Glucocorticoid pretreatment protects cardiac function and induces cardiac heat shock protein 72

GURO VALEN,1 TSUTOMU KAWAKAMI,1 PEETER TÄHEPÖLD,1 ALEXANDRA DUMITRESCU,1 CHRISTIAN LÖWBEER,2 AND JARLE VAAGE3

1Crafoord Laboratory of Experimental Surgery, 2Department of Thoracic Surgery, Karolinska Hospital, S-171 76 Stockholm; and 3Department of Clinical Chemistry, Huddinge University Hospital, 141 86 Huddinge, Sweden

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Valen, Guro, Tsutomu Kawakami, Peeter Tähepöld, Alexandra Dumitrescu, Christian Löwbeer, and Jarle Vaage. Glucocorticoid pretreatment protects cardiac function and induces cardiac heat shock protein 72. *Am J Physiol Heart Circ Physiol* 279: H836–H843, 2000.—Acute administration of glucocorticoids reduces inflammation. Increasing knowledge of the mechanisms of action indicate that pretreatment with glucocorticoids could have organ-protective effects. We investigated whether pretreatment with methylprednisolone (MP) protected the heart against ischemia-reperfusion dysfunction, and we hypothesized that this protection might be due to induction of the cardioprotective heat shock protein 72 (HSP72). Rats were given vehicle or MP-40 mg/kg im as a double injection starting either 24 or 120 h (5 days) before their hearts were excised for Langendorff perfusion (n = 6–11 hearts in each group). MP improved left ventricular function and coronary flow during reperfusion after 30 min of global ischemia and reduced infarct size. Cardiac HSP72 gradually increased in a 24-h time course after MP treatment, and the increase was sustained 5 days (immunoblotting). HSP72 mRNA was either reduced or unchanged, indicating a posttranscriptional regulation. Pretreatment with hydrocortisone or dexamethasone (n = 7–8 hearts of each) similarly increased cardiac HSP72 24 h afterward. This paper demonstrates that glucocorticoids increase cardiac HSP72 and protect organ function against ischemia-reperfusion injury.

methylprednisolone; dexamethasone; hydrocortisone; ischemia-reperfusion

**APPLICATION OF MOLECULAR BIOLOGY techniques has provided new insights into a range of molecular mechanisms of glucocorticoid action. The most well-characterized anti-inflammatory effects of glucocorticoids are the redirection of leukocytes, repression of proinflammatory cytokines and leukocyte adhesion molecules, and induction of antioxidants (1, 2, 12, 23). Glucocorticoids bind to cytoplasmatic glucocorticoid receptors containing two subunits of the heat shock protein 90 kDa family (HSP90) (2, 5). Thereafter HSP90 dissociates, allowing rapid nuclear translocation of the activated receptor-steroid complex. In the nucleus, the complex binds to glucocorticoid response elements in the promoter region of steroid-responsive target genes, resulting in either transcription or repression of the targeted gene (2). Glucocorticoids also inhibit activation of the transcription factors nuclear factor-κB (NFκB) and activator protein 1 (AP-1), and they reduce their DNA-binding activity (17). Additionally, steroids may cause posttranscriptional up- or downregulation of target substances (1, 8).

Glucocorticoids given acutely are beneficial against inflammation in various experimental models, and they have even been employed to reduce the inflammatory effects of extracorporeal circulation during open-heart surgery (6, 17, 19, 22). However, the acute effects of glucocorticoids cannot be through activation/repression of steroid-targeted genes. Treatment regimens aiming at exploiting more long-term molecular actions of glucocorticoids could potentially be even more beneficial than acute administration. In isolated rat lungs subjected to oxidative stress, both acute administration as well as pretreatment with methylprednisolone (MP) attenuated airway and vascular responses, the most profound effect found by pretreatment (9, 10).

Heat shock proteins of the 70-kDa family (HSP70) are cytoprotective proteins involved in protein traffic and folding, translocation of proteins across membranes, and gene regulation (11, 15). The HSP70 family consists of the constitutive HSP73 (73 kDa) and the inducible HSP72 (72 kDa). HSP70 have powerful cardioprotective actions that may be related to the removal of misfolded proteins after injury and the reestablishment of a normal cardiac protein synthesis (11, 15). Additionally anti-inflammatory effects of heat shock proteins via inhibition of NFκB/AP-1-regulated inflammatory genes may be of importance (17). Induction of HSP70 by hyperthermia or brief episodes of ischemia and reperfusion (ischemic preconditioning) reduces infarct size and improves cardiac function (13, 18). Transgenic mice expressing high levels of HSP70 are protected against ischemia-reperfusion injury (14, 16). Unfortunately, it is presently difficult to exploit.
the cardioprotective actions of HSP70 in patients, because they cannot be heated to 41–42°C, and ischemic preconditioning per se is not a potentially large-scale therapy. A pharmacological agent inducing expression of HSP70 without undesirable side effects has not yet been characterized.

The aim of the present study was twofold. First, we investigated whether glucocorticoids employed as pre-treatment could protect isolated rat heart function when subjected to global ischemia and reperfusion. Second, we investigated whether the functional protection afforded could be due to induction of heat shock proteins of the 70-kDa family.

MATERIALS AND METHODS

Rat Heart Perfusion

The investigation conforms with the Guide for the Care and Use of Laboratory Animals published by the National Institutes of Health and was approved by the regional ethics committee of animal research. Male Sprague-Dawley rats (200–300 g) were anesthetized with diethyl ether, and 200 IU heparin was injected into the femoral vein. The hearts were then rapidly excised through a median sternotomy and placed in ice-cold buffer during preparation for aortic cannulation. The hearts were retrogradely perfused with gassed (5% CO2, 95% O2) Krebs-Henseleit buffer (in mmol/l: 118.5 NaCl, 25.0 NaHCO3, 4.7 KCl, 1.2 KH2PO4, 1.2 MgSO4 · 7H2O, 11.1 glucose · H2O, and 1.8 CaCl2 · 2H2O) as a modified Langendorff preparation. The perfusion pressure (100 cmH2O) was kept constant. Water jackets around the perfusionary reservoirs and heart chamber kept the temperature at 37°C throughout the experiments. A balloon was inserted into the left ventricle via the left atrium for isovolumetric recordings of left ventricular systolic (LVSP) and end-diastolic (LVEDP) pressures. Coronary flow (CF) was measured by timed collections of the coronary effluent. Heart rate (HR) was counted from the pressure curves. Left ventricular developed pressure (LVDP) was calculated (LVDP = LVSP – LVEDP). Global ischemia was induced by clamping the inflowing. Only hearts with LVSP between 60 and 160 mmHg, LVEDP 0 mmHg, CF 8–16 ml/min, and HR 240–360 beats/min at the end of a 25-min stabilization were included.

Experimental Protocol

Rats (n = 10) were injected with MP (40 mg/kg im, Solumedrol, Pharmacia Upjohn) 24 and 12 h before Langendorff perfusion with 30 min of global ischemia followed by 60 min of reperfusion, and these preparations were compared with preparations using vehicle injection only (n = 10). Other rats were MP treated 120 and 108 h before heart excision (n = 11), and the cardiac function was compared with vehicle only (n = 6). Additional hearts were sampled 24 h (13 MP, 13 vehicle) or 120 h (7 MP, 7 vehicle) after the start of injections and were compared with untreated controls (n = 7) to assess tissue HSP72 by immunoblotting. To follow the time course of HSP72 induction, hearts were sampled 0.5, 1, 2.5, 6, and 12 h after the first injection of MP. Other rats were given a second injection and sampled after 12.5, 13, 14.5, 18, and 24 h total observation time (n = 3 at each time point). The hearts were divided in two for analysis of protein or mRNA. Two other glucocorticoids, hydrocortisone (150 mg/kg, Solucortef, Pharmacia Upjohn) and dexamethasone (0.29 mg/kg, Decadron, Merek Sharpe & Dome) were given intramuscularly 24 and 12 h before heart sampling for immunoblotting were performed and were compared with the vehicle-only group (n = 8 in each group). Samples of the coronary effluent were collected before ischemia and after 20 min of reperfusion for measurements of creatine kinase (CK) and lactate dehydrogenase (LD) release from hearts of vehicle- or MP-treated animals (24-h model), and hearts were sampled at the end of reperfusion for assessment of infarcted tissue by triphenyl tetrazolium chloride (TTC, Sigma Chemical, St. Louis, MO) (n = 7–8 of each).

Evaluation of Infarct Size

After 60 min of reperfusion, the hearts of the animals treated with MP or vehicle were perfused with 10% TTC at a total volume of 3 ml delivered at 100 cmH2O. The hearts were fixed in 4% formaldehyde for 24 h and thereafter preserved in 10% sucrose in PBS. The hearts were cut manually into 1-mm transverse slices. The sections were visualized in a computer-imaging system (LEICA Qwin, Leica Imaging Systems, Cambridge, UK), and infarct size was marked and calculated in Adobe Photoshop 5.0. An unstained epicardial ring appeared in all hearts. After we calculated the size of this ring in various experimental groups and found it constant independent of intervention, we interpreted the outer ring as reduced epicardial LDH activity unrelated to the intervention, and we excluded the ring from the calculation of infarct size. The area of infarcted myocardium was calculated as the percentage of the total ventricular area minus cavities, and the mean value of all sections in one heart was treated as one value and used for statistics.

CK and LD

One-milliter samples of the coronary effluent were collected in precooled tubes, and albumin was added to a final concentration of 40 g/l to stabilize the samples. The samples were rapidly frozen at −70°C and kept until analysis. LD was measured with spectrophotometry using LD optimized BM/Hitachi 917/Keysys reagent (Boehringer Mannheim, Mannheim, Germany) on the Hitachi 917 analyzer (Naka, Japan). CK was measured with spectrophotometry using CK NACTivated BM/Hitachi 917 Systems Pack reagent (Boehringer Mannheim) on the Hitachi 917 analyzer. CK and LD in the coronary effluent were calculated as amount released per minute [CK or LD (μkat/ml) × CF (ml/min) = μkat/min] (European μkat/l = 1/60 American U/l).

Immunoblotting

Rat hearts were weighed and frozen at −70°C until processing. Frozen hearts were homogenized using a Polytron apparatus PT 1200 CL (Kinematica, Switzerland) with 1 ml of cold lysis buffer (50 mmol/l Tris · HCl, pH 7.5, 2% SDS, 1 mmol/l phenylmethylsulfonyl fluoride) added per 100 mg of tissue. Insoluble material was removed by centrifugation at 15,000 g (5 min, 4°C). Protein content was determined using the bicinchoninic acid reagent (Pierce, Rockford, IL) with bovine serum albumin as a standard. The lysates were mixed with Laemmli buffer and boiled for 5 min, and proteins were separated by SDS-PAGE in 10% gels (34 μg protein/lane). The gels were either stained with Coomasie blue or transferred to presoaked nitrocellulose membranes (Hybond-C pure; Amersham Life Science) for 90 min. The membranes were blocked in PBS, Dulbecco’s medium (GIBCO BRL, Life Technology) with 0.1% Tween and 5% nonfat dry milk for 1 h at room temperature followed by overnight incubation at 4°C with anti-HSP72 or anti-HSP73 mouse monoclonal antibodies (StressGen Biotechnologies, Victoria, Canada) diluted
1:1,000. Goat anti-mouse IgG-alkaline phosphatase (StressGen Biotechnologies) diluted 1/1,000 and an alkaline phosphatase conjugate substrate kit (Bio-Rad Laboratories, Hercules, CA) were used for visualization. The blots were scanned into Adobe Photoshop 5.0 (Adobe systems, San Jose, CA), and the optical densities of the bands were calculated.

mRNA Extraction and cDNA Synthesis

Frozen tissue was homogenized in a microdismembrator, and mRNA was extracted using a Dynabeads mRNA direct kit (Dynal, Oslo, Norway) following the manufacturer's procedure. Single-stranded cDNA synthesis was performed by Superscript II (Life Technologies, Paisley, UK) according to the manufacturer's instructions, using random hexamers (Life Technologies) as primers in the presence of RNAsin (Promega, Madison, WI).

PCR Reaction

Gene expression of HSP72 in rat heart biopsies was evaluated with a semiquantitative RT-PCR, where relative changes of mRNA were measured in relation to a reference gene in the linear phase of the PCR reaction. Each reaction was run in a volume of 25 μl. A master mix consisting of all reaction components [6.25 mmol/l dNTP, 1.5 mmol/l MgCl2, 0.02 U Taq polymerase (all Life Technologies), and 5 μCi [33P]dATP (NEN, DuMedical Scandinavia)] per reaction was used. The primer for H3, 5'-CCG CCA AGA ACC AGG TG (base position 818–837) and 3'-ACG CCG CCA GTG TT (base position 1106–1125) gave an amplification product of 308 base pairs. The PCR reaction was performed with 94°C for 2 min; 30 s, 60°C for 30 s, 72°C for 45 s followed by cycles of 30 s at 94°C, 30 s at 60°C, 45 s at 72°C. The H3 samples were run a total of 22 cycles, and HSP72 were run 26 cycles after determining the linear phase of the PCR reaction. Control PCR of the mastermix without cDNA with the primers were routinely done in all samples, and no contamination was detected. All PCR reactions were run at least twice. The PCR products were separated by electrophoresis on a 5% polyacrylamide gel and evaluated in a phosphorimager (BioImaging Analyzer System BAS 1000, Fuji). After optical density of the bands was measured, the ratio between test gene and H3 was calculated.

Statistics

Hemodynamic data were evaluated with an ANOVA test with a Scheffe’s post hoc test, whereas the band densities of HSP72 protein, infarct size, and release of cardiac enzymes were evaluated with a Student’s t-test. P < 0.05 was considered significant. Data are presented as means ± SE.

RESULTS

Cardiac Function

Left ventricular function: 24 h after the start treatment. Hearts were isolated from rats 24 h after the start of injection with a double bolus dose of MP given intramuscularly and subjected to global ischemia (Langendorff model). LVEDP increased during reperfusion in hearts of vehicle-treated animals. Hearts of MP-treated animals tended to have a reduced increase of LVEDP (P < 0.09) (Fig. 1A).

LVDP was depressed during the reperfusion of control hearts. This depression was attenuated by pretreatment with MP (Fig. 1B).

CF was reduced during reperfusion in controls. MP tended to increase CF during reperfusion without reaching significance (P < 0.08). HR and LVSP were not different between groups (not shown).

Left ventricular function: 120 h after start of treatment. When hearts were isolated 5 days after MP or vehicle injections, MP attenuated the increase of LVEDP during reperfusion (Fig. 1D).

LVDP (Fig. 1E), LVSP, and HR were not different between groups. The depression of CF during reperfusion was attenuated by pretreatment with MP (Fig. 1F).

Infarct Size

In hearts of vehicle-treated animals, ~31% of total myocardial tissue was calculated as unstained by TTC after 60 min of reperfusion. MP reduced this area to 20% (P < 0.04) (Fig. 2).

Release of CK and LD

After 20 min of reperfusion after global ischemia, release of LD and CK into the coronary effluent increased (Fig. 3). Pretreatment with MP starting 24 h before heart isolation tended to reduce the release of CK and LD compared with vehicle, but this was not significant (Fig. 3).

Heat Shock Protein 72

Time course. The time course of possible HSP72 induction was investigated in protein extracts from rat hearts euthanized at different time points after MP injection, using immunoblotting with a mouse monoclonal antibody and comparing with a monoclonal antibody against the constitutive HSP73. HSP73 did not change after treatment with MP, and a representative immunoblot is shown in Fig. 4A. HSP72, however, increased with time after MP treatment, and a typical time course is shown in Fig. 4B. When the optical densities of the immunoblot bands were calculated, a gradual increase of HSP72 protein was found with a maximal level 18–24 h after the start of the double injection (Fig. 4C). Untreated controls were similar to vehicle-treated animals (Fig. 4C). When the gels were stained with Coomassie blue, the protein contents in the different lanes were alike (not shown). In an attempt to characterize the mechanism of HSP72 increase, a semiquantitative RT-PCR employing histone H3 as a reference gene to evaluate the amount of cDNA and relative changes in test gene was performed. Radiolabeled nucleotides were incorporated for detection of PCR products in the linear phase of the PCR reaction. A polyacrylamide gel with RT-PCR products evaluat-
ing the time course of cardiac mRNA for HSP72 serially after the start of the MP injection is shown in Fig. 4D. When reading the optical densities of the test gene band and calculating the ratio to the H3 gene band, we found that HSP72 mRNA tended to decrease in conjuction with MP injection (Fig. 4E). Otherwise the gene expression of HSP72 was relatively unchanged.

Start of treatment (24 or 120 h after). To quantify the increase of HSP72 protein, the hearts of 13 rats treated with MP and 13 rats treated with vehicle were sampled 24 h after treatment was started corresponding to time of Langendorff perfusion. Immunoblotting was performed on protein extracts, and a representative immunoblot is shown in Fig. 5A. When quantifying the optical density of the bands in all hearts, we found MP to increase HSP72 (Fig. 5E). MP pretreatment starting 120 h earlier also increased the HSP72 band densities (Fig. 5, B and E). To evaluate whether the HSP72 induction was a general glucocorticoid effect, rats were treated with hydrocortisone or dexamethasone as a double injection 24 and 12 h before heart excision and were compared with vehicle-treated controls. Hydrocortisone (Fig. 5, C and E) and dexamethasone (Fig. 5, D and E) increased HSP72 24 h after the start of the treatment (Fig. 5E).

**DISCUSSION**

The main findings of the present study were that pretreating rats with MP upregulated cardiac HSP72 expression through a posttranscriptional mechanism. Two other glucocorticoids, dexamethasone and hydrocortisone, also increased HSP72 protein, indicating that this is a general glucocorticoid effect. The constitutive HSP73 was unchanged. Additionally, pretreatment with MP starting 24 h before the hearts were excised protected left ventricular function against global ischemia and reperfusion. LVSP and LVEDP were not significantly influenced after MP pretreatment, but LVDP was improved when evaluating the whole time course of reperfusion. Five days after the same treatment, no improvement of developed pressure was found, but an attenuation of the ischemia-induced increase of LVEDP as well as an increase of CF occurred concurrent with a persistant increase of HSP72. The protection of different functional parame-

Fig. 1. A: left ventricular end-diastolic pressure (LVEDP) in Langendorff-perfused rat hearts subjected to 30 min of global ischemia and 60 min of reperfusion after pretreatment with methylprednisolone (MP, 40 mg/kg im) or vehicle (VEH) 24 and 12 h before the hearts (−24 h, left) were excised. B: left ventricular developed pressure (LVDP) in the same hearts. C: coronary flow (CF) in the same hearts. D: LVEDP in rat hearts treated with MP 120 and 108 h (5 days) before hearts (−120 h, right) were excised. E: LVDP in the same hearts. F: CF in the same hearts. All values are means ± SE. BI, before ischemia. n = 6–11 hearts in each group.
ters in the different time frames may indicate that glucocorticoid-responsive factors other than HSP, synthetized or degraded within the 1–5 days of observation, may have been important for the functional protection. Both CK and LD release increased during reperfusion, indicating that irreversible injury took place in the present model of global ischemia. MP tended to reduce release of both enzymes. TTC staining at the end of reperfusion in hearts isolated 24 h after the start of MP treatment showed reduced infarctions in these hearts, suggesting that the protection afforded may be primarily against necrosis rather than stunning.

MP given acutely is beneficial against oxidative stress in the central nervous system, heart, liver, lungs, and kidneys (6, 9, 10, 17, 19, 22). However, glucocorticoids as a pretreatment for the preservation of organ function has not been widely investigated. We have previously shown that MP given to rats before subjecting their isolated lungs to oxidative stress attenuated airway and vascular responses (9, 10), which is in accordance with the present findings. We found no other papers investigating organ function after glucocorticoid pretreatment. We chose the dose of 40 mg/kg and double injections because this treatment protocol was efficient in preserving the function of rat lungs, and because the dose is within clinical range. The acute anti-inflammatory effects of MP must be mediated by other mechanisms than the pretreatment effects. In isolated, perfused rat lungs exposed to oxidative stress, the effects of MP pretreatment and MP given acutely were different and synergistic (9, 10). The well-characterized, long-term anti-inflammatory effects of glucocorticoids, such as influence on leukocyte traffic, and reduction of cytokines and leukocyte adhesion molecules (1, 2) could not be expected to have a large impact on cardiac function or cell viability in the present isolated heart model lacking blood-to-cell interactions.

To investigate the mediator of the beneficial functional effects, we hypothesized that the glucocorticoids increased HSP72 expression. We have previously tar-

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Fig. 2. Top, representative transverse section of triphenyl tetrazolium chloride (TTC)-stained hearts at the end of reperfusion of hearts isolated 24 h after the start of injection of vehicle or MP as a double bolus dose and Langendorff-perfused with 30 min of global ischemia and 60 min of reperfusion. Bottom, calculated infarct areas of the corresponding groups (n = 7–8, *P < 0.04).

Fig. 3. Release of creatine kinase (CK, A) and lactate dehydrogenase (LD, B) into the coronary effluent of isolated rat hearts pretreated with VEH or MP-24 starting 24 h before their hearts were excised (n = 7 of each, means ± SE). Samples were collected immediately before 30 min of global ischemia and after 20 min of reperfusion. There were no significant differences between groups.
geted known glucocorticoid responsive genes such as antioxidants (catalase, superoxide dismutase, and glutathione peroxidase) and inducible nitric oxide synthase as possible mediators of cardioprotection in this model, but without finding any significant influences to explain the protected cardiac function (22). HSP72 expression in cardiac tissue was assessed by immunoblotting. We found HSP72 to increase gradually with time during a 24-h observation period, and HSP72 was still increased 5 days after treatment, at which time cardiac function was still protected. To investigate whether this was a general glucocorticoid effect, the glucocorticoids hydrocortisone and dexamethasone were injected in the same treatment regimen. Hydrocortisone is the glucocorticoid produced physiologically by the adrenal glands, whereas dexamethasone is a synthetic glucocorticoid. The concentrations of hydrocortisone and dexamethasone were selected as those clinically recommended for treatment of shock. These glucocorticoids also induced expression of HSP72, indicating that this is a general glucocorticoid action. One previous paper (20) demonstrated induction of HSP72 by dexamethasone in a model of cultured rat small intestine cells.

A semiquantitative RT-PCR employing radiolabeled nucleotides for detection in the linear phase of the PCR reaction and histone H3 as a reference gene was employed to evaluate gene expression of HSP72 in a 24-h time course after the start of injections. There were no large changes of relative mRNA but a tendency toward reduction shortly after both MP injections. Although

**Fig. 4.** A: immunoblot of protein extracts from rat hearts employing a mouse monoclonal anti-heat shock protein (HSP) 73 antibody (product 73 kDa), where each band corresponds to a time course after injection of MP (40 mg/kg im) as a double bolus dose depicted on the x-axis on C. There was no change in expression of the constitutive HSP73. B: immunoblot of the same hearts as in A but with a monoclonal anti-HSP72 antibody (product 72 kDa) showing the time course of HSP72 induction after MP was injected (indicated with arrows in C). Lane 1 (from the left): unstimulated control; lane 2: rat treated with vehicle; the rest of the lanes are hours after MP injection corresponding to C. The last lane (24 h) corresponds to when the hearts were employed for Langendorff perfusion. C: optical density of the Western blot bands as shown in B (mean values of n = 3 at each time point). C, unstimulated controls; V, hearts treated with vehicle; the rest are hours after MP injection. D: representative polyacrylamide gel with radiolabeled RT-PCR products from the hearts in A and B. The first lane shows the 100-base pair DNA ladder. In each following well, the reference gene histone H3 (215 base pairs) is included to evaluate relative changes in the HSP72 gene (308 base pairs), as the amount of RNA was not the same in each extraction. E: changes in HSP72 mRNA when reading band densities as shown in D, and calculating ratio test gene/H3 (mean values of n = 3).

**Fig. 5.** Immunoblots of cardiac extracts of rats treated intramuscularly with glucocorticoids or VEH before sampling their hearts. Each band represents protein extracts from different hearts. A: 40 mg/kg of MP-24, 24 and 12 h before sampling, and corresponding vehicle. B: 40 mg/kg of MP, 120 and 108 h before sampling (MP-120). C: 150 mg/kg of hydrocortisone (HC) given 24 and 12 h before sampling. D: 0.29 mg/kg of dexamethasone (DEX) injected 24 and 12 h before sampling. E: optical densities of Western blot bands after glucocorticoid treatment. Values are means ± SE of a total of 13 (MP –24 and VEH –24), 7 (MP –120 and VEH –120), and 8 hearts (HC, DEX, and VEH) are shown.
mRNA tended to increase 6 h after the second injection, this does not explain the increased protein found hours earlier in the same hearts. Thus glucocorticoids induce HSP72 through a posttranscriptional mechanism. In general, the regulation of HSP72 is known to be both at a transcriptional and at a posttranscriptional level (8, 11, 15). Several HSP72 transcription factors that are translocated into the nucleus and induce transcription in response to different stimuli such as environmental stress (i.e., drugs, heat shock), non-stress conditions (i.e., growth factors), and disease states (i.e., ischemia, cancer) have been identified. However, activation of these is not necessarily involved in the induction of heat shock genes (8). At the posttranscriptional level, increased (8) HSP70 mRNA stability as assessed by a run-on assay has been found after heat shock. Possibly MP induced HSP72 through increasing mRNA stability in the present study. Another possibility for the increased cardiac contents of HSP72 after glucocorticoid pretreatment is through a HSP70-to-HSP90 interaction. The connection between the HSP90 of the glucocorticoid receptor and HSP70 is partially obscure. HSP70 is not a structural component of the glucocorticoid receptor. However, the nonactivated steroid receptor utilizes HSP70 and other accessory proteins for assembly into a heteromeric receptor complex, whereafter it can be activated (3, 5, 7). The mature glucocorticoid receptor will contain less HSP70 and accessory proteins. Possibly some of the increased HSP72 in the present study may be derived from dissolving preformed HSP70-accessory protein complexes during activation of the steroid receptor due to increased amounts of glucocorticoids.

The protection found in hearts of animals pretreated with MP could be due to induction of HSP72. Up to now, it has been difficult to exploit the cardioprotective actions of HSP70, because employing the classical stimuli for their induction, heating, or preconditioning with ischemia is not feasible in patients. Pharmacological agents inducing their expression without unacceptable side effects have not previously been found. We did not investigate other organs than the heart in the present study, but it is likely that a general whole body HSP72 induction may have taken place. As glucocorticoids are clinically employed drugs with well-characterized actions, they may potentially be used for cardioprotection before cardiac surgery or percutaneous transluminal coronary angioplasty, in patients with unstable angina, and imminent myocardial infarction. Potentially glucocorticoid treatment and induction of heat shock proteins could be employed in all organs subjected to inflammation, stress, and injury. It may possibly be given to organ donors and before major surgery.

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REFERENCES


