Effects of Normothermic Versus Mild Hyperthermic Forebrain Ischemia in Rats

W. Dalton Dietrich, PhD, Raul Busto, BS, Isabel Valdes, BS, and Yolanda Loor

We compared the neuropathological consequences of global forebrain ischemia under normothermia versus mild hyperthermia. Twenty-one rats underwent 20 minutes of four-vessel occlusion during which brain temperature was maintained at either 37°C (normothermia, n=9) or 39°C (hyperthermia, n=12). Quantitative neuropathological assessment was conducted 1 or 3 days later. At 1 day following the ischemic insult, normothermic rats demonstrated neuronal injury mainly confined to the most dorsolateral striatum. By 3 days, ischemic cells were present throughout the striatum and CA1 hippocampus in normothermic animals. Compared with normothermic rats, intraischemic hyperthermia significantly increased the extent and severity of brain damage at 1 day after the ischemic insult. Areas of severe neuronal necrosis and frank infarction included the cerebral cortex, CA1 hippocampus, striatum, and thalamus. Morphologic damage was also detected in the cerebellum and pars reticulata of the substantia nigra. An overall mortality rate of 83% was demonstrated at 3 days in the hyperthermic ischemic group. We conclude that intraischemic hyperthermia 1) markedly augments ischemic brain damage and mortality compared with normothermia, 2) transforms ischemic cell injury into frank infarction, and 3) accelerates the morphological appearance of ischemic brain injury in regions usually demonstrating delayed neuronal necrosis. These observations on mild hyperthermia may have important implications for patients undergoing cardiac or cerebrovascular surgery as well as patients following cardiac arrest or those with stroke-in-evolution. (Stroke 1990;21:1318–1325)
halothane and a mixture of 70% nitrous oxide and 30% oxygen. The following day, the femoral vessels were cannulated under halothane anesthesia, and polyethylene ligatures were placed around each common carotid artery. Rats were then intubated and mechanically ventilated to maintain \( \text{Paco}_2 \) and \( \text{PaO}_2 \) within normal limits. Surgical wounds were sutured and infiltrated with 1% xylocaine. Following a 1.25-hour stabilization period, 20 minutes of severe incomplete ischemia was produced by tightening the carotid ligatures bilaterally and maintaining mean arterial blood pressure at 80 mm Hg by gradual withdrawal of blood.

Two ischemic groups were investigated, rats in which intraischemic brain temperature was maintained in the range 36.5–37° C (normothermia, \( n = 9 \)) or 38.5–39° C (mild hyperthermia, \( n = 12 \)) by adjustments of a thermostatically regulated heating lamp placed above the head. Brain temperature was measured indirectly with a probe placed in the temporalis muscle. In previous publications, we have shown that externally monitored head (temporalis muscle) temperature closely reflects brain temperature.17 Brain temperature in all rats was 37° C before and following the ischemic insult. Rectal (body) temperature was monitored and maintained at 36–37° C throughout the experiment by a second heating lamp. Non-ischemic sham-operated control rats, which had undergone vertebral artery cauterization, had brain temperature maintained at either 37° C (\( n = 2 \)) or 39° C (\( n = 2 \)) for 20 minutes to simulate ischemic brain temperatures.

Following the 20-minute ischemic period, carotid ligatures were removed and recirculation was initiated. Blood, kept at 36–37° C, was reinfused to restore the mean arterial blood pressure to 100–120 mm Hg. Rats were given antibiotics (cefazolin), returned to their cages, and kept in a dark, quiet environment. At 1 or 3 days following the ischemic insult, rats were transcardially perfused with 0.9% sodium chloride solution followed by a mixture of formaldehyde-glacial acetic acid-methanol. Heads were immersed in fixative overnight prior to brain removal. Brains were next embedded in paraffin, and 10-\( \mu \)m coronal semiserial sections were prepared and stained with hematoxylin and eosin.

At the level of the anterior commissure (striatum, frontal cortex), dorsal hippocampus (thalamus, parietal cortex), substantia nigra, and cerebellum the severity of the ischemic cell change was graded on a semiquantitative scale as 0, normal brain; 1, few (<10%) affected neurons; 2, many (10–50%) affected neurons; 3, most neurons (>50%) affected; or 4, frank infarction. Microscopic sections were analyzed by an investigator who was blinded to the experimental conditions. To characterize more completely the effect of mild hyperthermia on the production of ischemic neuronal injury, the ischemic neurons were counted in the striatum and CA1 hippocampus. In the striatum, separate counts were made for the dorsolateral and central portions. For each region, five microscopic fields (\( \times 400 \)) were selected at random. In the hippocampus, the CA1 sector was divided into three equal segments (medial, middle, and lateral), and ischemic cells were counted at a magnification of \( \times 100 \).

Physiological and histopathological data were analyzed by one- or two-way analysis of variance, and statistical significance was assessed by Scheffé’s 5 test and Dunn’s multiple comparison procedure. Semiquantitative data were compared using the Kruskal-Wallis one-way analysis of variance by ranks.

Results

Physiological variables are summarized in Table 1. Values obtained immediately prior to, during, or 30 minutes after the ischemic insult were within normal ranges. In addition, no significant differences were documented between the ischemic groups. In sham-operated rats in which brain temperature was maintained at either 37° or 39° C for 20 minutes, histopathological evaluation of brain tissue was unremarkable at 3 days (Figures 2D and 3D).

Normothermic rats regained spontaneous respiration and consciousness within 30–45 minutes after the ischemic insult. Within 2–3 hours normothermic rats were observed to move around their cages, all survived the ischemic insult, and postischemic seizures were not documented. In contrast to these findings, rats that underwent hyperthermic brain ischemia exhibited severe clinical findings. On the average, the recovery of spontaneous breathing was delayed compared with normothermic rats (1.5–2 hours). Hyperthermic rats generally remained unresponsive during the entire postischemic period and did not regain normal motor activity. Seizures were

<table>
<thead>
<tr>
<th>Variable</th>
<th>Before ischemia</th>
<th>During ischemia</th>
<th>After ischemia</th>
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<tbody>
<tr>
<td>PaCO(_2) (mm Hg)</td>
<td>37.7±1.2</td>
<td>37.5±1.7</td>
<td>40.3±1.3</td>
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<tr>
<td>PaO(_2) (mm Hg)</td>
<td>153.1±15.1</td>
<td>148.4±13.7</td>
<td>144.7±8.3</td>
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<tr>
<td>pH</td>
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<td>7.267±0.03</td>
<td>7.362±0.02</td>
</tr>
<tr>
<td>Mean arterial blood pressure (mm Hg)</td>
<td>140.1±6.7</td>
<td>80.0±0.5</td>
<td>138.3±5.0</td>
</tr>
</tbody>
</table>

Values are mean±SEM, \( n = 4–6 \) rats.

*Brain temperature was measured indirectly with probe placed in temporalis muscle.

**Mean arterial blood pressure was lowered to 80 mm Hg during ischemic insult by withdrawal of blood.

Dietrich et al  Normothermic vs. Hyperthermic Ischemia

TABLE 1. Physiological Variables of Rats Subjected to Normothermic or Hyperthermic Forebrain Ischemia
documented in one hyperthermic rat at 24 hours. An overall mortality rate of 83% was seen at posts ischemic day 3. Most commonly, respiration became increasingly irregular and rats expired between 24 and 48 hours. A single hyperthermic rat demonstrating severe motor abnormalities survived 3 days for histopathological evaluation.

Neuropathological findings in the cerebral cortex are summarized in Tables 2 and 3. In the normothermic rats that were perfused 1 day following the ischemic insult, ischemic cell damage was not a consistent finding. However, when present, patches of ischemic neurons were detected in superficial cortical layers or adjacent to penetrating arterioles (Figure 1A). At 3 days after the ischemic insult, all normothermic rats demonstrated some degree of cortical damage. Scattered ischemic neurons were detected throughout the somatosensory cortex, being most prominent in layers 3, 5, and 6 (Figure 1B). Increasing brain temperature 2°C during the ischemic insult significantly increased the extent and severity of cortical damage at posts ischemic day 1 (Figure 2C). Ischemic neurons were commonly detected within all cortical layers, with infarction of the somatosensory cortex being observed in 50% of the hemispheres. When present, damage was symmetrical in severity and location.

In normothermic rats, the hippocampus appeared unremarkable at 1 day in the majority of hemispheres (Figure 1C). At 3 days, CA1 hippocampal injury was seen in all normothermic rats (Figure 1D). In contrast to these findings, hippocampal damage was seen in all hyperthermic rats 1 day after the ischemic insult. Pyramidal neurons within the CA1 hippocampus appeared dark and shrunken with pyknotic nuclei (Figure 2, A and B). In a single hyperthermic rat, ischemic cell damage was not restricted to the CA1 area but also involved the CA2–CA4 areas. In this rat, histopathological alterations were consistent with early infarction (i.e., parenchymal necrosis). Dentate granule neurons were also damaged in the majority of hyperthermic rats.

In normothermic rats, ischemic cell injury was prominent in the dorsolateral segment of the striatum at 1 day, while a low frequency of ischemic neurons was detected in the central striatum (Figure 1E). By 3 days, severe ischemic injury involved both the dorsolateral and the central portions of the striatum (Figure 1F). In contrast to this temporal profile of neuronal injury, severe ischemic cell injury was present in both the dorsolateral and the central portions of the striatum at 1 day when intraschismic brain temperature was maintained at 39°C (Figure 2E). In all hyperthermic rats, the striatum appeared pale and edematous, and evidence of early infarction was detected in one rat bilaterally.

In normothermic rats, ischemic injury to the thalamus was consistently not seen at 1 day. By 3 days, a high frequency of ischemic cells was present within the reticular nucleus. Injury to other thalamic nuclei

<table>
<thead>
<tr>
<th>Group</th>
<th>Ischemic injury grade</th>
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Significance

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<td>5.14</td>
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TABLE 2. Histopathological Assessment of Ischemic Damage in Rats Subjected to Normothermic or Hyperthermic Forebrain Ischemia

n, number of hemispheres. Ischemic injury grade: 0, normal brain; 1, few (<10%) affected neurons; 2, many (10–50%) affected neurons; 3, most neurons (>50%) affected; 4, frank infarction. Significance assessed by Kruskal-Wallis one-way analysis of variance, based upon bihemispheral average ischemic injury grade for each rat. NS, not significant.
was not a consistent finding. Bilateral neuronal necrosis of the ventrolateral nucleus was seen in the majority of hyperthermic rats at 1 day. Higher magnification identified vacuolated neuropil, swollen astrocytic nuclei, and eosinophilic neurons with pyknotic nuclei (Figure 2, A and F).

Normothermic rats demonstrated brain stems that appeared unremarkable. In contrast, ischemic cell injury was documented in hyperthermic rats. Unique to this group was bilateral necrosis of the substantia nigra pars reticulata in five of six rats (Figure 3, A and B). Higher magnification of the pars reticulata revealed neuronal necrosis and signs of early infarction (Figure 3B). More diffuse ischemic cell damage was also detected in other brain stem regions including the red nucleus, the superior and inferior colliculi, the central gray nucleus, and the reticular nucleus. Central chromatolysis of larger neurons was observed throughout the brain stems of hyperthermic rats (Figure 3E). In addition, necrosis of choroid plexus epithelial cells was frequently observed in the lateral and third ventricles (Figure 3F).

In normothermic rats, few Purkinje neurons demonstrated ischemic cell injury, while granule neurons appeared unremarkable at 3 days. In hyperthermic rats, damage to Purkinje neurons was seen in five of six rats. In some rats, granule neurons also appeared necrotic (Figure 3C). In these rats, the dorsal aspects of the cerebellum were more consistently involved.

**Discussion**

This study demonstrated enhanced ischemic injury with infarction in mildly hyperthermic rats. In addition, ischemic injury was also documented in the cerebellar cortex and substantia nigra pars reticulata, regions normally resistant to this ischemic insult. Compared with normothermic rats, mortality in the hyperthermic group was significantly increased at 3 days. These data indicate that a 2°C increase in intraischemic brain temperature is sufficient to markedly augment neuropathological damage and worsen clinical outcome.

Previously published histopathological studies using models of global forebrain ischemia have documented ischemic cell injury within selectively vulnerable brain regions. In transient global ischemia models, widespread neuronal death develops in the striatum by 24 hours but does not develop in the CA1 hippocampus until 72 hours after ischemia. Evidence for delayed hippocampal injury has also been documented in clinical studies following cardiac arrest. In the present study, severe CA1 damage was seen in a majority of hemispheres 24 hours following the hyperthermic ischemic insult. Variations in intraischemic brain temperature also affected the maturation of ischemic injury in the central striatum. These findings demonstrate that intraischemic brain temperature, in addition to influencing ischemic severity, is an important factor in determining the temporal profile of ischemic neuronal death. This finding may have important implications in establishing “therapeutic windows” for posts ischemic pharmacotherapy.

Tissue infarction following periods of transient global ischemia has been previously described in the context of complete, prolonged, and hyperglycemic brain ischemia. Evidence for delayed hippocampal injury has also been documented in clinical studies following cardiac arrest. In the present study, increasing intraischemic brain temperature converted ischemic cell injury to frank infarction in cortical, hippocampal, striatal, and thalamic areas. In contrast, sham-operated rats in which brain temperature was elevated to 39°C for 20 minutes to simulate ischemic brain temperatures showed no histopathological alterations. Thus, our method of maintaining head temperature above normal does not appear to be primarily responsible for the severe damage observed in the hyperthermic group.

Although the exact mechanism responsible for increased ischemic injury and tissue infarction under hyperthermic ischemic conditions is not known, recent data demonstrate that posts ischemic microvascular abnormalities may play a role. By means of the four-vessel occlusion model, we have demonstrated the importance of ischemic brain temperature in posts ischemic blood–brain barrier alterations. In that study, extravasation of the protein tracer horseradish peroxidase (HRP) was detected in cortical, thalamic, hippocampal, and striatal regions 1 hour following 20 minutes of hyperthermic (39°C) brain ischemia. In cortical regions, columns of extravasated HRP were associated with pial and penetrating arterioles. In other regions, arterioles surrounded by perivascular spaces were permeable to HRP. In the context of the present discussion, acute
altered in vascular permeability could influence ischemic outcome by increasing brain swelling and the movement of blood-borne neurotoxic substances (i.e., glutamate, Ca\(^{2+}\)) into the extravascular space. The observation that ischemic hyperthermia leads to necrosis of choroid plexus epithelial cells suggests that the removal of waste products of neuronal metabolism may be severely affected by this insult. In addition to forebrain structures, ischemic injury was also detected in the cerebellum and several brain stem nuclei of the hyperthermic rats. Previously published hemodynamic studies using the four-vessel occlusion model have demonstrated that these brain regions undergo only moderate reductions in local cerebral blood flow.\(^{22,23}\) In the present study, other mechanisms besides hemodynamic factors may be

FIGURE 1. Paraffin-embedded brain sections stained with hematoxylin and eosin from normothermic (37 °C) rats. A: One day following ischemic insult focal regions of cortical vacuolation and ischemic neuronal damage are seen (p, pial surface), ×309. B: Three days following ischemic insult ischemic neurons are present in layer 5 of somatosensory cortex, ×1140. C: Normal-appearing CA1 pyramidal neurons 1 day after ischemia, ×1140. D: Ischemic neurons are present throughout CA1 hippocampus 3 days after ischemia, ×1140. E: Damaged neurons are restricted to dorsolateral striatum (arrowheads) 1 day after ischemia. Normal-appearing neurons are present in central striatum (arrows), ×1140. F: At 3 days, ischemic neurons are present throughout striatum (dorsolateral and central areas), ×1140.
FIGURE 2. Paraffin-embedded rat brain sections stained with hematoxylin and eosin. Histopathological consequences 1 day after hyperthermic (39°C) brain ischemia. A: Ischemic damage is present in CA1 hippocampus and ventrolateral thalamus, ×28. B: Higher magnification showing neuronal damage in CA1 hippocampus, ×1128. C: Infarction of somatosensory cortex, ×306. D: Normal-appearing cortical neurons in sham-operated rat in which brain temperature was elevated to 39°C for 20 minutes, ×306. E: Ischemic neurons are present throughout striatum (dorsolateral and central areas), ×1128. F: Ischemic neurons and parenchymal vacuolation are seen in ventrolateral thalamus, ×1128.

Responsible for these alterations. In this regard, damage to the substantia nigra pars reticulata occurs in rats made hyperglycemic prior to global forebrain ischemia.21 Recently, the destruction by excitotoxins of striatal neurons has been reported to damage the ipsilateral substantia nigra pars reticulata—a remote consequence that could be prevented by γ-aminobutyric acid (GABA) agonist.24 In the present study, transient ischemia carried out at 39°C may increase the vulnerability of striatal GABAergic neurons, resulting in an imbalance between neuronal excitation and inhibition and subsequent necrosis of the pars reticulata.

In both the normothermic and hyperthermic groups, ischemic injury to the cerebellar Purkinje neurons was documented. In previous experimental studies in which 10–30 minutes of four-vessel occlusion was investigated, variable degrees of cerebellar

...injury have been reported. Neuropathological studies of cardiac arrest patients have shown that the cerebellum is frequently involved. In our study, intraschismic hyperthermia increased severity of ischemic damage in the cerebellum, involving both Purkinje and granule cells. It is known that Purkinje neurons receive a glutamatergic input from the climbing fibers originating in the inferior olive. The increased vulnerability of the cerebellum to hyperthermic cerebral ischemia may therefore involve the exaggerated release of glutamate from climbing fibers.

These experimental findings may be clinically important since stroke patients may present with a range of head and body temperatures. Hyperthermia is known to cause symptoms of heat stress and to increase the potential for stroke and thromboembolic episodes. Based on our findings, stroke patients...
with mild fever may experience more severe pathological damage and increased mortality than normothermic patients. Thus, measures should be taken to decrease head or brain temperature in patients undergoing cardiac surgery or those at risk for cerebrovascular disease.

Acknowledgments

We wish to thank Susan Kraydieh and Yamile Castella for expert histological support. Mrs. Helen Valkowitz prepared the manuscript.

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KEY WORDS • cerebral ischemia • hyperthermia • rats
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*Stroke*. 1990;21:1318-1325
doi: 10.1161/01.STR.21.9.1318

*Stroke* is published by the American Heart Association, 7272 Greenville Avenue, Dallas, TX 75231
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Print ISSN: 0039-2499. Online ISSN: 1524-4628

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