Effects of Hypothermia on Blood Flow and Neural Activity in Rabbit Spinal Cord during Postischemic Reperfusion

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Abstract: The effects of hypothermia on blood flow and neural activity were investigated in rabbit spinal cord during the acute phase of ischemia/reperfusion. Rabbits were exposed to ischemia for 10 or 40 min by occluding the abdominal aorta, using a balloon catheter. The body temperature was maintained either at 38°C (normothermia) or 34°C (hypothermia). Hyperperfusion was observed within 10 min after the cessation of ischemia in all rabbits exposed to ischemia. The magnitude of hyperperfusion in spinal cord blood flow (SCBF) was not significantly different between the 10 and 40 min ischemia rabbits, but the time for 50% recovery from the hyperperfusion was longer in the 40 min ischemia group (26.1 ± 2.5 min) than in the 10 min group (15.1 ± 2.1 min). The amplitude of evoked spinal cord potential decreased during ischemia and recovered to the baseline level during 8 h of reperfusion in the 10 min ischemia group. However, in the 40 min ischemia group, the amplitude was 40 ± 8% of the baseline value after 8 h of reperfusion. Hypothermia prevented the delay of recovery from hyperperfusion and the reduction of evoked spinal cord potential. These results suggest that hypothermia plays a beneficial role in protecting tissue injury in the acute phase of ischemia/reperfusion in the spinal cord by shortening the time for recovery from postischemic hyperperfusion. [Japanese Journal of Physiology, 51, 71–79, 2001]

Key words: ischemia-reperfusion, spinal cord blood flow, evoked spinal cord potential, hypothermia.
spinal cord ischemia in rabbits, and estimated the influence of hypothermia on the ischemic injury of the spinal cord. We hypothesized that hypothermia will decrease the changes in SCBF and prevent the impairment of ESCP during several hours of reperfusion.

**MATERIALS AND METHODS**

Thirty-eight adult Japanese white rabbits of both sexes, weighing 2.5 to 3.5 kg, were used in this study. All procedures were reviewed and approved by the Committee for Animal Experimentation in the Faculty of Medicine, Tottori University, Japan, and conformed to Guiding Principles for the Care and Use of Animals in the Field of Physiological Sciences published by the Council of the Physiological Society of Japan.

**Animal preparation.** The rabbits were anesthetized with an intraperitoneal injection of 1 g/kg urethane. The depth of anesthesia was maintained in the ear vein for the administration of medications and physiological salt solution (4 ml/kg/h). The animals were then intubated, placed supine, and artificially ventilated (tidal volume, 10 ml/kg) with a mixture of oxygen (50 to 100 ml/min) and room air, following paralysis with pancuronium bromide (an initial dose of 0.3 mg/kg and a continuous dose of 0.3 to 0.4 mg/kg/h, i.v., Oragnon, Teknika, Oss, The Netherlands). The respirator (SN-480-6, Shinano, Tokyo, Japan) was adjusted to 20 to 40 cycles/min to maintain $P_{aCO_2}$ at 35 to 40 mmHg throughout the experiments. A 4 Fr balloon catheter (AI-7121, Arrow international Inc., Bernville Road, Reading, PA) was inserted through the right femoral artery in the abdominal aorta distal to the left renal artery. Another polyethylene catheter was inserted in the abdominal aorta through the left femoral artery for the measurement of arterial pressure and blood gas analysis (238 gas analyzer, Ciba Corning, Tokyo, Japan). Arterial blood pressure was measured in the abdominal aorta distal to the balloon catheter. The esophageal temperature was measured in the abdominal aorta distal to the balloon catheter. The esophageal temperature was measured with a thermometer (BAT/10LOP, RET-1, Physistemp Instruments Inc., Clifton, NJ) and maintained between 37.5 and 38.5°C by using a domestic heating-cooling pad and an electric heater. Heparin (500 IU) was administered intravenously to prevent blood coagulation 5 min before catheter insertion, and an additional dose of heparin was given as required. After these procedures were completed, the rabbits were mounted in a prone position on a stereotaxic frame. An incision was made through the skin overlying the thoracic and lumbar sections of the spine. Laminctomy was performed at the vertebral levels of T12, L6, and L7. The epidural temperature was also measured by using a needle type temperature probe (MT-4, Physistemp Instruments Inc., Clifton, NJ) placed on the dura surface at L6.

**Animal groups.** Animals were randomly assigned to one of the following groups. Group 1 $(n=5)$: control group. Animals were not exposed to ischemia or hypothermia. Group 2 $(n=10)$: 10 min ischemia group. Animals were exposed to 10 min ischemia, and their esophageal temperature was maintained within the normal range throughout the experiments. Group 3 $(n=9)$: 40 min ischemia group. Animals were exposed to 40 min ischemia, and their esophageal temperature was maintained within the normal range throughout the experiments. Group 4 $(n=5)$: hypothermia control group. Animals were not exposed to ischemia, but their esophageal temperature was reduced for 160 min, reaching 33.5–34.5°C during the hypothermic period. Group 5 $(n=9)$: 40 min ischemia with hypothermia group. Animals were exposed to 40 min ischemia, and their esophageal temperature was maintained within the normal range throughout the experiments. Group 4 $(n=5)$: hypothermia control group. Animals were not exposed to ischemia, but their esophageal temperature was reduced for 160 min, reaching 33.5–34.5°C during the hypothermic period. Group 5 $(n=9)$: 40 min ischemia with hypothermia group. Animals were exposed to 40 min ischemia, and their esophageal temperature was reduced for 160 min, reaching 33.5–34.5°C during the hypothermic period (Fig. 1).

**Measurement of spinal cord blood flow (SCBF).** The blood flow was continuously measured by using a laser Doppler flowmeter (ALF21, Advance, Tokyo, Japan). The instrument emits a continuous semiconductor laser beam on one channel, with a wavelength of 780 nm and an available power of 2.0 mW. The sample volume covers a radius of about 1.0 mm. The time constant for signal processing was set at 1.0 s. A laser Doppler probe was positioned on the dura at L6 by a micromanipulator to a spot hav-
ing no vessels with a diameter of larger than 0.1 mm. Intense direct illumination of the explored field was avoided. Data logging was performed digitally by using the MacLab system (MacLab/400, ML401, ADInstruments Pty Ltd., Castle Hill, NSW, Australia). The output of the SCBF recording was averaged for 30 s. Relative SCBF was expressed as a percentage of the preischemic baseline value in each animal.

**Measurement of evoked spinal cord potential (ESCP).** The procedure for ESCP measurement was modified from the method reported by Takahashi and co-workers [12]. Briefly, two silver ball electrodes (about 0.5 mm in diameter and about 3 mm apart between the two electrodes) were fixed to the median dorsal region of the dura mater at T12 and L7. These electrodes were used to stimulate the spinal cord and to record resultant potentials. The spinal cord was stimulated by using rectangular constant currents (SEN-3201 and SS-102J, Nihon Kohden, Tokyo, Japan) with a duration of 0.1 ms. The intensity of the currents was supramaximal (10 V and 3 to 30 times the threshold). The recorded band width was 50 to 1,000 Hz, and the analysis time was 10 ms. Electrical stimulation was applied for 5 s with a frequency of 10 Hz, and summation was done 50 times on recording devices (VC-11, QC-111J, and ATAC-201, Nihon Kohden). The peak-to-peak amplitude of the first potential was digitally recorded on the MacLab system and analyzed by an off-line computer (Fig. 2). The average baseline value of the amplitude was 16.2±2.0 μV in 38 rabbits.

**Ischemia-reperfusion protocol.** After a 60 min stabilization period, lumbar spinal cord ischemia was induced by rapidly inflating the balloon with 0.5 ml of air. Occlusion of the abdominal aorta decreased distal arterial blood pressure significantly (Table 1b) without a marked change in heart rate (Table 1a). Exposure to hypothermia decreased heart rate (Table 1a) during the ischemia period. After 8 h of reperfusion, the mean arterial blood pressure dropped slightly compared with the baseline value in all groups. Although \( P_{aCO_2} \) did not change significantly throughout the experiment in any group (Table 1c), exposure to 40 min of ischemia produced metabolic acidosis 5 min after reperfusion (Table 1d). Esophageal temperature was kept constant in the normothermia groups (groups 1, 2, and 3), and decreased to 33.6±0.1°C, from 38.5±0.1°C, during the hypothermia period in group 5 (Table 2a). Hypothermia decreased the spinal cord local temperature by 3.8°C (Table 2b).

**Changes in SCBF**

In the control group (group 1), SCBF did not change significantly throughout the experiments (Fig. 3). In the 10 min ischemia group (group 2), SCBF decreased to 10±2% of the baseline value during ischemia and increased to 240±17% of the baseline at
5 min after reperfusion (Fig. 3). Within 1 h after reperfusion, SCBF decreased almost to the baseline level. Then SCBF increased again, gradually reaching significantly higher than the baseline at 4 h after reperfusion.

During 40 min of ischemia in group 3, SCBF decreased to 76.3% of the baseline value (Fig. 4). At 10 min after reperfusion, SCBF increased to 218.6% of the baseline level. At 1 h after reperfusion, SCBF decreased to 113.16% of the baseline level and then increased slightly. Exposure to hypothermia decreased SCBF to 86.3% of the baseline in group 4. The SCBF recovered almost to the baseline level during rewarming (Fig. 5). In the 40 min of ischemia with hypothermia (group 5), SCBF decreased to 78.65% of the baseline during hypothermia before ischemia and further decreased to 7.63% of the baseline level during ischemia. There was no significant difference in the extent of ischemia among groups 2, 3, and 5. The SCBF increased to 176.12% of the baseline level at 10 min after reperfusion, decreased below the baseline from 1 to 3 h, and again

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**Table 1. Heart rate, mean arterial blood pressure, and blood gas analysis.**

<table>
<thead>
<tr>
<th></th>
<th>Baseline</th>
<th>During ischemia</th>
<th>5 min reperfusion</th>
<th>8 h reperfusion</th>
</tr>
</thead>
<tbody>
<tr>
<td>(a) Heart rate (beats/min)</td>
<td></td>
<td></td>
<td></td>
<td></td>
</tr>
<tr>
<td>Group 1</td>
<td>285±14</td>
<td></td>
<td>276±8</td>
<td>291±13</td>
</tr>
<tr>
<td>Group 2</td>
<td>306±9</td>
<td>295±11</td>
<td>305±8</td>
<td>303±9</td>
</tr>
<tr>
<td>Group 3</td>
<td>324±6</td>
<td>296±20</td>
<td>298±10</td>
<td>306±6</td>
</tr>
<tr>
<td>Group 5</td>
<td>313±5</td>
<td>210±10**</td>
<td>196±6**</td>
<td>298±9</td>
</tr>
<tr>
<td>(b) Distal MAP (mmHg)</td>
<td></td>
<td></td>
<td></td>
<td></td>
</tr>
<tr>
<td>Group 1</td>
<td>93±4</td>
<td></td>
<td>90±5</td>
<td>70±10**</td>
</tr>
<tr>
<td>Group 2</td>
<td>78±5</td>
<td>13±2**</td>
<td>72±4</td>
<td>70±5</td>
</tr>
<tr>
<td>Group 3</td>
<td>86±4</td>
<td>14±1**</td>
<td>74±6</td>
<td>68±7**</td>
</tr>
<tr>
<td>Group 5</td>
<td>83±10</td>
<td></td>
<td>75±4</td>
<td>68±2</td>
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<tr>
<td>(c) $P_{CO_2}$ (mmHg)</td>
<td></td>
<td></td>
<td></td>
<td></td>
</tr>
<tr>
<td>Group 1</td>
<td>40.2±1.4</td>
<td></td>
<td>41.0±2.6</td>
<td>38.6±1.4</td>
</tr>
<tr>
<td>Group 2</td>
<td>39.6±1.4</td>
<td></td>
<td>38.5±1.5</td>
<td>36.1±1.4</td>
</tr>
<tr>
<td>Group 3</td>
<td>35.6±1.2</td>
<td></td>
<td>38.0±1.9</td>
<td>39.3±1.4</td>
</tr>
<tr>
<td>Group 5</td>
<td>37.6±1.4</td>
<td></td>
<td>35.3±2.3</td>
<td>34.8±2.2</td>
</tr>
<tr>
<td>(d) pH</td>
<td></td>
<td></td>
<td></td>
<td></td>
</tr>
<tr>
<td>Group 1</td>
<td>7.45±0.01</td>
<td></td>
<td>7.46±0.01</td>
<td>7.44±0.02</td>
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<tr>
<td>Group 2</td>
<td>7.45±0.01</td>
<td></td>
<td>7.42±0.02</td>
<td>7.45±0.01</td>
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<tr>
<td>Group 3</td>
<td>7.51±0.01</td>
<td></td>
<td>7.34±0.03**</td>
<td>7.47±0.03</td>
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<tr>
<td>Group 5</td>
<td>7.51±0.02</td>
<td></td>
<td>7.28±0.03**</td>
<td>7.48±0.01</td>
</tr>
</tbody>
</table>

Values are means±SEM. *p<0.05, **p<0.01 vs. baseline value.

**Table 2. Body temperature analysis.**

<table>
<thead>
<tr>
<th></th>
<th>Baseline</th>
<th>During ischemia</th>
<th>1 h reperfusion</th>
<th>8 h reperfusion</th>
</tr>
</thead>
<tbody>
<tr>
<td>(a) Esophageal temperature (°C)</td>
<td></td>
<td></td>
<td></td>
<td></td>
</tr>
<tr>
<td>Group 1</td>
<td>38.1±0.1</td>
<td></td>
<td>38.3±0.1</td>
<td>38.0±0.1</td>
</tr>
<tr>
<td>Group 2</td>
<td>38.3±0.1</td>
<td></td>
<td>38.2±0.1</td>
<td>38.1±0.1</td>
</tr>
<tr>
<td>Group 3</td>
<td>38.2±0.2</td>
<td>38.0±0.2</td>
<td>38.1±0.1</td>
<td>38.2±0.1</td>
</tr>
<tr>
<td>Group 5</td>
<td>38.5±0.1</td>
<td>33.6±0.1**</td>
<td>37.8±0.2**</td>
<td>38.1±0.1</td>
</tr>
<tr>
<td>(b) Spinal cord temperature (°C)</td>
<td></td>
<td></td>
<td></td>
<td></td>
</tr>
<tr>
<td>Group 1</td>
<td>34.7±0.3</td>
<td></td>
<td>34.7±0.3</td>
<td>34.5±0.3</td>
</tr>
<tr>
<td>Group 2</td>
<td>35.3±0.2</td>
<td>34.9±0.4</td>
<td>35.5±0.1</td>
<td>35.0±0.2</td>
</tr>
<tr>
<td>Group 3</td>
<td>35.5±0.2</td>
<td>35.2±0.1</td>
<td>35.1±0.4</td>
<td>35.4±0.1</td>
</tr>
<tr>
<td>Group 5</td>
<td>34.8±0.3</td>
<td>31.0±0.3**</td>
<td>33.5±0.7*</td>
<td>35.0±0.2</td>
</tr>
</tbody>
</table>

Values are means±SEM. *p<0.05, **p<0.01 vs. baseline value.

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5 min after reperfusion, then SCBF increased again, gradually reaching significantly higher than the baseline at 4 h after reperfusion.

During 40 min of ischemia in group 3, SCBF decreased to 7.3% of the baseline value (Fig. 4). At 10 min after reperfusion, SCBF increased to 218.2% of the baseline level. At 1 h after reperfusion, SCBF decreased to 113.16% of the baseline level and then increased slightly. Exposure to hypothermia decreased SCBF to 86.3% of the baseline in group 4. The SCBF recovered almost to the baseline level during rewarming (Fig. 5). In the 40 min of ischemia with hypothermia (group 5), SCBF decreased to 78.65% of the baseline during hypothermia before ischemia and further decreased to 7.63% of the baseline level during ischemia. There was no significant difference in the extent of ischemia among groups 2, 3, and 5. The SCBF increased to 176.12% of the baseline level at 10 min after reperfusion, decreased below the baseline from 1 to 3 h, and again
rose above the baseline after 5 h of reperfusion. No significant difference was found in the peak SCBF between groups 2 and 3 or between groups 3 and 5. Figure 6 shows the recovery time by which the SCBF returned halfway to the baseline. The recovery time in group 3 (26.1 ± 2.5 min) was significantly longer than in group 2 (15.1 ± 2.1 min) and in group 5 (18.0 ± 2.5 min).

Changes in ESCP

In the control group (group 1), ESCP showed no significant change during the experimental period (Fig. 7). In the 10 min ischemia group (group 2), ESCP decreased to 41 ± 11% of the baseline level during ischemia. The ESCP recovered to the baseline level within 20 min after reperfusion. Slight fluctuations of the ESCP were observed during 8 h of reperfusion, but no significant difference was noted in ESCP between groups 1 and 2.

In the 40 min ischemia group (group 3), ESCP decreased to 6 ± 2% of the baseline level during ischemia (Fig. 8). At 1 h after reperfusion, ESCP recovered to 69 ± 15% of the baseline. The ESCP then decreased gradually in the next 7 h of reperfusion, reaching 40 ± 8% of the baseline level at the end of experiments. In the rabbits of group 4, exposure to hypothermia increased the amplitude of ESCP to 220 ± 23% of the baseline value. The ESCP recovered to the baseline as soon as the animal was rewarmed (Fig. 5). In the 40 min ischemia with hypothermia group (group 5), ESCP increased to 193 ± 15% of...
baseline level because of hypothermia and decreased to 2±0% of the baseline level during ischemia. During reperfusion, ESCP increased above the baseline level at 1 h, decreased to 91±12% by 2 h, and was maintained between 82 and 96% through the rest of the experiment.

Figure 9 shows the amplitude of ESCP at 8 h after reperfusion. The ESCP in group 3 (40±8%) was significantly smaller than that in group 2 (125±11%) or in group 5 (88±12%).

**DISCUSSION**

Major findings in the present study are as follows: (1) The magnitude of postischemic hyperperfusion in SCBF was not significantly different between the 10 and 40 min ischemia rabbits, but the time for recovery from the hyperperfusion was longer in the 40 min ischemia group than in the 10 min group (Fig. 6); (2) ESCP recovered to the baseline level during 8 h of reperfusion in the 10 min ischemia group, but not in the 40 min ischemia group (Fig. 9); and (3) hypothermia prevented the delay of recovery from the hyperperfusion (Fig. 6) and the reduction of ESCP (Fig. 9) observed during reperfusion in the 40 min ischemia rabbits.

Several techniques have been used to measure spinal cord blood flow, including hydrogen clearance [14], 133Xe clearance [15], microsphere method [16], 14C-antipyrine autoradiography [17], and laser-Doppler flowmetry [18]. Among these, laser-Doppler flowmetry offers sensitive, stable, and reproducible estimates of microcirculation in the spinal cord [19]. It has the advantage of continuous monitoring for tissue blood flow during nonsteady states. The other techniques are useful to obtain the absolute value of the blood flow or to examine regional heterogeneity in the blood flow. However, they can measure the blood flow only intermittently, which is a disadvantage for monitoring rapid changes in it. As we have shown in the present study, SCBF increases to more than 200%, from less than 10%, of the baseline value and decreases to almost the baseline level within 1 h during reperfusion. When this happens, it is difficult to estimate the time for recovery from the hyperperfusion by using intermittent measurements of SCBF. Thus we employed laser-Doppler flowmetry in the present study for a continuous monitoring of SCBF.

In the present study, ESCP was used to evaluate spinal cord function during ischemia/reperfusion. Takahashi and co-workers [12] used ESCP to evaluate the tissue damage as a result of spinal cord compression and found that the change in ESCP correlated
well with findings in magnetic resonance imaging or morphological experiments. In this technique, the recorded potentials represent the nerve activity of the whole spinal cord, since the spinal cord is stimulated directly with supramaximal currents. The first potential has been reported to be transmitted mainly from the lateral column and partly from the dorsal and anterior columns [20, 21]. Thus ESCP has the advantage of monitoring both the sensory and motor functions simultaneously in this animal experiment model.

Spinal ischemia is a serious clinical problem during surgical operations for thoracobdominal aortic aneurysm. Attention should be paid to making the operation time short, since prolonged spinal ischemia may result in paraplegia in the lower extremities [22, 23], although that can occur even with short (less than 30 min) ischemia [24]. It is generally accepted that not only ischemia itself, but also posts ischemic reperfusion plays a certain role in the formation of neural damage. The present results demonstrated that the recovery time from the posts ischemic hyperperfusion was longer in 40 min than in 10 min ischemia rabbits. The mechanism of posts ischemic hyperperfusion is still unclear. Many factors have been listed, including loss of autoregulation [4], neurogenic factors [25], accumulation of lactate [26] and adenosine [27], and release of nitric oxide [28] or superoxide [29]. Some of these factors are cytotoxic and impair cellular function. In the present experiments, the prolonged recovery was accompanied by reduced ESCP in the 40 min ischemia rabbits. Therefore a greater amount of cytotoxic factor(s) seems to be induced by prolonged hyperperfusion after the longer exposure to ischemia in the rabbit spinal cord.

Hypothermia has been demonstrated in various studies to have beneficial effects in preventing impairment in spinal cord function because of ischemia [5–8]. It is known that hypothermia decreases metabolic activity [30], prevents ATP degradation [31], reduces glutamate release [32], inhibits protease activation [33], and decreases hydroxyl radical formation [1]. The degradation of ATP may cause intracellular edema through an impairment of Na-K exchange, glutamate release may increase intracellular calcium to a toxic level by activation of NMDA receptors, and hydroxyl radical formation may impair the cellular membrane of the spinal neurons. Thus we hypothesized that hypothermia will prevent the impairment of spinal cord function through some of these mechanisms. The effects of mild (34°C), moderate (32 to 28°C), and profound hypothermia (15°C) on ischemia-induced spinal cord damage have been examined in previous studies. It is well known that cardiac arrhythmia occurs when body temperature decreases to lower than 32°C [34]. Thus in the present study we employed 34°C as the temperature during hypothermia from a clinical point of view. Body cooling to 34°C itself increased the amplitude of ESCP (Fig. 5). This is consistent with a previous report [35], in which it is suggested that the increase in ESCP amplitude is attributable to an increase in the electrical charged density at the recording area. Although the mechanism of the effects of hypothermia was not examined in detail, the present results supported this hypothesis, since hypothermia was effective to prevent deterioration in SCBF and ESCP during reperfusion. Hypothermia shows a beneficial effect by preventing an elevation of cerebrospinal fluid pressure as a result of brain edema [36]. This did not happen in the present study because laminectomy, which prevents an increase in cerebrospinal fluid pressure, was performed to fix stimulating and recording electrodes. Studies have been carried out to estimate the effects of medications, such as thiopental, ketamine, lidocaine, naloxone, magnesium, methylprednisolone, and tissue factor pathway inhibitor, on transient spinal cord ischemia [37–40]. Some of these medications showed beneficial effects on spinal cord exposed to ischemia/reperfusion in animal experiments, but none is accepted as a clinical treatment for patients with spinal cord ischemia or patients who undergo aortic reconstructive surgery. Thus it is very important not only from a physiological, but also from a clinical point of view to clarify the underlying mechanism for hypothermia in the protection of spinal cord function during ischemia/reperfusion.

In conclusion, exposure to 40 min ischemia caused serious changes in blood flow and neural functioning in rabbit spinal cord. The time for recovery of the blood flow from posts ischemic hyperperfusion was significantly longer in the 40 min ischemia group than in the 10 min ischemia group. In the 40 min ischemia group, ESCP did not recover to the baseline level during 8 h of reperfusion. Hypothermia shortened the time for recovery from the hyperperfusion and prevented the reduction in ESCP amplitude during reperfusion, suggesting a beneficial role of hypothermia in the acute phase of ischemia/reperfusion in the spinal cord.

The authors thank Professor Y. Ishibe, Department of Anesthesiology and Reanimatology, Faculty of Medicine, Tottori University, for his valuable advice.

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