Heat shock protein expression protects against cerebral ischemia and monoamine overload in rat heatstroke

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Yang, Yi-Ling, and Mao-Tsun Lin. Heat shock protein expression protects against cerebral ischemia and monoamine overload in rat heatstroke. Am. J. Physiol. 276 (Heart Circ. Physiol. 45): H1961–H1967. 1999.—This study attempted to ascertain whether the ischemic damage to neurons and monoamine overload in brain that occur during rat heatstroke can be attenuated by heat shock protein (HSP) 72 induction. Effects of heatstroke on mean arterial pressure (MAP), cerebral blood flow (CBF), brain dopamine (DA) and serotonin (5-HT) release, and neural damage score were assayed in rats 0, 16, or 48 h after heat shock (42°C for 15 min) or chemical stress (5 mg/kg sodium arsenite ip). Brain HSP 72 in rats after heat shock or chemical stress was detected by Western blot, and brain monoamine was determined with a microdialysis probe combined with high-performance liquid chromatography. Heatstroke was induced by exposing the animal to a high ambient temperature (43°C); the moment at which MAP and CBF decreased from their peak values was taken as the time of heatstroke onset. Prior heat shock or chemical stress conferred significant protection against heatstroke-induced hyperthermia, arterial hypotension, cerebral ischemia, cerebral DA and 5-HT overload, and neuronal damage and correlated with expression of HSP 72 in brain at 16 h. However, at 48 h, when HSP 72 expression returned to basal values, the above responses that occurred during the onset of heatstroke were indistinguishable between the two groups (0 h vs. 48 h). These results lead to the hypothesis that the brain can be preconditioned by thermal or chemical injury, that this preconditioning will induce HSP 72, and that HSP 72 induction will correlate quite well with anatomic, histochemical, and hemodynamic protection in rat heatstroke.

There is mounting evidence that neurons produce heat shock protein (HSP) in response to a variety of environmental stresses, resulting in protection from subsequent lethal damage (29, 32, 36–38). HSP 72 induction increases the number of surviving neurons in rat hippocampal neuron primary culture as well as the tolerance of hippocampal neurons to ischemic injury (10, 19, 30). This raises the possibility that pretreatment that increases brain HSP 72 content before heatstroke may limit the development of arterial hypotension, cerebral ischemia, cerebral monoamine overload, and neuronal damage that occur during the onset of heatstroke (12).

To address the question properly, in the present study we compared the temporal profiles of mean arterial pressure (MAP), cerebral blood flow (CBF), brain dopamine (DA) and serotonin (5-HT) release, and neural damage score in rats with or without brain HSP 72 expression in heatstroke.

MATERIALS AND METHODS

HSP 72 induction. HSP expression was induced in male Wistar rats (Animal Resource Center, National Science Council, Taipei, Taiwan, ROC) weighing 250–300 g by either heat shock (39) or chemical stress (28). For heat shock treatment, rats under general anesthesia (1 h after 40 mg/kg pentobarbital sodium ip) were heated gently with an electric pad. The colonic temperature of the heated animals was kept at 42 ± 0.5°C for 15 min. They were returned to room temperature during the recovery period. For chemical stress treatment, the unanesthetized rats were treated with sodium arsenite (5 mg/kg ip). These groups of animals were subjected to heatstroke experiments at 0, 4, 8, 16, or 48 h after the start of heat shock or chemical stress treatment.

HSP 72 detection. The animals were killed by decapitation at the end of the experiment for detection of HSP 72. The brains were quickly removed and stored at 0°C. The corpus striatum was dissected from the brain and placed into Eppendorf tubes. For protein extraction, the samples were weighed, rapidly thawed in 6 vols of homogenizing buffer consisting of 20 mM Tris-HCl (pH 7.6), 100 mM KCl, 5 mM NaCl, 2 mM EDTA, 1 mM EGTA, 0.25 M sucrose, 2 mM dithiothreitol, and 2 mM phenylmethylsulfonyl fluoride at pH 7.2, and then homogenized by a sonicator. After centrifugation at 5,000 g for 15 min at 4°C, the protein assay was carried out by the Bradford method. The samples (40 µg/lane) were incubated for 5 min at 95°C in Laemmli buffer, separated on 10% SDS-polyacrylamide discontinuous gel, and then electrophoretically transferred to a polyvinylidene difluoride membrane. The membrane was blocked for 1 h at room temperature with PBS containing 10% skim milk. Mouse monoclonal antibodies against HSP 72 (Oncogene Science) were used as primary antibodies (1:1,000 dilution). Incubation time with the primary antibody at room temperature was 1 h. Band detection was performed with an enhanced chemilumines-
Heatstroke induction. Six groups of animals were used: 1) rats 0 h after heat shock; 2) rats 16 h after heat shock; 3) rats 48 h after heat shock; 4) rats 0 h after chemical stress; 5) rats 16 h after chemical stress; and 6) rats 48 h after chemical stress. Under urethane (1.4 g/kg ip) anesthesia, the femoral artery of these animals was cannulated with polyethylene tubing (PE-50) for measurement of systemic arterial blood pressure and heart rate. Pulsatile arterial pressure, MAP, and heart rate were monitored continuously with a pressure transducer and a chart recorder (model 2400, Gould). The animals were then placed in a Kopf stereotaxic frame (Grass) in the flat skull position. A flow probe was implanted into the right corpus striatum using the atlas and coordinates of Paxinos and Watson (24) for measurement of CBF. At the same time, for measurement of extracellular monoamine release a microdialysis probe was implanted stereotaxically into the left corpus striatum. Heatstroke was induced by exposing these groups of animals to an ambient temperature of 43°C; the moment in which MAP and CBF began to decrease from their peak levels was taken as the onset of heatstroke (8, 16; see Fig. 2). All experimental protocols were approved by the Animal Research Committee of the Yang-Ming University School of Medicine. Adequate hydration was maintained in the animals after administration of urethane (1.4 g/kg ip). At the end of the experiments, the animals were killed for evaluation of neuron damage, HSP 72 detection, or histological verification of the path of the probes.

CBF measurement. CBF was monitored with a Laserflo BPM2 laser-Doppler flowmeter (Vasamedic, St. Paul, MN). A 24-gauge stainless steel needle probe (diameter 0.58 mm, length 40 mm) was implanted into the corpus striatum as indicated in Heatstroke induction. The Laserflo BPM2 records at a bandwidth of 30 Hz and a low band pass of 20 KHz. The display is digital only, collects eight data points per second, and was set to give a moving average of data every 0.3 s. Blood perfusion was recorded until the flux value leveled off at a stable reading, which usually occurred after ~1 min. That value was then recorded and used as the flux measurement for the period.

In addition, an autoradiographic technique was used to quantify CBF in the corpus striatum (31). Approximately 50 µCi of [14C]antipyrine in 1 ml of normal saline were infused at a constant rate via the femoral venous catheter for a period of 1 min, during which time arterial samples were collected on filter paper disks for assay of arterial concentration. At exactly 1 min, the animal was decapitated and the brain was removed, frozen, and assayed for [14C] concentration by the autoradiographic technique. Autoradiographic sections of rat brain and a calibrated plastic [14C] standard were used to determine tissue concentrations of [14C] by densitometric measurements.

Measurement of extracellular monoamine release. After a microdialysis probe (active length 3.5 mm) was inserted into the left corpus striatum, it was perfused with artificial cerebrospinal fluid (in mM: 149 NaCl, 2.8 KCl, 1.2 CaCl2, 1.2 MgCl2, 0.125 ascorbic acid, and 5.4 d-glucose, pH 7.2–7.4) using microliter syringe pumps (model SJ-3206, Harvard) at a flow rate of 1.35 µl/min. After 2 h of stabilization, dialysate samples from the corpus striatum were collected into 700-µl Eppendorf tubes at 20-min intervals. Samples were assayed by an HPLC system. Extracellular monoamine concentrations were assayed by HPLC combined with an electrochemical detection system as described previously (13). The HPLC system comprised a Beckman 126 pump (Beckman Instruments) and a CMA-200 microautosampler (CMA/Microdialysis, Stockholm, Sweden), and a microbore reversed-phase column was filled with Inertsil ODS-2 (GSK-C18, 5-mm OD, 150 × 1.0-mm ID). The performance of each microdialysis probe was calibrated by dialysis of a known amount of the standard mixture, and recovery of all analyses was then determined. Brain concentrations of 5-HT and other monoamines were calculated by determining each peak height ratio relative to the internal standard and were also corrected by each probe's performance. The internal standard 3-methoxytyramine and standard mixtures were prepared fresh daily. The mobile phase was prepared by adding 60 ml of acetonitrile, 0.42 g of SDS (2.2 mm), 200 g of sodium citrate (30 mM), 10 mg of EDTA (0.027 mM), and 1 ml of diethylamine in double-distilled water. The solution was adjusted to pH 3.5 by concentrated orthophosphoric acid, and its final volume was adjusted to 1 liter. We filtered the mixture with a 0.22-µm nylon filter under reduced pressure and degassed it by pumping helium gas for 20 min. The flow rate was 0.05–0.06 ml/min, maintaining column pressure at ~7.6 mPa.

In vitro tests were run to determine the recovery of 5-HT or other monoamines in the dialysis solution at the flow rate used. At room temperature, dialysis probes were immersed in dialysis solution containing 5-HT or DA. At a flow rate of 1.35 µl/min, the relative recovery was 14 ± 1.4% (n = 8). Basal monoamine levels were considered stable after observation of four consecutive samples with no upward or downward trend. These basal values were averaged, and all samples were expressed as a percentage of the mean baseline to avoid large variations in the abscissa values.

Evaluation of neuron damage and probe placement. In separate experiments, 5 min after the onset of heatstroke, rats were killed, and brains were perfused, fixed in 10% neutral Formalin, and embedded in paraffin blocks. Coronal sections (6 µm) through the striatum were stained with hematoxylin and eosin for microscope evaluation. Neuronal damage was graded from the grading system of Pulsinelli et al. (26, 27) in which 0 = normal, 1 = few neurons damaged, 2 = many neurons damaged, and 3 = all neurons damaged. All probe placements were in the regions of the corpus striatum as verified by microscopic evaluation.

Statistics. The numerical values cited are means ± SE. Repeated-measures analysis of variance was used for factorial experiments, whereas Duncan's multiple-range test (multi-time point experiments) was used for post hoc multiple comparisons among means. Student's t-test was used when only two groups were compared. The criterion for statistical significance was set at P < 0.05.

RESULTS

Figure 1, top, shows that heat shock (42°C body temperature for 15 min), in the presence of an increase in body temperature, induced HSP 72 expression in the striatum that was detected 4 h after treatment, peaked between 8 and 16 h, and returned to baseline by 48 h. In this series of experiments, heat shock was conducted in rats 1 h after intraperitoneal administration of pentobarbital sodium. Normothermic control rats receiving the same dose of pentobarbital sodium did not express HSP 72 in the corpus striatum. Chemical stress (sodium arsenite injection), in the absence of an increase in body temperature, also induced HSP 72 expression in the corpus striatum that was detected 4 h after injection, peaked between 8 and 16 h, and returned to baseline by 48 h (Fig. 1, bottom). Every animal in the
treatment groups showed a consistent HSP 72 response to the priming insults as shown in Fig. 1.

Tables 1 and 2 summarize means ± SE values for all groups of animals. Heatstroke was induced by exposing the animals to an ambient temperature of 43°C. Heatstroke rats without striatal HSP 72 expression showed higher colon temperature, heart rate, and striatal DA and 5-HT release but lower mean arterial pressure, striatal blood flow, and survival time compared with those of normothermic control rats. Figure 2 shows typical examples of the effects of heatstroke on colon temperature, mean arterial pressure, heart rate, striatal DA and 5-HT release, striatal blood flow, and survival time. Induction of striatal HSP 72 expression, 16 h after heat shock or chemical stress, significantly reduced the hyperthermia, arterial hypotension, striatal DA and 5-HT overload, and striatal neuronal damage. These results are consistent with previous findings that HSP 70 expression can alter the resistance of the heart (42) and the brain (4) to subsequent ischemic or nonischemic injury. With the loss of HSP 72 observed at 48 h, no further protection was induced.

In separate experiments, 5 min after the onset of heatstroke, animals were killed for determination of both local CBF and neuronal damage score. The data are summarized in Table 3. After the onset of heatstroke, animals with no HSP 72 expression displayed higher values of striatal neuronal damage score but lower values of striatal blood flow compared with those of normothermic control rats. In addition, histopathological verification revealed that heatstroke caused cell body shrinkage, pyknosis of the nucleus, loss of Nissl substance, and disappearance of the nucleolus in the corpus striatum (Fig. 3B). However, with the expression of HSP 72 in the corpus striatum observed at 16 h, neuroprotection was ensured (Fig. 3C).

**DISCUSSION**

In the present study using a well-described rat model (12), we induced thermal or chemical preconditioning and clearly demonstrated production of HSP 72 in the brain at 16 h that returned to baseline at 48 h. Using preconditioned animals, we induced heatstroke to find that prior heat shock or chemical stress conferred significant protection against heatstroke-induced hyperthermia, arterial hypotension, striatal ischemia, striatal DA and 5-HT overload, and striatal neuronal damage. These results are consistent with previous findings that HSP 70 expression can alter the resistance of the heart (42) and the brain (4) to subsequent ischemic or nonischemic injury. With the loss of HSP 72 observed at 48 h, no further protection was induced in the present studies. It should be stated that there are many consequences of hyperthermic insults, including the induction of other HSP as well as diverse physiological changes (32). Because the thermal insult is attenuated in the preconditioned animals in our studies, this raises

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**Table 1. Effects of heatstroke on colon temperature, mean arterial pressure, heart rate, striatal DA and 5-HT release, striatal blood flow, and survival time in rats with or without heat shock protein induction in striatum**

<table>
<thead>
<tr>
<th>Groups</th>
<th>T&lt;sub&gt;co&lt;/sub&gt;, °C</th>
<th>MAP, mmHg</th>
<th>HR, beats/min</th>
<th>DA, % baseline</th>
<th>5-HT, % baseline</th>
<th>SBF, % baseline</th>
<th>ST, min</th>
<th>HSP 72 Expression</th>
</tr>
</thead>
<tbody>
<tr>
<td>Normothermic control rats</td>
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<tr>
<td>0 min before testing</td>
<td>36.5 ± 0.2</td>
<td>86 ± 2</td>
<td>331 ± 13</td>
<td>100 ± 4</td>
<td>100 ± 5</td>
<td>100 ± 4</td>
<td>&gt;300</td>
<td>Negative (0/10)</td>
</tr>
<tr>
<td>80 min after testing</td>
<td>36.4 ± 0.2</td>
<td>83 ± 2</td>
<td>325 ± 17</td>
<td>101 ± 5</td>
<td>102 ± 4</td>
<td>98 ± 3</td>
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<tr>
<td>Rats 0 h after heat shock</td>
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<tr>
<td>70 min before HS onset</td>
<td>36.3 ± 0.3</td>
<td>90 ± 5</td>
<td>355 ± 19</td>
<td>100 ± 6</td>
<td>101 ± 7</td>
<td>102 ± 5</td>
<td>18 ± 6</td>
<td>Negative (0/10)</td>
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<tr>
<td>10 min after HS onset</td>
<td>43.0 ± 0.3&lt;sup&gt;*&lt;/sup&gt;</td>
<td>45 ± 4&lt;sup&gt;*&lt;/sup&gt;</td>
<td>555 ± 22&lt;sup&gt;*&lt;/sup&gt;</td>
<td>636 ± 23&lt;sup&gt;*&lt;/sup&gt;</td>
<td>440 ± 39&lt;sup&gt;*&lt;/sup&gt;</td>
<td>32 ± 6&lt;sup&gt;*&lt;/sup&gt;</td>
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<td>Rats 16 h after heat shock</td>
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<tr>
<td>70 min before HS onset</td>
<td>36.8 ± 0.7</td>
<td>88 ± 7</td>
<td>340 ± 21</td>
<td>108 ± 7</td>
<td>105 ± 9</td>
<td>103 ± 6</td>
<td>&gt;300&lt;sup&gt;†&lt;/sup&gt;</td>
<td>Positive (10/10)</td>
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<tr>
<td>10 min after HS onset</td>
<td>41.5 ± 0.7&lt;sup&gt;†&lt;/sup&gt;</td>
<td>85 ± 3&lt;sup&gt;†&lt;/sup&gt;</td>
<td>575 ± 40&lt;sup&gt;†&lt;/sup&gt;</td>
<td>106 ± 9&lt;sup&gt;†&lt;/sup&gt;</td>
<td>110 ± 10&lt;sup&gt;†&lt;/sup&gt;</td>
<td>106 ± 8&lt;sup&gt;†&lt;/sup&gt;</td>
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<td>Rats 48 h after heat shock</td>
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<tr>
<td>70 min before HS onset</td>
<td>36.2 ± 0.5</td>
<td>96 ± 7</td>
<td>350 ± 21</td>
<td>105 ± 7</td>
<td>109 ± 9</td>
<td>105 ± 8</td>
<td>20 ± 8</td>
<td>Negative (0/10)</td>
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<tr>
<td>10 min after HS onset</td>
<td>42.8 ± 0.4&lt;sup&gt;†&lt;/sup&gt;</td>
<td>42 ± 3&lt;sup&gt;†&lt;/sup&gt;</td>
<td>560 ± 35&lt;sup&gt;†&lt;/sup&gt;</td>
<td>648 ± 25&lt;sup&gt;†&lt;/sup&gt;</td>
<td>432 ± 40&lt;sup&gt;†&lt;/sup&gt;</td>
<td>35 ± 8&lt;sup&gt;†&lt;/sup&gt;</td>
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</table>

Values are means ± SE of 10 rats/group; nos. in parentheses denote no. of rats with heat shock protein (HSP) induction over total no. of rats tested. T<sub>co</sub>, colon temperature; MAP, mean arterial pressure; HR, heart rate; DA, dopamine; 5-HT, serotonin; SBF, striatal blood flow; ST, survival time; HS, heat stroke. *P < 0.05, significantly different from corresponding control values (70 min before HS onset), ANOVA; †P < 0.05, significantly different from corresponding control values (rats without HSP 72 expression), ANOVA.
the possibility that the resulting pathology would be less. Although tissue injury was formerly attributed to hyperthermia itself, it has recently been observed that normal volunteers heated passively and cancer patients treated with whole body hyperthermia can endure a rectal temperature of 41–42°C with no, or minimal, tissue injury (3, 25). Heatstroke can occur when the rectal temperature increases to only 40°C (1).

### Table 2. Effects of heatstroke on colon temperature, mean arterial pressure, heart rate, striatal DA and 5-HT release, striatal blood flow, and survival time in rats with or without striatal HSP 72 induction

<table>
<thead>
<tr>
<th>Groups</th>
<th>Tco, °C</th>
<th>MAP, mmHg</th>
<th>HR, beats/min</th>
<th>DA, % baseline</th>
<th>S-HT, % baseline</th>
<th>SBF, % baseline</th>
<th>ST, min</th>
<th>HSP 72 Expression</th>
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<tbody>
<tr>
<td>Rats 0 h after chemical stress</td>
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<tr>
<td>70 min before HS onset</td>
<td>36.6 ± 0.4</td>
<td>87 ± 8</td>
<td>331 ± 24</td>
<td>106 ± 7</td>
<td>101 ± 6</td>
<td>100 ± 6</td>
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<tr>
<td>10 min after HS onset</td>
<td>42.7 ± 0.5*</td>
<td>47 ± 6*</td>
<td>568 ± 25*</td>
<td>609 ± 30*</td>
<td>450 ± 41*</td>
<td>34 ± 5*</td>
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<td>Rats 16 h after chemical stress</td>
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<tr>
<td>70 min before HS onset</td>
<td>36.7 ± 0.2</td>
<td>91 ± 5</td>
<td>338 ± 22</td>
<td>101 ± 6</td>
<td>102 ± 7</td>
<td>98 ± 5</td>
<td>20 ± 4</td>
<td>Negative (0/6)</td>
</tr>
<tr>
<td>10 min after HS onset</td>
<td>41.2 ± 0.4*</td>
<td>82 ± 5†</td>
<td>548 ± 23*</td>
<td>110 ± 7†</td>
<td>114 ± 6†</td>
<td>148 ± 9†</td>
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<tr>
<td>Rats 48 h after chemical stress</td>
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<td></td>
<td></td>
<td>&gt;300†</td>
<td>Positive (6/6)</td>
</tr>
<tr>
<td>70 min before HS onset</td>
<td>36.6 ± 0.2</td>
<td>88 ± 7</td>
<td>351 ± 24</td>
<td>102 ± 8</td>
<td>100 ± 6</td>
<td>101 ± 6</td>
<td></td>
<td></td>
</tr>
<tr>
<td>10 min after HS onset</td>
<td>42.2 ± 0.4*</td>
<td>48 ± 7*</td>
<td>553 ± 27*</td>
<td>580 ± 26*</td>
<td>462 ± 39*</td>
<td>36 ± 5*</td>
<td>19 ± 3</td>
<td>Negative (0/6)</td>
</tr>
</tbody>
</table>

Values are means ± SE of 6 rats/group; nos. in parentheses denote no. of rats with HSP induction over total no. of rats tested. *P < 0.05, significantly different from corresponding control values (70 min before HS onset), ANOVA; †P < 0.05, significantly different from corresponding control values (rats without HSP 72 expression), ANOVA.

Fig. 2. Time course of changes of colon temperature (Tco), mean arterial pressure (BP), heart rate (HR), striatal dopamine (DA) and serotonin (5-HT) release, and cerebral blood flow (CBF) in striatum produced by high ambient temperature (Ta) in a rat 16 (●) or 0 (○) h after heat shock. The former displays striatal HSP 72 expression, whereas the latter has none.
2); moreover, tissue injury continues to develop after cooling to normal body temperature in 25% of heatstroke patients (2, 7, 20, 34). Our previous results (5, 8, 9, 12, 14, 16, 33) further demonstrated that cerebral ischemia and overloading of DA and 5-HT in the brain, rather than hyperthermia, is the main reason for heatstroke formation in animals.

It has been shown that both intracranial hypertension (because of cerebral edema and cerebral vascular congestion) and arterial hypotension result in a reduction in cerebral perfusion pressure in animals with heatstroke (15, 33). Reduction of cerebral perfusion pressure to below the autoregulatory level will induce cerebral ischemia in animals with heatstroke (14). Our recent results (15) also showed that a decline in stroke volume or ventricular depolarization resulting from an increased plasma level of interleukin-1 is an important mechanism signaling arterial hypotension in rat heatstroke. Indeed, the heatstroke-induced arterial hypotension, intracranial hypertension, cerebral ischemia, cerebral DA and 5-HT overload, and cerebral neuronal damage were significantly attenuated by blockade of interleukin-1 receptors in rats (12, 18). Other lines of evidence showed that heat shock and chemical stress with heavy metal salts or sulfhydryl reagents, all of which induce the expression of HSP 70, concomitantly inhibit the production of interleukin and other cytokines in human monocytes and mouse macrophages activated by lipopolysaccharide (6). Putting these observations together, it seems that induction of brain HSP 72 by prior heat shock or chemical stress reduces the augmented production of interleukin-1 and other cytokines in the plasma and results in protection against heatstroke-induced arterial hypotension, cerebral ischemia, cerebral monoamine overload, and cerebral neuronal damage in rats.

It should be stated that, after the onset of heatstroke, ischemic injury or monoamine overload was observed to occur in different brain structures including the corpus striatum, hypothalamus, cortex, and thalamus (8, 9, 17). In addition, prior heat shock was shown to induce

<table>
<thead>
<tr>
<th>Groups</th>
<th>Striatal Neuronal Damage Score (0–3)</th>
<th>SBF, ml·100 g⁻¹·min⁻¹</th>
<th>HSP 72 Expression in Striatum</th>
</tr>
</thead>
<tbody>
<tr>
<td>Normothermic controls</td>
<td>0 (10)</td>
<td>138 ± 6 (10)</td>
<td>Negative (10)</td>
</tr>
<tr>
<td>0 h after heat shock</td>
<td>2.7 ± 0.2 (10)</td>
<td>18 ± 2 (10)</td>
<td>Negative (10)</td>
</tr>
<tr>
<td>16 h after heat shock</td>
<td>1.0 ± 0.3* (10)</td>
<td>160 ± 4* (10)</td>
<td>Positive (10)</td>
</tr>
<tr>
<td>48 h after heat shock</td>
<td>2.5 ± 0.3 (10)</td>
<td>22 ± 3 (10)</td>
<td>Negative (10)</td>
</tr>
<tr>
<td>0 h after chemical stress</td>
<td>2.4 ± 0.4 (6)</td>
<td>20 ± 3 (6)</td>
<td>Negative (6)</td>
</tr>
<tr>
<td>16 h after chemical stress</td>
<td>1.2 ± 0.2* (6)</td>
<td>146 ± 16* (6)</td>
<td>Positive (6)</td>
</tr>
<tr>
<td>48 h after chemical stress</td>
<td>2.6 ± 0.4 (6)</td>
<td>24 ± 4 (6)</td>
<td>Negative (6)</td>
</tr>
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</table>

Values are means ± SE for no. of rats in parentheses. *P < 0.05, significantly different from corresponding control values (rats without HSP 72 expression), ANOVA.

Fig. 3. Photomicrographs of striatum of a normothermic control rat without HSP 72 expression in striatum (A), a heatstroke rat without HSP 72 expression in striatum (0 h after heat shock treatment; B), and a heatstroke rat with HSP 72 expression in striatum (16 h after heat shock treatment; C). Five minutes after onset of heatstroke, rat without HSP 72 expression in striatum showed cell body shrinkage, pyknosis of nucleus, loss of Nissl substance, and disappearance of nucleolus in striatum. With induction of HSP 72 in striatum observed at 16 h, neuroprotection was induced.
HSP 72 induction in different brain structures (including corpus striatum, hypothalamus, cortex, and thalamus), liver, heart, and kidney (39, 40). In the present study, the corpus striatum was chosen as a representative region for measurement of HSP 72 induction, blood flow, and neuronal damage in rat heatstroke. However, it can be inferred from the present results that in addition to the corpus striatum, the mechanisms existing in other brain structures (such as the hypothalamus, cortex and thalamus) and vital organs are also responsible for these physiological consequences of preconditioning.

In fact, the processes involved in cell injury during ischemia and reperfusion are complex. The generation of free radicals, hydrogen peroxide, and cellular calcium overload are implicated in the process of ischemic neuronal damage (11, 23, 41). In this regard, many investigators demonstrated that HSP 72 induction by either heat shock or immobilization protects the rat or rabbit heart against the calcium overload triggered by calcium depletion-repletion (21, 22, 35). In a rat myocardial culture model, heat shock is capable of inducing acquired thermotolerance and limiting myocyte injury on subsequent H2O2 exposure (35). However, it is not known whether the same mechanisms can be applied to explain the neuroprotective action exerted by HSP expression in brain during heatstroke.

In summary, the present results show that heat shock and chemical stress, which induce the expression of HSP 72 in the brain, concomitantly inhibit the arterial hypertension, cerebral ischemia, cerebral DA and 5-HT overload, and cerebral cell injury that occur during the onset of heatstroke in rats. This leads to the hypothesis that the brain can be preconditioned by thermal or chemical injury, that this preconditioning will induce HSP 72, and that HSP 72 induction will correlate well with anatomic, biochemical, and hemodynamic protection in rat heatstroke.

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