Hypothermia Reduces 72-kDa Heat-Shock Protein Induction in Rat Brain After Transient Forebrain Ischemia

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Background and Purpose: We examined the influence of concurrent moderate hypothermia (30°C) and transient forebrain ischemia on the induction of 72-kDa heat-shock protein and neuronal damage in male Wistar rats.

Summary of Report: Experimental groups included: normothermic with 8 minutes of transient forebrain ischemia (group 1, n=1), hypothermic without ischemia (group 2, n=9), and hypothermic (30°C) with 8 minutes of transient forebrain ischemia (group 3, n=5). Intense 72-kDa heat-shock protein immunoreactivity was demonstrated in rat forebrain 48 hours after induction of normothermic forebrain ischemia (group 1); it was not detected in the brain of animals subjected to hypothermia without ischemia (group 2), and hypothermia during ischemia (group 3) significantly inhibited its expression compared with that in normothermic ischemia animals (group 1).

Conclusions: These observations suggest that 72-kDa heat-shock protein induction is not the mechanism by which moderate hypothermia protects against ischemic cell damage.

Evidence is accumulating that lowering the brain temperature during ischemia confers a marked protective effect on metabolic, neurological, and physiological outcome. The mechanisms by which hypothermia protects neurons from ischemic damage are unknown. The 72-kDa heat-shock protein (HSP-72) and other proteins common to the heat-shock response are induced in rodent brain following transient ischemia, and HSP-72 has been associated with protection in neural tissue subjected to various types of injury. Thus, HSP-72 may play a role in the hypothermic protection of brain after an ischemic insult. We therefore investigated the effect of hypothermia on HSP-72 induction in rats subjected to 8 minutes of forebrain ischemia.

Materials and Methods

The regional expression of HSP-72 immunoreactivity in rat brain after normothermic forebrain ischemia, moderate hypothermia (30°C) without ischemia, and hypothermic (30°C) forebrain ischemia was studied and related to neuronal injury.

A total of 25 male Wistar rats weighing 220–330 g were used in all experiments. The model of transient forebrain ischemia used in the present study has been described in detail by Smith et al. Briefly, after induction of anesthesia with halothane and 70% N2O-30% O2, the animals were intubated and mechanically ventilated to maintain blood gases within physiological range. Forebrain ischemia, confirmed by the absence of electroencephalographic activity, was induced and maintained for 8 minutes using a combination of bilateral carotid artery clamping and reduction of mean arterial blood pressure (<50 mm Hg). Arterial blood pressure was reduced by means of combined hypovolemia and Arfonad (Roche Laboratories, Nutley, N.J.) administration. The electroencephalogram and electrocardiogram were recorded during 8 minutes of ischemia and the subsequent recovery period.

The animals were divided into the following three groups: In group 1 (normothermic forebrain ischemia), the core temperature (rectal) of the animal was maintained at 37°C by means of a recirculating water bath (n=7). The HSP-72 data from selected animals in group 1 have been reported. Additional rats were subjected to sham operation without the
induction of ischemia (n=2). Because the peak of forebrain HSP-72 immunoreactivity has been reported to occur at 48 hours of recirculation in gerbils, we killed the rats at 48 hours of recirculation with the assumption that HSP-72 immunoreactivity in rats was similar to that in gerbils.

In group 2 (hypothermia without ischemia), whole-body hypothermia (30°C rectal temperature) was maintained for 3 hours (n=9). No surgical procedures were performed on these rats. Hypothermia was induced using evaporation cooling (alcohol and a fan), and the core temperature was regulated as described in group 1. The animals were anesthetized with halothane and 70% N₂O-30% O₂, using a face mask. The hypothermic rats were killed at 48 hours (n=2) or 24 hours (n=7) after hypothermic induction. The 24-hour time point was selected because no HSP-72 staining was detected at 48 hours, and we sought an earlier time point at which to detect HSP-72. Two additional control rats, not subjected to surgical procedures, were killed 48 hours after induction of anesthesia.

In group 3 (hypothermic forebrain ischemia), ischemia and surgical procedures were identical to those performed in group 1, except that hypothermia (30°C) was instituted and maintained 1 hour before and during ischemia and for 2 hours of reperfusion (n=5). Hypothermia was induced and regulated as in group 2. These rats were killed 48 hours after ischemia.

The HSP-72 immunohistochemical technique was essentially as described in detail by Vass et al. All rats were given an overdose of pentobarbital and fixed by transcardial perfusion with 100 mM sodium phosphate buffer (pH 7.4), followed by 4% paraformaldehyde in the buffer. Brains were removed and kept in the same fixative overnight at 4°C, then transferred to 10 mM phosphate buffered saline. Coronal slices (3 mm) were made using a rodent brain matrix. Coronal sections (50 μm) were cut on a vibratome and reacted immunohistochemically using a mouse monoclonal antibody C92 to HSP-72 (RPN 1197; Amersham, Cleveland, Ohio). Biotinylated sheep anti-mouse immunoglobulin G was used as the second antibody. Sections were incubated with streptavidin "bridge" 1:200 in phosphate buffered saline for 1 hour, followed by biotinylated horseradish peroxidase 1:400 in phosphate buffered saline for 1 hour. Peroxidase was detected with diaminobenzidine. Sections were gelatin-mounted on slides for light microscopic evaluation. Control sections were run in each experiment without primary antibody to FIGURE 1. Panel a: Intensive 72-kDa heat-shock protein (HSP-72) immunoreactivity was present in CA1-CA2, CA3, and CA4 areas of the hippocampus in five of seven rats from group 1 (normothermic group). Magnification, x21. Panel b: CA1-CA2 hippocampal areas containing damaged or necrotic neurons were devoid of or low in HSP-72 immunoreactivity in two of seven rats from group 1 (normothermic group). Magnification, x21.
FIGURE 2. Neurons throughout the forebrain (cortex, hippocampus, thalamus, and amygdala) of group 3 animals (hypothermic ischemia group) were very weakly stained or devoid of 72-kDa heat-shock protein (HSP-72) immunoreactivity. Magnification, x21.

rule out cross-reactivity with anti-mouse secondary antibody and endogenous rat immunoglobulin.

The remaining coronal slices from these rat brains were fixed in 10% buffered formalin for a minimum of 1 week and embedded in paraffin, and sections (6 μm) adjacent to those cut on the vibratome were stained with hematoxylin and eosin for histopathologic evaluation.

Results

Normothermic ischemia produced neuronal HSP-72 induction in the hippocampus in all seven rats in group 1. Induction of HSP-72 in neurons, detected in the CA1-CA2 sections of hippocampus, was dependent on whether the neurons were morphologically intact or damaged. Pronounced HSP-72 staining in morphologically intact neurons was found in CA1-CA2 pyramidal cells (n=5; Figure 1A). The CA1-CA2 hippocampal areas containing damaged or necrotic neurons were devoid of or low in HSP-72 (n=2; Figure 1B). Weak immunoreactivity was detected in neurons exhibiting structural change on hematoxylin and eosin sections. In all rats, the majority of CA3-CA4 hippocampal and dentate gyrus neurons showed no histological damage, but were positively stained for HSP-72. CA4 hippocampus exhibited intense HSP-72 induction in all rats.

Areas outside the hippocampus also exhibited widespread neuronal HSP-72 induction. Staining of HSP-72 in normal neurons was found in cortices III-V (cingula, insular, piriform, frontal, parietal perirhinal, temporal, occipital, and entorhinal neurons), thalamus (lateral, posterior, and medial thalamic neurons), striatum, and amygdaloid nuclei (lateral and basolateral, lateral, and medial amygdaloid neurons). Elsewhere, HSP-72 was detected in subiculum, paraseptal, and presubicular of the hippocampus; septal nucleus; olfactory; orbital cortex; habenula neuron; and hypothalamic and subthalamic nuclei. No difference in HSP-72 staining was detected in anatomical sites outside the hippocampus between animals exhibiting neuronal necrosis and animals with intact neurons in CA1-CA2 hippocampus.

Rats subjected to hypothermia without ischemia (group 2) exhibited no HSP-72 staining throughout the forebrain (data not shown). No obvious damaged or necrotic neurons were found in these rats (data not shown).

Induction of HSP-72 was dramatically reduced in the hypothermic ischemia rats (group 3) compared with the normothermic ischemia group (group 1). Neurons throughout the entire forebrain were very weakly stained or devoid of HSP-72 staining. Figure 2 illustrates the weak HSP-72 staining in hippocam-
pus, cortex, thalamus, and amygdala. This lack of staining in hypothermic ischemia rats can be contrasted to the intense HSP-72 staining observed in the corresponding regions (Figures 1A and 1B) obtained in normothermic ischemia animals. Few damaged or necrotic neurons were detected in the hypothermic ischemia rats, and the degree of damage was similar to that found in control or sham groups.

Control and sham-operated rats showed no HSP-72 immunoreactivity in the forebrain.

Discussion

In the present study, we tested the hypothesis that hypothermic protection against cerebral ischemic cell damage may be mediated by the induction of HSP-72. Our data indicate that moderate hypothermia, while reducing ischemic cell injury, significantly reduces the expression of HSP-72 throughout the brain.

We have no data about the possible expression of HSP-72 at times other than 48 hours after hypothermia–ischemia. However, both in vivo and in vitro studies suggest that HSP-72 possesses a long (>48 hours) half-life. Thus, when the tissue is devoid of HSP-72 at 48 hours after the insult, it is unlikely that HSP-72 is expressed and disappears before 48 hours. Likewise, to our knowledge, there have been no reports of initial expression of HSP-72 at 48 hours after the provoking insult. Thus, our study suggests that hypothermic protection against ischemic cell damage is not associated with the induction of HSP-72.

Our in vivo data are consistent with in vitro data, in which induction of heat-shock proteins, as well as glucose-regulated proteins in chemically stressed L929 cells, was shown to be inhibited by moderate hypothermia. Though protein analogues were incorporated and protein turnover was increased in these cells at low temperatures, stress proteins were not induced at ≤30°C. Thus, both in vivo and in vitro, hypothermia (30°C) inhibits the expression of heat-shock proteins.

We cannot extract information from our study concerning the step at which HSP-72 induction was inhibited. Hypothermic suppression of HSP-72 expression may occur at the transcriptional level where mRNA for HSP-72 is not expressed, or at the translational level. To address this question, stress protein mRNA must be measured and compared with the immunohistochemical detection of HSP-72.

Acknowledgment

The authors wish to acknowledge the assistance of Patricia Ruffin in manuscript preparation.

References


Key Words • heat-shock proteins • hypothermia • neuronal damage • rats
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Stroke. 1992;23:104-107
doi: 10.1161/01.STR.23.1.104

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