The Effect of Graded Posts ischemic Spinal Cord Hypothermia on Neurological Outcome and Histopathology after Transient Spinal Ischemia in Rat

Manabu Kinoshana, M.D.,* Yutaka Taira, M.D., Ph.D.,* Martin Marsala, M.D.†

Background: Previous data have shown that posts ischemic brain hypothermia is protective. The authors evaluated the effect of posts ischemic spinal hypothermia on neurologic function and spinal histopathologic indices after aortic occlusion in the rat.

Methods: Spinal ischemia was induced by aortic occlusion lasting 10 min. After ischemia, spinal hypothermia was induced using a subcutaneous heat exchanger. Three studies were conducted. In the first study, the intrathecal temperature was decreased to 34, 30, or 27°C for 2 h beginning with initial reperfusion. In the second study, hypothermia (target intrathecal temperature 27°C) was initiated with reflow and maintained for 15 or 120 min. In the third study, the intrathecal temperature was decreased to 27°C for 2 h starting 5, 60, or 120 min after normothermic reperfusion. Animals survived for 2 or 3 days, at which time they were examined and perfusion fixed with 4% paraformaldehyde.

Results: Normothermic ischemia followed by normothermic reflow resulted in spastic paraplegia and spinal neuronal degeneration. Immediate posts ischemic hypothermia (27°C for 2 h) resulted in decreased motor dysfunction. Incomplete protection was noted at 34°C. Fifteen minutes of immediate cooling (27°C) also provided significant protection. Delay of onset of post-reflow hypothermia (27°C) by 5 min or more failed to provide protection. Histopathologic analysis revealed temperature-dependent suppression of spinal neurodegeneration, with no effect of delayed cooling.

Conclusions: These findings indicate that the immediate period of reperfusion (0–15 min) represents a critical period that ultimately defines the degree of spinal neuronal degeneration. Hypothermia, when initiated during this period, showed significant protection, with the highest efficacy observed at 27°C. (Key words: Aneurysm; cooling; ischemia; occlusion; paraplegia.)

MILD hypothermia (30–34°C) provides protection against transient spinal cord ischemia as measured by behavioral, electrophysiologic, and histopathologic indices. Using a technique of selective spinal cord cooling (spinal temperature, 20–25°C), we and others have shown comparable protective effects against protracted (40–60 min) spinal ischemia in the dog and rabbit. More recently, we developed a technique for localized spinal cord cooling in the rat and found that mild hypothermia (34°C) during ischemia prevents glutamate release during ischemia and produces significant spinal protection in the posts ischemic interval (for as many as 2 days). A similar effect of deep intrasicaemic spinal hypothermia (20°C) on spinal ischemia-evoked glutamate release in a dog model has been reported.

These observations suggest that hypothermia may prevent the initiation of the cascade of events that leads to subsequent neuronal deterioration. An important question, however, is whether cooling can alter the development of degenerative processes once the cascade has begun. In this regard, cooling of the brain after reflow can suppress subsequent neuronal degeneration in rats and gerbils. Whether such posts ischemic cooling is effective in spinal cord ischemia is not known.

Using a model of spinal ischemia in the rat, in conjunction with a subcutaneous spinal cord cooling tech-
Fig. 1. (A) The subcutaneously implanted copper heat exchanger overlying the paravertebral muscle and extending from the lower thoracic to lumbosacral spinal segments in the rat. To measure corresponding paravertebral muscle temperature, a thermocouple needle was placed into the paravertebral muscle through the opening in the central part of the heat exchanger. (B) Intrathecal temperature profiles measured in animals subjected to a cooling protocol to induce 34, 30, and 27°C spinal hypothermia or maintained at 38°C for a 2 h period of posts ischemic reperfusion. (C) A significant correlation was seen between the temperatures measured concurrently in the lumbar intrathecal space and paravertebral muscle (L3 vertebral level) before, during, and after 2 h of 34, 30, or 27°C of posts ischemic spinal hypothermia.

and 2) the parameters of the temporal window in which such reflow protection can be effective.

Materials and Methods

General Preparation/Induction of Spinal Ischemia

Details of the aortic occlusion model have been reported previously. Briefly, male Sprague-Dawley rats (300–350 g) were anesthetized in an acrylic box with 4% halothane in an oxygen-and-room air mixture (1:1). After induction, rats were maintained with 1.5–2% halothane delivered by mask. To monitor distal arterial pressure and to inject heparin, a polyethylene catheter (PE-50) was inserted in the tail artery. To induce spinal ischemia, a left femoral artery was isolated and a 2-French-gauge Fogarty catheter was placed in the descending thoracic aorta so the tip of the catheter reached the level of the left subclavian artery (10.5 to 11 cm from the site of catheter insertion). To control arterial blood pressure above the level of aortic occlusion at 40 mmHg during the period of aortic occlusion, a 20-gauge Teflon catheter (Angiocath™, Becton Dickinson, Sandy, UT) connected to an external blood reservoir (37.5°C) was inserted 1 cm into the left carotid artery. When all cannulas were placed, 200 units heparin was injected into the tail artery. To induce spinal ischemia, the balloon catheter was inflated with 0.05 ml saline and the blood was allowed to flow to the external reservoir. The completeness of the occlusion was evidenced by an immediate and sustained loss of any detectable pulsation and a decrease in pressure below the level of aortic occlusion. After ischemia, the balloon was deflated and removed, and the blood was reinfused in 60 s. Protamine sulphate (4 mg) was administered subcutaneously. After a predetermined reperfusion period, all catheters were...
TRANSIENT SPINAL ISCHEMIA: EFFECT OF POSTISCHEMIC COOLING

Table 1. Summary of Experimental Groups

<table>
<thead>
<tr>
<th>Study/Group</th>
<th>Number of Rats</th>
<th>Post Ischemic Cooling Cycle (min)</th>
</tr>
</thead>
<tbody>
<tr>
<td></td>
<td></td>
<td>On*</td>
</tr>
<tr>
<td>Study 1†</td>
<td></td>
<td>6</td>
</tr>
<tr>
<td>38°C × 120 min (control)</td>
<td></td>
<td>120</td>
</tr>
<tr>
<td>34°C × 120 min</td>
<td></td>
<td>6</td>
</tr>
<tr>
<td>30°C × 120 min</td>
<td></td>
<td>6</td>
</tr>
<tr>
<td>27°C × 120 min</td>
<td></td>
<td>6</td>
</tr>
<tr>
<td>Study 2‡</td>
<td></td>
<td>6</td>
</tr>
<tr>
<td>38°C × 15 min</td>
<td></td>
<td>6</td>
</tr>
<tr>
<td>27°C × 15 min</td>
<td></td>
<td>6</td>
</tr>
<tr>
<td>27°C × 120 min</td>
<td></td>
<td>6</td>
</tr>
<tr>
<td>Study 3‡</td>
<td></td>
<td>5</td>
</tr>
<tr>
<td>27°C × (5–125 min)</td>
<td></td>
<td>5</td>
</tr>
<tr>
<td>27°C × (60–180 min)</td>
<td></td>
<td>6</td>
</tr>
<tr>
<td>27°C × (120–240 min)</td>
<td></td>
<td>6</td>
</tr>
</tbody>
</table>

* Time zero in this table indicates the instant of spinal reperfusion.
† In all experimental groups animals survived for 3 days. Only animals that showed fully developed paraplegia with no signs of recovery at 48 h were perfusion fixed at this time point.
‡ In this study 120 min of spinal cord cooling was initiated after 5 min, 60 min, or 120 min of normothermic reflow.

removed, incisions were closed, anesthesia was discontinued, and animals were allowed to recover.

Induction of Spinal Cord Hypothermia
To induce spinal hypothermia, a subcutaneous cooling technique was used. A heat exchanger was constructed from copper tubing. The heat exchanger was inserted into the tissue of the back via a subcutaneous tunnel extending from S2 to the T4–T5 spinal segments (fig. 1A). To induce spinal cord hypothermia, cold water (8 or 9°C) was perfused through the heat exchanger at rate of 100 ml/min using a peristaltic pump. To control and maintain the degree of spinal cord hypothermia, a thermocouple to measure paravertebral muscle temperature was placed below the heat exchanger and the output was used to turn the peristaltic pump on or off (using an external microprocessor-based temperature controller).

Experimental Groups and Design
The investigation consisted of three major components. Table 1 summarizes the groups associated with each section.

Study 1. Graded posts ischemic cooling (34°C, 30°C, or 27°C) was initiated immediately after 10 min of normothermic ischemia and maintained for the initial 2 h of reperfusion. (Group designations: normothermic, 38°C × 120 min; 34°C × 120 min, 30°C × 120 min, and 27°C × 120 min; n = 6 in each group.)

Study 2. Posts ischemic cooling to 27°C was initiated immediately after 10 min of normothermic ischemia and was maintained for 15 or 120 min of reperfusion. (Group designations: 38°C × 15 min; 27°C × 15 min; and 27°C × 120 min; n = 6 in each group.)

Study 3. Posts ischemic cooling to 27°C was initiated at 5, 60, or 120 min of normothermic reflow after a 10-min interval of normothermic spinal ischemia, and the hypothermia was maintained for a period of 2 h after the start of cooling. (Group designations: 27°C × [5–125 min; n = 5]; 27°C × [60–180 min; n = 6]; 27°C × [120–240 min; n = 6].)

In animals with delayed cooling (study 3), rats were allowed to recover for the 50 or 110 min of normothermic reperfusion and then anesthetized again to permit the placement of the heat exchanger and subsequent spinal cord cooling, which was initiated at exactly 60 or 120 min after normothermic reflow.

At the end of the cooling-rewarming cycle in all experimental groups, the heat exchanger was removed, the incision was closed, and the animals were allowed to recover and survived for 2 or 3 days. During this period, the recovery of neurologic function was assessed periodically. After the experimental periods, all animals were perfusion fixed and the spinal cord was processed for light histopathologic analysis. For ethical reasons, animals displaying spastic or flaccid paraplegia with no signs of recovery for the initial 2 days of survival were perfusion fixed at this point. In paraplegic animals, Crede’s maneuver was used periodically (every 12 h) to empty the bladder.

Assessment of Neurologic Function
During reperfusion (5, 24, 48, and 72 h), recovery of motor and sensory functions was assessed using the following grading system by an observer without knowledge of the treatment groups (M.M.).

Motor function was quantified by assessing ambulation and placing and stepping responses. For statistical purposes, ambulation (walking with lower extremities) was graded as follows: 0 = normal; 1 = toes flat under the body when walking, but ataxia present; 2 = knuckle walking; 3 = movement in lower extremities but unable to knuckle walk; or 4 = no movement, drags lower extremeties. The placing and stepping reflex was assessed by dragging the dorsum of the hind paw over the edge of a surface. This normally evokes a coordinating lifting and placing response (e.g., stepping) which was graded as 0 = normal; 1 = weak; or 2 = no stepping. A motor deficit index was calculated for each rat at each
time interval. The final index was the sum of the scores (walking with lower extremities plus the placing and stepping reflex). The presence of spasticity or flaccidity was determined by the presence of an exaggerated flexion response to a pinch of the hind paw. Flaccidity was defined as no tone in response to limb extension or pinch.

Sensory function was assessed by the reaction to hind paw pinch. An exaggerated sensory response was defined as the evocation of vigorous squeaking and agitation in response to light stroking of the flank. Nonresponsiveness was defined as the failure to evoke any motor or vocal response to pinch.

Perfusion Fixation and Histopathologic Analysis

At the end of the experimental period, rats were killed with pentobarbital (100 mg/kg given intraperitoneally) and phenytoin (25 mg/kg given intraperitoneally). The rats were then transcardially perfused with 100 ml heparin-prepared saline followed by 150 ml paraformaldehyde, 4%, in phosphate buffer (pH 7.4). Twenty-four hours later, the spinal cords were removed and postfixed in the same fixative for 2–4 days. After this period, the spinal cords were removed and L3, L4, and L5 spinal segments were dissected. Each of the dissected spinal segments was divided into two pieces and processed separately for Nissl staining (proximal part) or silver impregnation (distal part). For Nissl staining, the samples were postfixed in 1% buffered OsO₄ and embedded in Araldite (Ted Pella, Inc., Redding, CA). Semithin sections (1 μm) were prepared and stained with p-phenylenediamine. For silver impregnation, samples were immersed overnight in 30% sucrose and frozen sections (30 μm; 20 subserial sections from each segment) were cut. A previously described silver impregnation technique was used to detect terminal neurodegenerative changes.¹⁴,¹⁵

For quantitative evaluation, Nissl-stained sections were used. Each fifth section (1 μm) from all three spinal segments was saved. Saved sections from L3, L4, and L5 spinal segments (10 sections per segment) were processed separately. Each section was scored on a four-point scale (0–3), with grade 0 = no damage; grade 1 = fewer than 5% neurons affected; grade 2 = 5–50% of neurons affected; grade 3 = more than 50% of neurons affected. Average values from each segment were added so the final score for one spinal cord ranged from 0 (no detectable damage) to 9 (more than 50% of neurons damaged in the L3, L4, and L5 spinal segments).

Scores were tabulated and analyses were prepared by the observer (M.M.) without knowledge of either the behavioral outcome or the treatment group.

Measurement of Intrathecal Temperature

To characterize intrathecal temperature produced by the cooling protocol we used, an intrathecal thermocouple (8.5 cm), incorporated into a PE-10 catheter, was implanted into the intrathecal space in a separate group of rats (n = 12). To place the PE-10 catheters, a previously described technique for the placement of intrathecal catheters was used.¹⁶ Briefly, rats were anesthetized with 2.5% halothane in a room air-and-oxygen mixture (1:1), and the back of the head and neck was shaved. The animals were placed in a stereotaxic head holder with the head flexed forward. Anesthesia was maintained with 1.5% halothane delivered by mask. A midline incision was made on the back of the neck. The muscle was freed at the attachment to the skull and retracted with a flat elevator, exposing the cisternal membrane. The membrane was opened with a stab blade and retracted with a dural hook. The PE-10 catheter was inserted through the cisternal opening and passed into the lumbar intrathecal space. The incision on the neck was closed and the external portion of the PE-10 catheter was fixed to the skin with a suture. The temperature output from the intrathecal and paravertebral thermocouple and the rectal temperature probe was monitored continuously and recorded before and during normothermic spinal ischemia and then for 2 h of hypothermic reperfusion at 34, 30, or 27°C. After 2 h, all animals were killed.

Statistics

Statistical analysis of physiologic data was performed by one-way analysis of variance for multiple comparisons followed by Dunnnett’s post hoc test. Data were expressed as the mean ± SD. For the analysis of neurologic outcome and spinal histopathologic indices, nonparametric tests were used. For each study (studies 1–3), tests for overall temperature-dependent and time-dependent main effects were performed using the Kruskal-Wallis test. Significant main effects (P < 0.05) were probed further through sequential comparisons of each test condition with the adjacent test condition (e.g., 37 vs. 34°C, 34 vs. 30°C, and so forth) using the comparison of the experimental Mann-Whitney U test (an unpaired two-group test). To correct for the increased probability of type 1 error with multiple comparisons, we set our significance level for post hoc Mann-Whitney U test
comparisons at $P = 0.01$. Nonparametric data were expressed as the median using a scattergram.

### Results

**Intrathecal, Paravertebral, and Systemic Temperature Changes after Spinal Cooling**

Initiation of cooling caused a gradual decrease in the intrathecal temperature, with the intrathecal temperature $34^\circ C$ reached 2 min after initiation of the cooling, $30^\circ C$ at 7.5 min and $27^\circ C$ at 12.5 min. These intrathecal temperatures were maintained for 2 h of reperfusion (fig. 1B). A significant correlation between intrathecal and paravertebral muscle temperature was measured (fig. 1C). At the end of the 2-h cooling cycle, rectal temperature decreased from $37.2 \pm 0.2^\circ C$ to $34.5 \pm 0.8^\circ C$. Paravertebral muscle temperatures measured in each cooling protocol were used to initiate and maintain the target intrathecal temperature in the behavioral part of the study as follows.

- Target intrathecal temperature $27^\circ C = 22^\circ C$ paravertebral muscle temperature
- Target intrathecal temperature $30^\circ C = 27^\circ C$ paravertebral muscle temperature
- Target intrathecal temperature $34^\circ C = 31^\circ C$ paravertebral muscle temperature.

**Physiologic Variables**

During the preischemic and intraischemic periods, body temperature ranged from $38.5$ to $37.1^\circ C$ (table 2). Baseline distal arterial pressure was $89 \pm 15$ mmHg and decreased to $4 \pm 2$ mmHg after 10 min of aortic occlusion. No significant differences between groups were detected.

**Effect of Postischemic Cooling on the Recovery of Function**

**Study 1: Effect of 2 h of Postischemic Graded Spinal Hypothermia on Outcome**. In this study, a progressive reduction in postischemic spinal cord temperature resulted in a progressive improvement in motor scores. Statistical analysis showed an overall significant effect (Kruskal–Wallis test; $P = 0.0002$). Sequential intergroup analysis revealed temperature-dependent protection with the highest potency seen at $27^\circ C$ (Mann–Whitney U test; $27^\circ C \times 120$ min vs. $30^\circ C \times 120$ min; $P = 0.0051$; fig. 2A).

**Study 2: Effect of the Duration of Postischemic Cooling ($27^\circ C$) on Outcome**. In this study, statistical analysis also showed an overall significant effect (Kruskal–Wallis test; $P = 0.0012$). Sequential intergroup analysis showed better protection in the $27^\circ C \times 120$ min group when compared with the $27^\circ C \times 15$ min group (Mann–Whitney U test; $P = 0.005$). Because one animal in the $27^\circ C \times 15$ min group was paraplegic, no significant difference was seen compared with the $37^\circ C \times 15$ min group (Mann–Whitney U test; $P = 0.026$; fig. 2B).

**Study 3: Effect of a Delay in Initiation of Postischemic Cooling ($27^\circ C$) on Outcome**. Initiation of cooling 5 min, 60 min, or 120 min after normothermic reflow provided no protection (fig. 2C).

**Histopathologic Analysis**

Consistent with previously published data using this ischemic model, a clear correlation was seen between

### Table 2. Body Weight and Temperature in All Experimental Groups

<table>
<thead>
<tr>
<th>Study/Group</th>
<th>n</th>
<th>Body Weight (g)</th>
<th>Paravert. Muscle Temperature (°C)</th>
<th>Rectal Temperature (°C)</th>
</tr>
</thead>
<tbody>
<tr>
<td></td>
<td></td>
<td></td>
<td>Preischemia</td>
<td>End-ischemia</td>
</tr>
<tr>
<td></td>
<td></td>
<td></td>
<td>Preischemia</td>
<td>End-ischemia</td>
</tr>
<tr>
<td>Study 1</td>
<td>6</td>
<td>366.4 ± 21.3</td>
<td>38.4 ± 0.2</td>
<td>38.1 ± 0.2</td>
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<tr>
<td></td>
<td>6</td>
<td>361.7 ± 23</td>
<td>38.4 ± 0.1</td>
<td>38.5 ± 0.1</td>
</tr>
<tr>
<td></td>
<td>6</td>
<td>360.4 ± 23</td>
<td>38.5 ± 0.1</td>
<td>38.6 ± 0.1</td>
</tr>
<tr>
<td>Study 2</td>
<td>6</td>
<td>365 ± 19</td>
<td>38.3 ± 0.2</td>
<td>38.3 ± 0.2</td>
</tr>
<tr>
<td></td>
<td>6</td>
<td>361.4 ± 15.6</td>
<td>38.3 ± 0.2</td>
<td>38.3 ± 0.3</td>
</tr>
<tr>
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<td>5</td>
<td>369.6 ± 27.3</td>
<td>38.3 ± 0.2</td>
<td>38.5 ± 0.2</td>
</tr>
<tr>
<td></td>
<td>6</td>
<td>374.3 ± 24.5</td>
<td>38.3 ± 0.2</td>
<td>38.3 ± 0.2</td>
</tr>
<tr>
<td></td>
<td>6</td>
<td>347.3 ± 8.9</td>
<td>38.4 ± 0.3</td>
<td>38.5 ± 0.1</td>
</tr>
</tbody>
</table>

Values are mean ± SD.
the loss of motor and sensory function and the extent of histopathologic changes. In animals that had spastic paraplegia, these changes were expressed by the presence of extensive necrosis between laminae II and VII in the lumbosacral segments (fig. 3A). Corresponding with the presence of spasticity, a number of normally appearing A-motor neurons were also seen (fig. 3B). Using silver impregnation, in some animals somatodendritic argyrophilia affecting small and medium interneurons in the intermediate zone also was observed.

In animals that displayed nearly complete recovery, most neurons, including interneurons and A-motor neurons, had a normal appearance (fig. 3C). Only in some sections“dark”staining neurons in the ventral horn were observed (fig. 3D). By using the silver impregnation technique, a sparse appearance of drop-like degeneration suggestive of terminal dendritic degeneration in lamina VII also was seen.

Figure 4 summarizes the quantitative histopathologic analyses. In study 1, statistical analysis showed an overall significant effect (Kruskal–Wallis test; P = 0.0001). Intergroup analysis showed temperature-dependent suppression of spinal neuronal degeneration, with the most effective suppression seen in the 27°C group (fig. 4A). Although better protection was seen in the 27°C × 120 min group than in the 30°C × 120 min group, statistical analysis showed no significant difference (Mann–Whitney U test; P = 0.037). In study 2, significantly better protection in the 27°C × 120 min group, compared with the 27°C × 15 min group, was seen (Mann–Whitney U test; P = 0.008). In study 3, no overall significant effect was observed.

**Discussion**

Several experimental and clinical studies have shown that preischemically induced spinal cord hypothermia provides a significant protective effect against even a prolonged period of aortic occlusion. In the current
study, we found that posts ischemic cooling, if initiated immediately after an injurious interval of normothermic ischemia, also provides significant protection.

Rat Spinal Cord Vasculature and Spinal Collateral System

The rat, similar to several larger animal species such as the monkey, dog, and cat, possesses a heterosegmental aorta with 8–14 radicular arteries that give rise to one anterior and two posterior spinal arteries. Interestingly, a significant difference exists between the size and the localization of the radicular arteries, with the major (Adamkiewicz artery) typically branching from the thoracoabdominal aorta at the T8–L2 segmental level.17 Similarly, several collateral systems may contribute significantly to the preservation of the distal flow (i.e.,
below the level of aortic occlusion), particularly after a single aortic cross-clamp. One of the major contributors may derive from the internal thoracic artery, which branches from the subclavian arteries and has well-developed anastomoses with the lower thoracic arterial system and may provide a retrograde flow to the segmental spinal cord vessels. In addition, a continuing collateral flow down through the ventral chest wall and the abdominal free wall anastomoses with the iliac arteries. Finally, both subclavian arteries supplying the vertebral artery may provide additional flow through the anterior spinal artery. These properties are remarkably similar to the human spinal vascular organization.

**Technique of Selective Spinal Cord Cooling and Spinal Temperature Changes**

In the current study, we used a technique of selective spinal cord cooling in the rat by using a small copper heat exchanger implanted into the subcutaneous space overlying lumbosacral spinal cord segments. In a previous study using this technique, we induced and maintained selective spinal cord hypothermia (as low as \(27^\circ\mathrm{C}\)) for a period of at least 5 h without affecting systemic temperature. Importantly, we also showed a significant correlation between intrathecal and paravertebral muscle temperature, thus permitting independent continuous measurement of spinal temperature. However, it is important to note that by using this cooling technique, intrathecal temperature, as measured by a thermocouple placed on the dorsal or ventral spinal cord surface, can be decreased to \(27^\circ\mathrm{C}\) with a 2 or \(3^\circ\mathrm{C}\) temperature gradient measured between the dorsal and ventral surface of the lumbar spinal cord. Although it is likely that spinal core temperature will be slightly higher in spinal parenchyma than measured in the intrathecal space, we believe that spinal temperature changes will be in the range of temperatures measured between the dorsal and ventral surface of the spinal cord. In addition, it is important to stress that 12 min are needed to decrease the intrathecal temperature to \(27^\circ\mathrm{C}\). Thus, in the current study in the \(27^\circ\mathrm{C} \times 15\) min cooling group, the initial 12 min of postischemic cooling corresponded...
with a gradual decrease in spinal cord temperature from 38 to 27°C, and 27°C hypothermia was maintained for a subsequent 3 or 4 min.

Postischemic Hypothermia and a Critical Temporal Window of Influence

In the current study, spinal cord hypothermia, when it was initiated immediately after ischemia and maintained for 2 h of reperfusion, provided temperature-dependent protection of neurologic function when assessed at 48 h of normothermic reperfusion. The highest degree of protection was observed at 27°C when all animals showed normal motor behavior and sensory function. At 34°C, only partial protection was observed.

Delaying the onset of hypothermia for 5 min or more significantly reduced the protective effects of 27°C hypothermia (even though the cooling persisted for a total of an additional 2 h). Conversely, cooling initiated immediately after reflow even for only 15 min (target temperature 27°C reached at 12 min after the onset of cooling) provided significant protection at 48 to 72 h of survival. These observations define the presence of a critical 5- to 15-min window in which hypothermia can alter processes leading to a deleterious outcome.

These data appear to be similar to the experimental data obtained in global cerebral ischemia models. In those studies, 3 h of moderate (30°C) postischemic hypothermia (if initiated immediately after reperfusion) provided significant protection. However, this protective effect was lost when the cooling was initiated 30 min after normothermic reperfusion. Similarly, in a dog cardiac arrest model, immediate postischemic mild (34°C) cerebral hypothermia provided significant improvement of functional outcome, whereas a 15-min delay in the onset of the cooling had no effect. It is important to note that in recent studies 3 h of postischemic brain hypothermia (34°C) provided only transient protection during the initial 3 days of subsequent normothermic reperfusion and that permanent protection was achieved only if brain postischemic hypothermia (32°C) was maintained for at least 24 h of reperfusion.

In the current study, the animals that had significant recovery survived for a maximum of 3 days. Although neuroprotection was observed clearly at this time, long-term degeneration during subsequent survival cannot be ruled out. However, using an experimental and treatment design identical to that used in the current study, we recently observed a comparable protective effect that lasted for a minimum of 5 days of survival. Nonetheless, prolonged survival periods (several weeks or months) will be necessary to allow us to make definitive conclusions.

Spinal Neurohistopathologic Changes

Consistent with the described effect of postischemic hypothermia on the recovery of neurologic function, the temperature- and duration-dependent protective effect of postischemic cooling in the suppression of spinal neurodegenerative changes was observed. Thus, in animals that had spastic paraplegia (i.e., normothermic control), extensive necrotic changes (typically in the central gray matter between laminae IV-VII) were observed. Consistent with the selective loss of inhibitory neurons in this region and several surviving Aα-motor neurons, a spastic type of paraplegia typically was observed. Importantly, in a previous study using an identical ischemic interval (10 min) we observed fully developed spastic paraplegia that continued for 3 days of survival and corresponded with extensive necrotic changes in the lumbar sacral gray matter. These neurohistopathologic changes are comparable to changes observed at 48 h of survival in the current study in control normothermic animals. These observations further emphasize a potent neuroprotective effect of immediate postischemic hypothermia (27°C) that persists for at least 3 days after normothermic reflow.

A Mechanism of Hypothermia-mediated Protection

Although the mechanisms by which postischemic hypothermia might protect against ischemic insults in the brain and spinal cord are still unclear, it has been shown in a rat cerebral ischemia model that a secondary excitatory amino acid (EAA) release occurs after 8 h of reperfusion (in addition to the intraschismic EAA release). This would suggest that glutamate receptor activation and an ongoing Ca2+ influx may occur during the early and the late period of reflow, and this influx may potentiate neurodegeneration initiated by normothermic ischemia. By using long-term lumbar intrathecal microdialysis, we observed a similar biphasic glutamate release after an injurious interval (12 min) of spinal ischemia, with the first peak observed at 15–30 min of reperfusion, followed by a secondary release at 4–6 h. These data indicate that postischemic cooling, when initiated dur-

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ing early reperfusion, can prevent changes that account for the delayed EAA release.

In addition, research has also shown that postischemic hypothermia (28°C) suppresses phospholipase A2 and correspondingly suppresses the loss of membrane-bound phospholipids, suggesting an additional protective mechanism.

In conclusion, the current data show that 2 h of postischemic spinal cord cooling (27°C), when initiated immediately after an otherwise injurious interval of normothermic spinal ischemia, provides significant recovery of neurologic function and a corresponding suppression of histopathologic changes during a subsequent 2 or 3 days of normothermic survival. These studies define a critical temporal window when hypothermia, after a 10-min interval of ischemia, may prevent the initiation of a cascade of processes that would otherwise lead to irreversible neuronal dysfunction. Although the mechanism of this protection is not clear, it may be related in part to the suppression of intracellular enzymatic processes that mediate the neuronal degeneration or modulation of excessive excitatory amino acid release observed during the initial hours of normothermic reperfusion.

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