	92.2 $\pm$ 28.7
<i>Heat shock</i>			
50	94.2 $\pm$ 34.8	1.4 $\pm$ 1.4	76.3 $\pm$ 34.2
100	110.2 $\pm$ 36.9	3.9 $\pm$ 2.2	90.2 $\pm$ 34.9
150	125.3 $\pm$ 36.8	6.5 $\pm$ 3.6	104.8 $\pm$ 33.6
200	137.4 $\pm$ 34.2	10.6 $\pm$ 6.1	116.9 $\pm$ 28.2
250	167.7 $\pm$ 28.7	15.6 $\pm$ 13.9	130.6 $\pm$ 14.9

LVDP, Left ventricular developed pressure.

Fig 4. Postischemic left ventricular developed pressure in heat-stressed and control hearts subjected to (A) 4 hours of cold cardioplegic arrest at 4°C and reperfusion (n = 5) and (B) 10 minutes of global normothermic ischemia at 37°C and reperfusion (n = 4). Values represent the mean $\pm$ SD (* P < .05 vs control).

Fig 2. Twenty-four hours after the heat shock procedure, there was an initial increase in Hsp70 expression, which did not rise or decline over the next 5 hours.

Mechanical function. Systolic, diastolic, and developed pressures for each balloon volume are displayed in Table I for hearts subjected to cardioplegic arrest and in Table II for those subjected to normothermic ischemia. The diastolic stiffness coefficients after ischemia are displayed in Fig 3. There was no difference between the heat-shocked and the control hearts of the 2 protocols. The left ventricular developed pressures of hearts reperfused for 20 minutes after 4 hours of hypothermic cardioplegic arrest indicated better function in the heat-shocked hearts than in the controls, as shown in Fig 4, A. Similarly, developed pressures for

heat-shocked and control hearts reperfused for 20 minutes after 10 minutes of normothermic ischemia are displayed in Fig 4, B. The postischemic developed pressure was significantly higher for heat-shocked hearts than for control hearts. The maximal left ventricular developed pressure for both groups was higher for heat-shocked than for control hearts after both cold cardioplegic and normothermic ischemia (Fig 5).

Discussion

This study demonstrates that cold cardioplegic arrest enhanced the concentration of Hsp70 in the heat-shocked myocardium. This rapid increase in Hsp70 content was not observed after short periods of normothermic ischemia.

We have previously shown that the degree of myocardial protection against ischemic injury is proportional to the concentration of Hsp70. Both cardiac output and endothelial function were improved when the time interval between heat shock and the onset of ischemia ranged from 24 to 30 hours. Analysis of Hsp70 expression showed maximal levels at 24 hours, which correlated with better mechanical function.⁹ Therefore, this enhanced level of Hsp70 after hypothermia may contribute to the endogenous protective mechanisms of the heart. The data showing the natural time course of Hsp70 after heat shock confirm that this raised level of Hsp70 can be attributed to hypothermic cardioplegic arrest, inasmuch as the experiment without cardioplegic arrest shows no subsequent rise in Hsp70 expression. A further increase in the level of Hsp70 was not observed after normothermic ischemia and reperfusion. This suggests that prolonged hypothermic ischemia plays a vital role in this phenomenon to a greater extent than either ischemia or reperfusion. The increase in Hsp70 after hypothermic ischemia was observed within 20 minutes of reperfusion. The short time interval required for the presence of this enhanced level of Hsp70 suggests that this protein may not necessarily be the result of gene transcription or translation. However, heat shock genes are unusual in that they do not contain any introns or intervening sequences, allowing for rapid transcription of the gene and messenger RNA processing. In response to intracellular stress, the synthesis of genes containing introns is inhibited. Because of the lack of introns in *hsp* genes, replication of the active *hsp* mRNA genes is quickly facilitated, which is essential for rapid deployment of the protein.¹⁴

Rapid expression of Hsp70 has been documented by Liu and associates,¹⁵ demonstrating the presence of Hsp70 within 15 minutes after the onset of heat shock pretreatment, which showed a notable increase after 1 hour of reperfusion. Han and colleagues¹⁶ demonstrated a rapid 46% increase in Hsp70 levels within 20 minutes in cells continuously exposed to magnetic fields. Demidov and coworkers¹⁷ observed an increase in Hsp70 levels in the right atria of patients after cardiopulmonary bypass; the postbypass level of Hsp70 was highest when the duration of cardioplegic arrest was greater than 2 hours. Clarification of the mechanisms responsible for the rapid expression of stress proteins is an important challenge if the observed effects are to be fully explained. The use of hypothermia for organ preservation is based on a reduction in metabolic rate.¹⁸ Although the effects of hypothermia can be both damaging and beneficial, the net result on ischemic tissue is protective. Myocardial hypothermia reduces the energy requirements of the organ by

Fig 5. Maximal left ventricular developed pressure in heat-shocked and control hearts subjected to 10 minutes of global normothermic ischemia at 37°C and reperfusion (n = 4) or subjected to 4 hours of cold cardioplegic arrest at 4°C and reperfusion (n = 5). Values represent the mean $\pm$ SD (* P < .05).

depressing mechanical, electrical, and metabolic activity. This protective effect is potentiated by the addition of hyperkalemic cardioplegia.¹⁸ In conclusion, the results of this study show that hypothermic cardioplegic arrest is capable of inducing Hsp70 in the heat-shocked heart; however, the exact mechanisms have yet to be identified. Our findings highlight an additional pathway for the protective effect of hypothermic cardioplegic arrest.

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REFERENCES

- Currie RW, Karmazyn M, Kloc M, Mailer K. Heat-shock response is associated with enhanced postischemic ventricular recovery. *Circ Res* 1988;63:543-9.
- Richard V, Kaeffer N, Thuillez C. Delayed protection of the ischemic heart—from pathophysiology to therapeutic applications. *Fundam Clin Pharmacol* 1996;10:409-15.
- Patel B, Khaliq A, Jarvis-Evans J, Boulton M, Arrol S, Mackness M, et al. Hypoxia induces HSP 70 gene expression in human hepatoma (HEP G2) cells. *Biochem Mol Biol Int* 1995;36:907-12.
- Kabakov AE, Gabai VL. Heat shock proteins and cytoprotection: ATP-deprived mammalian cells. Heidelberg: Springer-Verlag; 1997.
- Das DK, Maulik N, Moraru II. Gene expression in acute myocardial stress: induction by hypoxia, ischemia, reperfusion, hyperthermia and oxidative stress. *J Mol Cell Cardiol* 1995;27:181-93.

6. Jayakumar J, Smolenski RT, Gray CC, Goodwin A, Kalsi KK, Amrani M, et al. Influence of heat stress on myocardial metabolism and functional recovery after cardioplegic arrest: a 31P N.M.R. study. *Eur J Cardiothorac Surg* 1998;12:467-74.
7. Pell TJ, Yellon DM, Goodwin RW, Baxter GF. Myocardial ischemic tolerance following heat stress is abolished by ATP-sensitive potassium channel blockade. *Cardiovasc Drugs Ther* 1997;11:676-86.
8. Saad AH, Hahn GM. Activation of potassium channels: relationship to the heat shock response. *Proc Natl Acad Sci U S A* 1992;89:9396-9.
9. Amrani M, Corbett J, Boateng SY, Dunn MJ, Yacoub MH. Kinetics of induction and protective effect of heat-shock proteins after cardioplegic arrest. *Ann Thorac Surg* 1996;61:1407-11.
10. Amrani M, Allen NJ, O'Shea J, Corbett J, Dunn MJ, Tadjkarimi S, et al. Role of catalase and heat shock protein on recovery of cardiac endothelial and mechanical function after ischemia. *Cardioscience* 1993;4:193-8.
11. Smolenski RT, Jayakumar J, Seymour A-ML, Yacoub MH. Energy metabolism and mechanical recovery after cardioplegia in moderately hypertrophied hearts. *Mol Cell Biochem* 1998;180:137-43.
12. Wong K, Boheler KR, Bishop J, Petrou M, Yacoub MH. Clenbuterol induces cardiac hypertrophy with normal functional, morphological and molecular features. *Cardiovasc Res* 1998;37:115-22.
13. Amrani M, Corbett J, Allen NJ, O'Shea J, Boateng SY, May AJ, et al. Induction of heat-shock proteins enhances myocardial and endothelial functional recovery after prolonged cardioplegic arrest. *Ann Thorac Surg* 1994;57:157-60.
14. Gray CC, Amrani M, Yacoub MH. Heat stress proteins and myocardial protection: experimental model or potential clinical tool? *Int J Biochem Cell Biol* 1999;31:559-73.
15. Liu X, Engelman RM, Moraru II, Rousou JA, Flack JE, Deaton DW, et al. Heat shock: a new approach for myocardial preservation in cardiac surgery. *Circulation* 1992;86(5 Suppl):II358-63.
16. Han L, Lin H, Head M, Jin M, Blank M, Goodman R. Application of magnetic field-induced heat shock protein 70 for presurgical cytoprotection. *J Cell Biochem* 1998;71:577-83.
17. Demidov ON, Tyrenko VV, Svistov AS, Komarova YY, Karpishenko AI, Margulis BA, et al. Heat shock proteins and cardiosurgery patients. *Eur J Cardiothorac Surg* 1999;16:444-9.
18. Brody WR, Reitz BA. Topical hypothermic protection of the myocardium. *Ann Thorac Surg* 1975;20:66-71.

Notice of correction

In the May 2001 issue of the *Journal*, in the article by Fiser and associates titled "Aerosolized Prostacyclin (Epoprostenol) as an Alternative to Inhaled Nitric Oxide for Patients With Reperfusion Injury After Lung Transplantation" (2001;121:981-2), an error was made. On page 981 the name of one author, Stuart M. Lowson, MD, was omitted. The correct listing of authors is available online at www.mosby.com/jtcvs.