HIGHLIGHTED TOPIC | A Physiological Systems Approach to Human and Mammalian Thermoregulation

Heat shock protein 72 overexpression protects against hyperthermia, circulatory shock, and cerebral ischemia during heatstroke

W. C. Lee,1,* H. C. Wen,2,* C. P. Chang,3,4 M. Y. Chen,1 and M. T. Lin4

1Division of Biotechnology, Animal Technology Institute Taiwan, Chunan, Miaoli; 2Department of Radiological Technology, Yuanpei University of Science and Technology, Hsinchu; 3Department of Biotechnology, Southern Taiwan University of Technology, Tainan; and 4Department of Medical Research, Chi-Mei Medical Center, Tainan, Taiwan

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Lee, W. C., H. C. Wen, C. P. Chang, M. Y. Chen, and M. T. Lin. Heat shock protein 72 overexpression protects against hyperthermia, circulatory shock, and cerebral ischemia during heatstroke. J Appl Physiol 100: 2073–2082, 2006; doi:10.1152/japplphysiol.01433.2005.—This study extends our earlier studies in rats by applying our heatstroke model to a new species. Additionally, transgenic mice are used to examine the role of heat shock protein (HSP) 72 in experimental heatstroke. Transgenic mice that were heterozygous for a porcine HSP70i gene (+HSP72), transgene-negative littermate controls (−HSP72), and normal Institute of Cancer Research strain mice (ICR) under pentobarbital sodium anesthesia were subjected to heat stress (40°C) to induce heatstroke. In −HSP72 or ICR, the values for mean arterial pressure, the striatal blood flow, and the striatal PO2 after the onset of heatstroke were significantly lower than those in preheat controls. The core and brain temperatures, the extracellular concentrations of ischemic and injury markers in the striatum, and the striatal neuronal damage scores were significantly greater than those in the preheat controls. In +HSP72 or ICR, the body temperatures, cell ischemia content, and injury marker in the striatum were significantly higher, and the mean arterial pressure, striatal blood flow, and striatal PO2 concentration were significantly lower during heatstroke than in +HSP72. Accordingly, the latency and the survival times for +HSP72 significantly exceeded those of −HSP72 or ICR. These results demonstrate that the overexpression of HSP72 in multiple organs improves survival during heatstroke by reducing hyperthermia, circulatory shock, and cerebral ischemia and damage in mice. transgenic mice; heat stress; glutamate; glycerol; cerebral blood flow

HEATSTROKE IS A LIFE-THREATENING disease that results from exposure to high ambient temperature (4). The diagnosis of heatstroke is based on hyperpyrexia (elevated core body temperature of over 40°C), multi-organ damage and dysfunction (such as arterial hypotension, acute myocardial infarction, hepatic and renal failure), and predominant central nervous system dysfunction that causes delirium, convulsion, or coma (22, 44). In many respects, heatstroke resembles sepsis, and evidence is growing that endotoxemia and cytokines may be implicated in its pathogenesis (4, 10, 38). Hemorrhagic diathesis is invariably present in victims of severe heatstroke, and autopsy findings reveal hemorrhage and necrosis with widespread microthrombi in many vital organs (6, 9, 39, 55, 57, 62). These results indicate that disseminated intravascular coagulation and excessive activation of inflammation are involved in the pathogenesis of heatstroke. Immediate initiation of rapid and effective cooling is crucial in a patient with heatstroke (1). Currently, no medications for treating heatstroke are available (25).

The lactate-to-pyruvate ratio is a well-known marker of cellular ischemia, whereas glycerol is a marker of how severely cells are affected by ongoing pathology (13–15, 59). Excessive accumulation of glutamate has been reported in ischemic brain tissue (43, 48). Our previous results established that cerebral ischemia and injury during heatstroke in anesthetized rats are associated with increased production of glycerol, lactate-to-pyruvate ratio, and glutamate in the brain (5, 8, 24). Additionally, the brain or blood PO2 is reduced after the onset of heatstroke. Thus excessive accumulation of cellular glycerol and glutamate as well as an excessive lactate-to-pyruvate ratio in the brains of anesthetized rats may be secondary to cerebral ischemia and hypoxia injury. Moreover, all heat-stressed, anesthetized rats displayed systemic inflammation and activated coagulation, as evidenced by increased tumor necrosis factor-α, prothrombin time, activated partial thromboplastin time, fibrinogen degradation products, and D-dimer, as well as decreased platelet count and protein C concentration during heatstroke (28). Biochemical markers also revealed cellular ischemia and injury, as evidenced by increased plasma levels of blood urea nitrogen (BUN), creatinine, glutamic oxaloacetic transaminase, glutamic pyruvic transaminase, and alkaline phosphatase and increased cerebral levels of glyceral, glutamate, and lactate-to-pyruvate ratio, as well as decreased cerebral levels of partial pressure of oxygen and local blood flow during heatstroke. In severe hyperthermic, anesthetized rats, a loss of compensatory vasoconstriction tone to the mesenteric arterial bed and, hence, a dramatic mesenteric vasodilation, preceded any fall in arterial blood pressure (23). These observations together indicate that anesthetized rats exhibited hypotension, overproduction of cytokines, hypercoagulable state, intracranial hypertension, and cerebral ischemia and injury during heatstroke.

When unanesthetized mice were subjected to acute heat stress by exposing them to whole body hyperthermia treatment,
the stress response indicators, such as mortality, hypothermia, apoptosis, inflammatory cytokines, heat shock protein (HSP) 70, nitrite, inducible nitric oxide synthase (iNOS), and kinin B1 receptor in various tissues were observed (7, 29, 30). One objective of the current study is to apply our previously published model of rat heatstroke to mice to take advantage of transgenic models. The establishment of a mouse model of heatstroke supports the examination of changes in HSP, hyperthermia, hypotension, cerebral ischemia, and neuronal damage in HSP72 transgenic mice and correlates these changes with those occurring at the tissue level.

Evidence has accumulated that preconditioning with HSP72 can induce thermotolerance. For example, the prior overproduction of HSP72 under thermal or chemical stress (34, 60, 68) increases thermotolerance by reducing the heat stress-induced hyperthermia, arterial hypotension, intracranial hypertension, brain hypoperfusion, decreased baroreceptor reflex sensitivity, cerebral ischemia, and cerebral injury in the rat. The time course of this protection was strongly correlated with the temporal profile of HSP72 expression in multiple organs, including both brain and heart. Whenever the core temperature of a healthy volunteer reached or exceeded 39°C, serum HSP70 levels were elevated (61). In contrast, patients who were classified as having serious heatstroke had hyperthermia (above 40°C), confusion, delirium, or even coma, without increased serum levels of HSP70. Increased levels of HSP70 appeared to correlate with a better outcome for the patient. Equally interesting was that the patients with serious heatstroke exhibited the higher levels of HSP70 autoantibody. The role of HSP72 in protecting against heatstroke-induced circulatory shock and cerebral ischemia has not yet been established using transgenic mice that overexpress HSP72.

This work has two objectives. The first is to assess whether arterial hypotension, hyperthermia, and cerebral ischemia and injury could be induced in anesthetized mice attendant with heatstroke. The second is to investigate the enhanced thermotolerance of HSP72 in this well-established model of heatstroke using transgenic mice that overexpress HSP72.

METHODS

Animals. Transgenic mice that were heterozygous for a porcine HSP70i gene (+ HSP72) and transgene-negative littermate controls (− HSP72) were obtained from Dr. W. C. Lee at Animal Technology Institute Taiwan (ATITT, Chunan, Miaoli, Taiwan). Institute of Cancer Research (ICR) strain mice were purchased from the National Taiwan University Animal Resource Center (Taipei, Taiwan). Three groups of mice, each weighing 28 ± 0.5 g (~8 wk of age), were used. Groups of four mice were housed separately in a group in nalgene polycarbonate cages (46 cm × 24 cm × 20 cm) that had been fitted with HEPA filter cage tops and wood-chip bedding. Rodent laboratory chow and water were provided ad libitum as mice were acclimated to the ambient temperature (Ta) of 30 ± 2°C for a minimum of 2 wk before experimentation (12:12-h light/dark cycle; lights on at 0600). All protocols were approved by the Animal Ethics committee of the Chi Mei Medical Center (Tainan, Taiwan) in accordance with the guide for the Care and Use of Laboratory Animals of the National Institute of Health as well as the guidelines of the Animal welfare Act.

The empirical trial used for the diagnosis of classic human heat stroke is hyperthermia, central nervous system alteration, and a history of heat stress (4, 22). Our previous studies have demonstrated that when anesthetized rats are exposed to an ambient temperature of 42 or 43°C, the observed responses fulfill the empirical trial used for the diagnosis of human heatstroke model. Therefore, in the present experiments, adequate anesthesia was maintained to abolish the corneal reflex and pain reflexes induced in mice by tail pinching. It was administered by an intraperitoneal injection of pentobarbital sodium (50 mg/kg). At the end of the experiments, the control mice and any mice that had survived heatstroke were killed with an overdose of pentobarbital sodium.

Induction of heatstroke. Before heatstroke was induced, the core temperature of each pentobarbital sodium-anesthetized mouse was maintained at ~36°C using a folded heating pad. During heat stress, this pad was removed and heatstroke was induced by exposing the animals to a Ta of 40°C (with a relative humidity of 60% in a temperature-controlled chamber). The moment at which the mean arterial pressure (MAP) fell to 25 mmHg from its peak level was taken to be the onset of heatstroke (19–21, 42). Immediately after the onset of heatstroke, the animals were allowed to recover at room temperature (30°C). As shown in Table 1, the latency for the onset of heatstroke (interval between the start of heat exposure and the onset of heatstroke) was found to be 60 ± 3 min for the normal ICR mice (n = 8). For comparison, both the − HSP72 and the + HSP72 mice were exposed to heat for exactly 60 min and then allowed to recover at room temperature (30°C).

Construction of plasmid vector and generation of transgenic mice. The procedures for generating transgenic mice were those described elsewhere in a previous report (58). Transgenic mice were generated using a chimeric transgene (+ HSP720) that consisted of a porcine HSP70.2 gene (Genbank accession no. AY466608) inserted into vector pCX-EGFP (46). The pCX-EGFP construct places the porcine HSP70.2 gene under the control of the β-actin promoter (PACTIN) and a reporter V5 gene in cardioxyl basin (Fig. 1). The chimeric transgene was cut from the plasmid by SalI and HindIII digestion, purified, and used to generate transgenic mice. The linear DNA preparation was diluted in TE buffer (10 mM Tris·HCl, 0.1 mM EDTA, pH 7.4) to 2 ng/μL. DNA fragments were microinjected into the pronuclei of fertilized eggs to produce transgenic mice. Prepuberal breed ICR mice were used as embryo donors and recipients. The genomic DNA of transgenic positive mice was screened by performing the PCR with primers F8: 5′-GAC GCC AAC GGC ATC CTG AAC-3′ and HSP70V5R: 5′-GCC GAA TTA CCT GAG AGAATC GAG ACC GAG-3′. The PCR reaction was conducted at 95°C for 45 s; 58°C for 45 s, and 72°C for 45 s, repeated for 35 cycles. The length of the PCR product was 0.6 kb. F2 transgenic mice were used to proceed all experimentation. A total of 280 fertilized eggs that contained pACTIN-HSP70-V5 transgene were impregnated in 10 foster mothers by pronuclear microinjection. Of these porcine HSP720 recipients, seven foster mothers were successfully impregnated and a total of 58 pups were obtained. Transgenic and nontransgenic

<table>
<thead>
<tr>
<th>Treatment Groups</th>
<th>Latency, minutes</th>
<th>Survival Time, min</th>
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<tbody>
<tr>
<td>ICR normothermic mice</td>
<td>450 ± 3 (10)</td>
<td>450 ± 2 (10)</td>
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<tr>
<td>[−] HSP72 normothermic mice</td>
<td>450 ± 2 (10)</td>
<td>450 ± 3 (10)</td>
</tr>
<tr>
<td>[+] HSP72 normothermic mice</td>
<td>450 ± 3 (10)</td>
<td>450 ± 4 (10)</td>
</tr>
<tr>
<td>ICR heatstroke mice</td>
<td>60 ± 3* (10)</td>
<td>16 ± 2* (10)</td>
</tr>
<tr>
<td>[−] HSP72 heatstroke mice</td>
<td>59 ± 4* (10)</td>
<td>17 ± 2* (10)</td>
</tr>
<tr>
<td>[+] HSP72 heatstroke mice</td>
<td>67 ± 2* (10)</td>
<td>293 ± 4* (10)</td>
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Data are the means ± SE followed by numbers of mice in parentheses. All groups exposed to heat at 40°C for 59 min. Ta, ambient temperature. *P < 0.05, significance of difference from corresponding values (treatments 1–3, control mice one-way ANOVA, Student’s t test; P < 0.05, significance of difference from corresponding values (treatment 5, control mice one-way ANOVA, followed by Duncan’s test). All groups of animals kept at 30°C were terminated by overdose of urethane at about 450 min.
mice were screened by PCR as stated above. One male pup was verified as carrying the pACTIN-HSP70-V5 transgene (data not shown). The ICR mouse was served as the control.

**Surgery and physiological parameter monitoring.** The right femoral arteries of mice were cannulated with polyethylene tubing (PE-50) under general anesthesia to monitor blood pressure. The animals were positioned in a stereotaxic apparatus (Kopf 1406; Grass Instrument, Quincy, MA) to insert probes for measuring the cerebral blood flow, oxygenation, and temperature in the striatum. The following stereotaxic coordinates of Paxinos and Franklin (47) were used: anterior, interaural, 0.14 mm; lateral, 1.6 mm from the midline; and height, 3.4 mm from the top of the skull.

A 100-μm thermocouple and two 230-μm fibers were attached to the oxygen probe. This combined probe measured oxygen, temperature, and microvascular blood flow. The measurement involves OxyLite and OxyFlo instruments. OxyLite 2000 (Oxford Optronix, Oxford, UK) is a two-channel device (simultaneously measuring PO2 and temperature at 2 sites), and OxyFlo 2000 is a two-channel laser Doppler perfusion monitoring instrument. The combination of these two instruments simultaneously provides data on tissue blood flow, oxygenation, and temperature.

**Measurements of extracellular ischemia and damage markers in striatum.** A microdialysis probe (4 mm in length; CMA/12, Carnegie Medicine, Stockholm, Sweden) was stereotaxically implanted into the left corpus striatum according to the atlas and coordinates of Paxinos and Franklin (47). The probe was pulled out of the skull. The tip of the probe was inserted into the striatum as verified microscopically. Figure 2 presents a typical example of the tip of a probe.

**HSP72 detection.** Animals were killed by decapitation at the end of each experiment to detect of HSP72. Brain, heart, liver, spleen, lung, kidney, skeletal muscle, tail, and testis obtained from [+]HSP72 mice, [−]HSP72 mice, and normal ICR mice were placed into microcentrifuge tubes and stored at −20°C. For protein extraction, the samples were weighed, rapidly thawed in five volumes of homogenizing buffer comprised of 0.3 M sucrose, 50 mM Tris·HCl, and 0.17 mM pefabloc SC, and then homogenized by a sonic Vibra cell (Sonic and Material). After centrifugation at 13,200 g for 5 min at 4°C, total protein concentrations were analyzed using a Bio-Rad protein assay kit (Bio-Rad, Hercules, CA). The samples (50 μg/lane) were incubated for 5 min at 95°C in laemmlı buffer and then separated on 10% SDS-polyacrylamide discontinuous gel. After electrophoresis, the gels were processed for use in an immunoblotting study. Polyclonal antibody that is specific for HSP72 (SPA-812, Stress Gen) and monoclonal antibody specific for actin (CB-111, Cashmire/Biotech) were used as the primary antibodies, and then anti-rabbit immunoglobulin G conjugated with alkaline phosphatase (Amersham Pharmacia) was used as the secondary antibody. The membranes were washed, incubated in an alkaline phosphatase developing buffer reagent plus kit, and exposed to an x-ray film. The expression of HSP72 and actin were semiquantified using a gel densitometric scanning program.

**Neuronal damage score.** At the end of each experiment the brain was removed, fixed in 10% neutral buffered formalin, and embedded in paraffin blocks. Serial (10 μm) sections through the striatum were stained with hematoxylin and eosin for microscopic evaluation. The extent of striatal neuronal damage was scored on a scale of 0−3, modified from the grading system of Pulsinelli et al. (50), in which 0 is normal, 1 means that ~30% of the neurons are damaged, 2 means that ~60% of the neurons are damaged, and 3 means that 100% of those neurons are damaged. Each hemisphere was evaluated independently without knowledge of the experimental conditions. When examined for neuronal damage in gray matter, only areas other than those invaded by probes were assessed. Our previous results (20, 37) have shown that the hippocampal, striatal, hypothalmic, and cortical neurons are all susceptible to cerebral ischemia after heatstroke. However, in this work, only the striatal regions were histologically examined for neuronal damage.

**Data presentation and statistical analysis.** As shown by our previous findings (19, 20, 36, 68), heatstroke causes both cerebral ischemia and neuronal damage in different brain structures, including cortex, striatum, hypothalamus, thalamus, and hippocampus. In the current study, the local blood flow and neuronal damage of the striatal region were measured. All probes were placed in regions of the striatum as verified microscopically. Figure 2 presents a typical example of the tip of a probe.

Data are presented as mean ± SE. ANOVA was used to conduct factorial experiments, and Duncan’s multiple-range test was performed to make post hoc multiple comparisons among means. The Wilcoxon’s signed rank test was used to compare the neuronal damage across two groups. The Wilcoxon tests convert scores to ranks, a sum of the ranks is calculated, and critical values of the sum are provided to test the null hypothesis at a given significant level. The data were given as “median,” first quartile, and third quartile. A P value of less than 0.05 was considered as statistically significant.

**RESULTS**

**Overexpression of HSP72 in multiple organs improves heat tolerance in transgenic mice.** Figure 3 displays the Western analyses of protein extracts from different tissues of normal ICR (C), [−]HSP72 (N), and [+]HSP72 (T) mice killed after acclimatization at room temperature (30°C) for at least 90 min. The fractions obtained from the brain, heart, liver, spleen, lung, kidney, skeletal muscle, and tail of [+]HSP72 mice (n = 8) had higher OD values of inducible HSP72 than those of normal
ICR (n = 8) or [–]HSP70 (n = 8) mice. Figure 4 shows Western analyses of protein extracts from different tissues of normal ICR, [–]HSP72, and [+]HSP72 mice killed after heat stress (40°C for 59 min) followed by 10 min at room temperature (30°C) exposure. Again, the fractions obtained from the various tissues had higher OD values of inducible HSP72 than those of normal ICR ([–]HSP70 mice (n = 8).

In separate experiments, these three groups of animals were held at room temperature (Ta = 30°C for 59 min) or exposed to heat stress (Ta = 40°C for 59 min) before remaining for 10 min at room temperature (Ta = 30°C). Table 1 summarizes the latency for the onset of heatstroke and the survival times. The table shows that the latency for the onset of heatstroke and the survival time were 59–60 min and 16–17 min, respectively, for normal ICR or [–]HSP72 mice. However, the values for both the latency for onset of heatstroke and survival time during heatstroke were significantly higher, 67 and 293 min, respectively, in [+]HSP72 mice.

*Overexpression of HSP72 in multiple organs reduced arterial hypotension, cerebral ischemia, and cerebral damage during heatstroke. Figure 5 shows the effects of heat exposure on core temperature (Tco), mean arterial pressure (MAP), heart rate (HR), striatal blood flow (SBF), and striatal PO2 in different groups of animals. In either [–]HSP72 or normal ICR mice, 10 min after the onset of heatstroke, the values for MAP, SBF, and striatal PO2 were significantly lower than those of their preheat controls (time 0). However, the values for Ta 10 min after the heatstroke were significantly higher than those of their preheat controls. The overproduction of HSP72 in [+]HSP72 mice significantly ameliorated heatstroke-induced hyperthermia, arterial hypotension, bradycardia, and striatal hypoxia.

In either normal ICR or [–] HSP72 mice, the striatal concentrations of glutamate and glycerol and lactate-to-pyruvate ratio, as well as brain temperature after the onset of heatstroke, were significantly higher than those of their preheat controls. The induction of HSP72 in [+]HSP72 mice significantly reduced the increased levels of glutamate, level of glycerol, lactate-to-pyruvate ratio in striatum, and brain temperature during heatstroke.

Table 2 summarizes the effects of heat exposure (40°C for 59 min), followed by standing for 10 min at room temperature (30°C) on the neuronal damage scores of the striatum from normal ICR mice, [–]HSP72 mice, or [+]HSP72 mice. The scores for striatal neuronal damage in ICR or [–]HSP72 heatstroke mice significantly (P < 0.05) exceeded those of the respective normothermic controls. However, the striatal neuronal damage scores in [+]HSP72 heatstroke mice were significantly lower than those of ICR or [–]HSP72) heatstroke.
Fig. 4. Western blots of protein extracts from normal ICR (C), [−]HSP72 (N), and [+]HSP72 (T) mice killed after heat stress (Ta = 40°C for 59 min) and 10 min room temperature (30°C). Top: proteins were probed using an alkaline phosphatase-conjugated mouse monoclonal primary antibody specific for inducible HSP72. Nitrocellulose was then developed using alkaline phosphatase buffer. Bottom: OD values of protein assay scanned by the densitometer for various tissues obtained from normal ICR (C), [−]HSP72 (N), and [+]HSP72 (T) mice. Data are expressed as means ± SE for 8 mice per group. *P < 0.05, **P < 0.01, ***P < 0.001, significantly different from the corresponding control values (C or N groups; ANOVA, followed by Duncan’s test).

Fig. 5. Effects of heat stress on core temperature (Tco), mean arterial pressure (MAP), heart rate (HR), striatal blood flow (SBF), and striatal PO2 in ICR (●), [−]HSP72 (○), and [+]HSP72 (△) mice. Points represent means ± SE for 8 mice/group. *P < 0.05, compared with preheat controls; †P < 0.05, compared with [−]HSP72 mice (ANOVA, followed by Duncan’s test).
mice. After the onset of heatstroke, normal ICR or \([-HSP72]\) mice exhibited cell shrinkage and pyknosis of the nucleus in the striatum (Fig. 7, a and b). However, the heatstroke-induced neuronal damage in striatum was markedly less in \([-HSP72]\) mice (Fig. 7c).

**DISCUSSION**

This study makes two points that make it of particular value to understanding heatstroke. First, to the best of our knowledge, this is the first study to use transgenic mice to study the role of HSP72 in this well-established model of heatstroke (35). Second, this work extends our laboratory’s earlier studies in rats (35) by successfully applying our heatstroke model (which was previously established in rats) to a new species, to expand significantly our ability to use novel transgenic/knockout models to study heatstroke responses. As described in the Introduction, the rodent heatstroke model can nearly mirror the full spectrum of human heatstroke. The anesthetized rat or mouse heatstroke model fulfilled the empirical triad used for the diagnosis of classic human heatstroke; this triad consists of hyperthermia, central nervous system alteration, and a history of heat stress (4, 22).

Previous studies have established that the sublethal heat stress-induced accumulation of inducible HSP70 is necessary for acquired thermotolerance, which is defined as the ability of a cell or organism to become resistant to heat stress (26, 27, 32, 33, 40, 41). The kinetics of thermotolerance induction have been linked to decay with parallel changes in the induction and degradation of HSP70 (26, 33). Our previous works also established an association between HSP72 induction and heat-
stroke-induced circulatory shock and cerebral ischemia (68). Prior heat shock confers significant protection against heatstroke-induced hyperthermia, arterial hypotension, cerebral ischemia, and neuronal damage, and correlates with the expression of HSP72 in rat brain at 16 h. However, at 48 h, when HSP72 expression is returned to its basal values, these responses of these two groups of animals (0 vs. 48 h) during heatstroke are indistinguishable from each other. However, these findings have generally been correlative, establishing no causal link between acquired thermotolerance and the overexpression of HSP70. In this work, the protective role of HSP72 is investigated using an in vivo model of heatstroke-induced arterial hypotension, cerebral ischemia, and hypoxia in transgenic mice that overexpress HSP72. Arterial hypotension, cerebral ischemia, and hypoxia during heatstroke in these transgenic mice are significantly lower than those in transgene-negative controls. The results herein demonstrate that the overexpression of HSP72 in transgenic mice increased thermal tolerance (as revealed by prolonged survival) by reducing arterial hypotension and cerebral ischemia and hypoxia. This investigation shows a cause-and-effect relationship between the overexpression of HSP72 and thermal tolerance, because it prevents the cellular and physiological changes that have been shown to accompany hyperthermic pretreatment. The results herein indicate that when exposed to heat stress, [+HSP72 mice exhibit prolonged survival, to an extent that is related to the maintenance of a suitable mean arterial pressure and cerebral blood flow, as well as reduced cerebral neuronal damage during heatstroke. The maintenance of an appropriate cerebral blood flow may be caused by higher cerebral perfusion pressure, resulting from lower intracranial pressure (due to reduction in cerebral edema and cerebroventricular congestion) and higher mean arterial pressure during the development of heatstroke (56). The maintenance of an appropriate mean arterial pressure in [+HSP72 mice during heatstroke may be related to an increase in augmentation of stroke volume and total peripheral vascular resistance (22).

In fact, the present results obtained using an in vivo study in mice are consistent with several previous studies. For instance, the transfection of a plasmid that contains the Drosophila HSP70 gene into a monkey fibroblast cell line causes the overproduction of HSP72 in these cells and improves heat
tolerance (31). Conversely, the inhibition of the synthesis of HSP70 protein with specific monoclonal antibodies also reduces heat tolerance in fibroblasts (51).

Notably, hyperthermia induces the expression of HSP72 mRNA in the regions of the brain that are involved in the central control of blood pressure (3). Heat shock also causes HSP70 expression in the peripheral vasculature (67) and cardiac myocytes (18), which confers cardiovascular protection during heatstroke. Our earlier data have also shown that induction of HSP72 by prior heat shock (68) and progressive exercise (17) reduces the augmented production of interleukin-1, tumor necrosis factor-α, or other cytokines in the plasma and thus protects against arterial hypotension during heatstroke. Hence, a substantial reversal in vascular dysfunction in the periphery, as well as the attenuation of cytokine overproduction, may be critical in the beneficial effect of overexpression of HSP72 during heatstroke. The overexpression of HSP72 may also protect against heatstroke-induced circulatory shock and cerebral ischemia by reducing oxidative stress and energy depletion (60). The presented results are somewhat consistent with many recent reports that indicate that heat shock proteins function as regulators of the immune response (56).

When mice were subjected to acute stress by exposing them to whole body hyperthermia treatment, HSP70 levels in the liver were increased significantly by administration of l-arginine, an effect correlated with reduced production of serum inflammatory cytokines (7). The administration of l-arginine rescued the mice from heat-induced death and inhibited overproduction of inflammatory cytokines such as interleukin-1β and tumor necrosis factor-α (7). Elevations in intracellular HSP70 levels have been shown to improve cell tolerance to inflammatory cytokines (18, 21, 42). Kluger et al. (21) also showed that heat preconditioning, in addition to increasing intracellular HSP70 levels, protected animals from an endotoxin dose that elicited fever in unconditioned rats. The endotoxin-induced TNF-α overproduction was greatly reduced in heat-conditioned animals. Although the precise mechanisms for the improvement in heat tolerance in association with HSP72 overproduction are unclear, the protective mechanism of HSP72 is believed to be involved in preventing the denaturation of protein and/or the processing of denatured proteins and protein fragments that are formed by stressors such as hyperthermia (40).

Evidence has accumulated that HSP70 can be generated by hypoxia (11, 12), ischemia (53), acidosis (63), energy depletion (53), immune responses (18, 49), ultraviolet radiation (2), and volatile anesthetics (54, 69). In this study, normal ICR, [−]HSP72, and [+]HSP72 mice were all anesthetized with pentobarbital sodium and exposed to heat stress (40°C) for the same period (59 min). As shown in Table 1, [+]HSP72 normothermic or heat-stressed mice had higher HSP72 expression levels in tissues than those of ICR or [−]HSP72 normothermic or heat-stressed mice, indicating that pentobarbital sodium did not affect the expression of HSP72 in these experiments.

Rodent models used currently involve rectal temperature probes, restraint, or anesthesia (16, 45, 52, 64–66). Rectal temperature probes, restraint, and anesthesia all affect the thermoregulatory profiles generated during and after heat stress. A mouse model has recently been developed to improve the quality of research because it uses biotelemetry to examine unrestrained, conscious mice during and after heat stress (29, 30). After mice are subjected to different levels of heat stress, a biphasic core temperature profile characterized by a ambient temperature-dependent hyperthermia and then a feverlike state is observed. For humane reasons, these studies were conducted in mice under pentobarbital sodium anesthesia. Anesthesia impairs the maintenance of normal body temperature regulation and potentially affects the study of heatstroke pathophysiology using this model. Nevertheless, this potential source of variation should have been accounted for by the appropriate controls used in the present study. Apparently, heat shock protein preconditioning confers protection against heatstroke in both mice (present results) and rats (60, 68).

Importantly, the protective effects of overexpression of HSP72 in [+]HSP72 mice may have been related to the enhanced cooling rate after removal of the heat, because the cooling rate is directly related to survival during heatstroke. In this study, the body weights of the different stains were identical. Rapid cooling is currently the most effective method of heatstroke therapy in humans, making the findings herein applicable to humans.

In summary, our results demonstrate that transgenic mice that overexpress HSP72 attenuated hyperthermia, circulatory shock, and cerebral ischemic injury exhibited during heatstroke. These findings reveal a causal relationship between the overexpression of HSP72 in multiple vital organs including the heart and the brain, and protection from hyperthermia, circulatory shock, and cerebral ischemic injury in this in vivo model of heatstroke. Therefore, HSP72 expression appears to be critical to the development of thermotolerance and protection from cellular damage associated with heat stress.

GRANTS

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REFERENCES

OVEREXPRESSION OF HSP72 PROTECTS AGAINST HEATSTROKE


