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Neuroprotective Effect of Epidural Electrical Stimulation against Ischemic Spinal Cord Injury in Rats

Electrical Preconditioning

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Background: **Electroconvulsion therapy is likely to serve as an effective preconditioning stimulus for inducing tolerance to ischemic brain injury. The current study examines whether electrical stimuli on the spinal cord is also capable of inducing tolerance to ischemic spinal cord injury by transient aortic occlusion.**

Methods: **Spinal cord ischemia was induced by occlusion of the descending thoracic aorta in combination with maintaining systemic hypotension (40 mmHg) during the procedure. Animals implanted with epidural electrodes were divided into four groups according to electrical stimulation and sham. Two groups consisted of rapid preconditioning (RE group, n = 8) and sham procedure (RC group, n = 8) 30 min before 9 min of spinal cord ischemia. In the two groups that underwent delayed preconditioning, rats were exposed to 9 min of aortic occlusion 24 h after either pretreatment with epidural electrical stimulation (DE group, n = 8) or sham (DC group, n = 8). In addition, rats were exposed to 6–11 min of spinal cord ischemia at 30 min or 24 h after epidural electrical stimulation or sham stimulation. The group P50 represents the duration of spinal cord ischemia associated with 50% probability of resultant paraplegia.**

Results: **Pretreatment with electrical stimulation in the DE group but not the RE group protected the spinal cord against ischemia, and this stimulation prolonged the P50 by approximately 15.0% in the DE group compared with the DC group.**

Conclusions: **Although the optimal setting for this electrical preconditioning should be determined in future studies, the results suggest that epidural electrical stimulation will be a useful approach to provide spinal protection against ischemia.**

TRANSIENT spinal cord ischemia with subsequent loss of neurologic function (spastic or flaccid paraplegia) represents a serious complication associated with transient aortic cross clamping as used in repair of aortic aneurysm. Ischemic spinal cord injury after thoracoabdominal aortic aneurysm repair remains a devastating complication because patients with postoperative paraplegia and paraparesis have decreased survival rates.[1] Consideration of the clinical importance of spinal ischemia has led to efforts to characterize the potency of numerous pharmacologic, surgical, and physical interventions in an effort to reduce spinal neuronal degeneration during periods of transient spinal cord ischemia.

Recently, the phenomenon of induced ischemic tolerance, *i.e.*, development of higher ischemic tolerance against otherwise injurious intervals of ischemia, has been described in tissue of several organs, including the brain, heart, and kidneys. Several studies have also demonstrated that ischemic tolerance in the spinal cord can develop after sublethal ischemic stress in rat,[2] rabbit,[3] and dog[4] spinal cord ischemic models. In addition, Cizkova *et al.*[5] demonstrated that there are two forms of ischemic tolerance, rapid and delayed tolerance. The former is established within 30 min of reperfusion, and the latter is established 24 h after ischemia. Such a wide time window for the interval between pretreatment and ischemia suggests that more than one mechanism is involved in protection by ischemic preconditioning protection of the spinal cord.

Although patients undergoing such surgery could receive ischemic preconditioning to modulate spinal cord ischemic tolerance before the lethal interval of spinal cord ischemia, its safety margins and potential for eliciting injury remains controversial in the clinical setting.[6] Therefore, it is still important to search for effective preconditioning stimuli with greater safety margins and established potential for clinical implementation. In the central nervous system, ischemic tolerance is elicited by a number of preconditioning stimuli, including brief ischemia,[7–9] spreading depression,[10,11] hypoxia,[12] hyperthermia,[13] and repetitive hyperbaric oxygen.[14] In addition, recent findings indicate that electrical stimulation on the spinal cord can serve as an effective preconditioning stimulus for inducing tolerance to traumatic spinal cord injury.[15] Electrical stimuli on the epidural space surrounding the spinal cord also is an established treatment in pain clinics and is performed with substantial safety. However, the potential of electrical stimuli for inducing ischemic tolerance has not been examined in the field of spinal cord ischemia. The current study examines whether electrical stimuli on the spinal cord is also capable of inducing tolerance to ischemic spinal cord injury induced by transient aortic occlusion and, if so, whether there are two forms of tolerance, rapid and delayed.

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Materials and Methods

The following investigations were performed under a protocol approved by the Institutional Animal Care Committee, University of the Ryukyus (Nishihara, Okinawa, Japan).

Implantation of the Epidural Electrodes

Male Sprague-Dawley rats (330–370 g) were anesthetized with isoflurane (5%) in an anesthesia induction box. Upon loss of responsiveness and spontaneous movement, the rat was removed from the induction box and anesthetized continuously with isoflurane (2–2.5%) in an air–oxygen mixture (1:1) during spontaneous respiration. With the rat in the supine position, the dorsal thoracolumbar spinal region was surgically prepared with 70% alcohol. A 2- to 3-cm midline skin incision was made at the level of the upper lumbar area (L1–L3), and vertebral muscles were bluntly dissected from the vertebrae (L1–L3). The spinal process at L2 was removed, and the vertebra at L3 was retracted to expose the intervertebral spaces (L1/2 and L2/3). Both intervertebral ligaments were carefully cut, and one epidural silver electrode was inserted into the epidural space 2 cm cephalad from L1/2, and another was inserted 2 cm caudally from L2/3. The epidural silver electrodes (0.1 mm in diameter; Unique Medical, Fukuoka, Japan) were insulated by polyethylene tubing (PE-5: 0.14 mm OD; Clay Adams, Parsippany, NJ), and the end of the silver wire that was inserted into the epidural space was melted by fire and changed into a ball shape. Muscles and fascia were sutured with 3-0 Nespiren (HV1303; Alfresa Co., Chuo, Osaka, Japan), and the skin was closed using 3-0 silk (HA-03, Alfresa Co.). Inhalation of isoflurane was stopped, and animals were observed during recovery in a warm box. All animals were typically allowed to recover for a minimum of 5 days before experimentation. Rats showing signs of motor weakness or paresis after recovery from anesthesia were killed. In harvesting a spinal cord for histopathologic analysis, we confirmed that the ends of the silver wire advanced cephalad and caudad were placed on the dorsal surface of the spinal cord at the levels of T10 and L5, respectively.

Determination of Epidural Electrical Stimulation Intensity

In a preliminary study (six rats) to determine epidural electrical stimulation intensity, compound muscle action potentials were recorded by Neuropack II (Nihon Koden, Tokyo, Japan) corresponding to the epidural electrical stimulation. After rats previously implanted with the epidural silver electrodes were anesthetized with isoflurane by an inhalation mask, a needle electrode was inserted into the left soleus muscle for recording compound muscle action potentials in the prone position. The stimulation intensity began at 0.4 mA and was gradually increased in 0.4-mA increments with 60 s between each stimulus until the maximal M wave on compound muscle action potentials was reached. The least stimulus intensity that produced a stable maximal M wave was used as the epidural electrical intensity. Data of this preliminary study showed a gradual increase in M-wave response corresponding to a gradual increase in epidural electrical intensity (figs. 1A and B). From these data, the optimal electrical stimulus intensity was determined as 5 mA.

Epidural Electrical Stimulation

Rats previously implanted with the epidural silver electrodes were anesthetized in a Plexiglas box with 4% isoflurane in room air. After induction, rats were maintained with 1.0% isoflurane delivered by an inhalation mask. Epidural electrical stimulation (a square wave pulse, 500 Hz, 5 mA, 10 s) was administered *via* the epidural silver electrodes. Control rats were anesthetized with isoflurane, but no current was delivered. All rats that received epidural electrical stimulation typically showed opisthotonus during stimulation and fasciculation on the hind limbs lasting approximately 5–10 s after the stimulation.

Induction of Spinal Cord Ischemia

Details of the aortic occlusion model have been reported previously.[16] In brief, animals previously implanted with an epidural electrode were anesthetized in a Plexiglas box with 5% isoflurane in room air. After induction, rats were maintained with 1–2% isoflurane delivered by an inhalation mask. For monitoring of distal arterial pressure and injection of heparin, a polyethylene catheter (PE-50) was inserted into the tail artery. For induction of spinal ischemia, a left femoral artery was isolated, and a 2-French Fogarty catheter was placed into the descending thoracic aorta so that the tip of the catheter reached the level of the left subclavian artery. To control the proximal arterial blood pressure at 40 mmHg during the period of aortic occlusion, a 20-gauge Teflon catheter (BD Angiocath™, Becton Dickinson Infusion Therapy Systems Inc., Sandy, UT) connected to an external blood reservoir (37.5°C) was inserted into the left carotid artery. To control and maintain the degree of spinal cord normothermia during aortic occlusion, water (38.5°–38.8°C) was perfused through the heat exchanger[17] at 100 ml/min. At completion of all cannulations, heparin (200 U) was injected into the tail artery. To induce spinal ischemia, the balloon catheter was inflated with 0.05 ml saline, and blood was allowed to flow into the external reservoir. The efficiency of the occlusion was evidenced by an immediate and sustained loss of any detectable pulse pressure and decrease of distal arterial pressure. After ischemia, the balloon was deflated, and blood was reinfused over a period of 60 s. Protamine sulfate (4 mg) was then administered subcutaneously. Arterial blood gases, pH, and hematocrit were measured

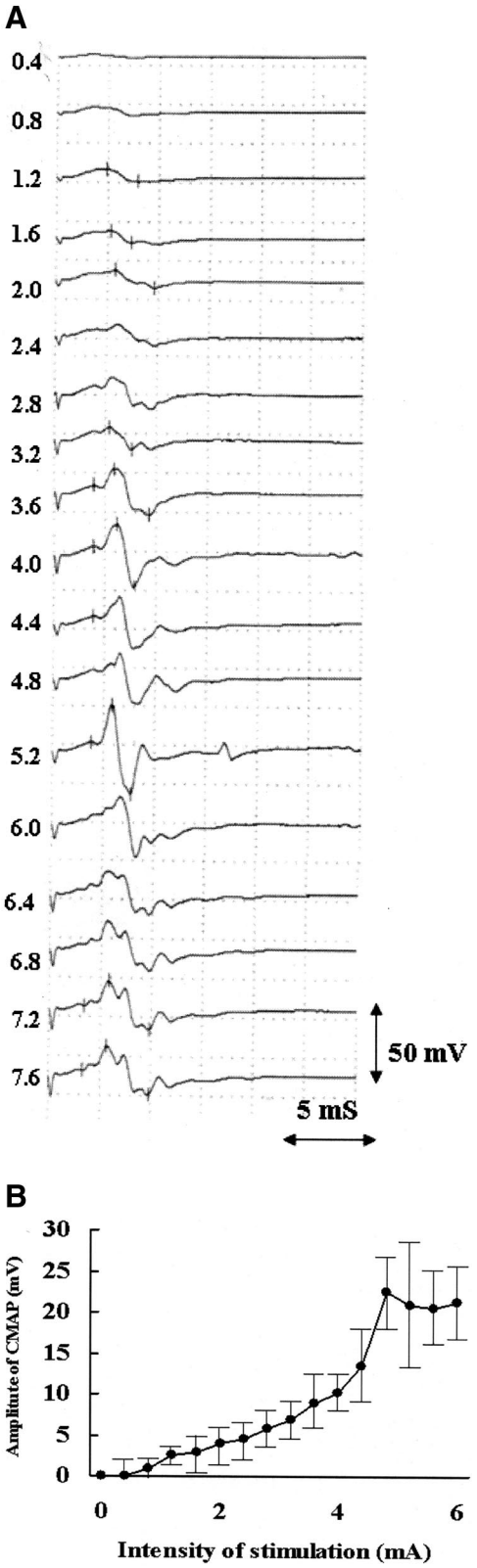

Fig. 1. (*A*) Compound muscle action potentials (CMAPs) recorded from the left soleus muscle corresponding to the currents of epidural electrical stimulations. (*B*) Changes in amplitudes of CMAPs recorded from the left soleus muscle corresponding to currents of epidural electrical stimulations. Ceiling effects were seen over approximately 5 mA current of epidural electrical stimulation.

5 min before and 10 min after the ischemia. All arterial lines were then removed, incisions were closed, and animals were allowed to recover.

Assessment of Neurologic Function

Rats were tested for functional deficit at day 7 after spinal cord ischemia, according to their Basso-Beattie-Bresnahan (BBB) score.[18] Two persons who were unaware of the respective treatment groups or of previous functional scores observed each animal. In the BBB open field locomotor score, the open field walking score measures recovery of hind limb movements in rats during free open field locomotion as described by Basso *et al.*[18] A score of 0 was given if there was no spontaneous movement, and a score of 21 indicated normal locomotion. A score of 14 points was assigned when the animal displayed plantar stepping with full weight support and complete forelimb–hind limb coordination.

Experimental Design

Study 1: Assessment of the Effect of Epidural Electrical Stimulation on Neurologic Outcome after 9 min of Aortic Occlusion. For assessment of the effect of epidural electrical stimulation by a electrical stimulator (SEN 3301; Nihon Koden) on neurologic outcome after 9 min of aortic occlusion, rats were randomly divided into four groups, and all underwent implantation of epidural electrodes. The rapid preconditioning (RE) group (n = 8) comprised rats exposed to 9 min of aortic occlusion 30 min after the pretreatment by epidural electrical stimulation, whereas in the rapid control (RC) group (n = 8), the procedure was the same except for sham treatment with epidural electrical stimulation. In the delayed preconditioning (DE) group (n = 8), exposure to 9 min of aortic occlusion occurred 24 h after pretreatment by epidural electrical stimulation, and in the delayed control (DC) group (n = 8), the procedure was identical except for sham treatment of epidural electrical stimulation.

Study 2: Measurement of SCBF during Aortic Occlusion. To investigate the effect of the epidural electrical stimulation on spinal cord blood flow (SCBF) throughout the experiment, 24 rats previously implanted with epidural electrodes were anesthetized in a Plexiglas box with 5% isoflurane in room air. After induction, rats were maintained with 1–2% isoflurane delivered by an inhalation mask. After all cannulas were inserted completely for spinal cord ischemia, rats were changed from the supine position to the prone position. Animals were divided into four groups as described in study 1: an RC group (n = 6), an RE group (n = 6), a DC group (n = 6), and a DE group (n = 6). SCBF was measured with a laser probe (0.8 mm in diameter; ALF21.; Advance Co., Ltd., Tokyo, Japan) implanted into the epidural space through a burr hole in the lateral aspect of the L1 vertebral body. SCBF was then contin-

uously monitored before and during spinal cord ischemia and for 30 min of reperfusion using 5-s averaging cycles.

Study 3: Quantal Bioassay for Effect of Epidural Electrical Stimulation on the Relation between the Interval of Aortic Occlusion and Neurologic Function—Rapid and Delayed Ischemic Tolerance. In this study, rats were randomly divided into four groups according to the following procedure. In the rapid tolerance study control group (RC group, n = 17), the epidural electrode was inserted, and the animal was exposed to aortic occlusion without the pretreatment of epidural electrical stimulation. In the electrical stimulation group (RE group, n = 17), the same procedure was followed, but animals were exposed to aortic occlusion 30 min after pretreatment with epidural electrical stimulation. In the delayed tolerance study control group (DC group, n = 22), animals were exposed to aortic occlusion without pretreatment by epidural electrical stimulation, but in the electrical stimulation group (DE group, n = 19), exposure to aortic occlusion occurred 24 h after pretreatment with epidural electrical stimulation.

For the quantal bioassay of the effect of epidural electrical stimulation on the relation between the interval of aortic occlusion and neurologic function, the duration of aortic occlusion was selected to span all grades of neurologic function ranging from walking (BBB score: 14–21) to paraplegia or paraparesis (BBB: 0-13). Taira and Marsala[16] showed that there was a good relation between the duration of aortic occlusion and the extent of spinal cord damage. The duration of aortic occlusion for individual rats was varied from 4 to 11 min.

Perfusion Fixation and Histopathologic Analysis

At the end of the survival period (7 days) in study 1, rats were terminally anesthetized with pentobarbital (100 mg/kg intraperitoneal) and phenytoin (25 mg/kg intraperitoneal). Animals were then transcardially perfused with 100 ml heparinized saline followed by 150 ml paraformaldehyde, 4%, in phosphate buffer (pH = 7.4). After 24 h, spinal cords were removed and postfixed in the same fixative for 2–14 days. After this period, spinal cords were removed and L3, L4, and L5 spinal segments were dissected. A spinal cord segment was embedded in paraffin, and serial transverse sections (10 μm) were obtained. Slides were stained using the Nissl method and evaluated for evidence of cellular degeneration and necrosis. Cells that contained Nissl substance in the cytoplasm, loose chromatin, and prominent nucleoli were considered to be normal neurons, and ischemic neurons were identified by loss of Nissl substance and by the presence of pyknotic homogenous nuclei. In each of the three sections (L3, L4, and L5), the number of normal neurons between laminae V and IX were counted in both sides of spinal cord gray matter under high-power microscopic magnification (200×) by an observer unaware of group assignment. The total number of normal neurons was counted for each section at L3, L4, and L5. The total number of normal neurons of both sides at L3, L4, and L5 was divided by 6 to obtain a unilateral average.

Statistics

Statistical analysis of physiologic data and the number of normal neurons in the spinal cord was performed by one-way analysis of variance for multiple comparisons followed by the Dunnett *post hoc* test. BBB scores were compared by using the Kruskal–Wallis test followed by the Mann–Whitney U test. A P value less than 0.05 was considered significant.

For the quantal bioassay on the effect of epidural electrical stimulation on the relation between an interval of aortic occlusion and neurologic function at 7 days after spinal cord ischemia, the P50 that represented the interval of aortic occlusion associated with 50% probability of resultant paraplegia was analyzed and graphically demonstrated by computer construction of a dose–response curve.[19] The computer calculated a P50 that represented the interval of aortic occlusion that produced paraplegia in 50% of the rats. The quantal dose–response analysis method used in the current study was published previously.[20]

Results

Study 1

During the preischemic and intraischemic periods, the paravertebral temperature ranged between 38.3° and 37.5°C. No significant differences among experimental groups were detected except for tail arterial blood pressure during aortic occlusion (table 1). Neurologic outcome for motor function is shown in figure 2. All rats in both the RC and the RE groups had spastic paraplegia 7 days after spinal cord ischemia, and neurologic function assessed by the BBB score in the RE group was not significantly different from that in the RC group. In contrast, in the DE group, three rats displayed ataxia but with preserved ability to walk 7 days after spinal cord ischemia, and one rat had almost normal motor function. Electrical stimulation 24 h before aortic occlusion (DE group) resulted in significantly better neurologic function than such stimulation 24 h before occlusion (DC group).

In the RC and DC group, histopathologic analysis at 7 days after spinal cord ischemia revealed the presence of extensive necrosis between laminae V and IX in the lumbosacral segments. In animals that displayed nearly complete recovery in the DE group, most neurons, including interneurons (laminae V–VII) and spinal motor neurons (laminae IX), had a normal appearance (fig. 3). The number of normal neurons in the spinal cord at 7

Table 1. Physiologic Data

	RC Group	RE Group	DC Group	DE Group
n	8	8	8	8
Body weight, g	348 ± 11	351 ± 12	351 ± 13	350 ± 9
Before ischemia				
pH	7.40 ± 0.06	7.39 ± 0.07	7.40 ± 0.09	7.40 ± 0.07
$Paco_2$, mmHg	41 ± 3	38 ± 2	40 ± 4	40 ± 4
Pao_2, mmHg	228 ± 37	241 ± 22	230 ± 31	233 ± 27
Hematocrit, %	40 ± 2	39 ± 2	39 ± 4	39 ± 3
Distal MAP, mmHg	89 ± 19	71 ± 11	73 ± 15	81 ± 20
Paravertebral temperature, °C	38.1 ± 0.2	38.1 ± 0.2	38.1 ± 0.2	38.2 ± 0.2
During ischemia				
Distal MAP, mmHg	3 ± 2	4 ± 2	4 ± 1	5 ± 4
Paravertebral temperature, °C	38.1 ± 0.2	38.1 ± 0.2	38.1 ± 0.2	38.2 ± 0.2
After ischemia				
pH	7.19 ± 0.02	7.20 ± 0.04	7.19 ± 0.04	7.19 ± 0.03
$Paco_2$, mmHg	46 ± 3	48 ± 5	47 ± 4	46 ± 5
Pao_2, mmHg	204 ± 55	218 ± 40	210 ± 35	223 ± 37
Hematocrit, %	38 ± 2	37 ± 3	36 ± 5	37 ± 2
Distal MAP, mmHg,	92 ± 10	82 ± 21	91 ± 11	83 ± 24
Paravertebral temperature, °C	38.0 ± 0.4	37.9 ± 0.3	37.9 ± 0.4	38.0 ± 0.2

Data are presented as mean ± SD.

DC = delayed control; DE = delayed preconditioning; MAP = mean arterial blood pressure; $Paco_2$ = arterial carbon dioxide tension; Pao_2 = arterial oxygen tension; RC = rapid control; RE = rapid preconditioning.

days after spinal cord ischemia was significantly greater in the DE group than in the other groups (fig. 4).

Study 2

Spinal cord blood flow in the RC group decreased from baseline (100%) to 5.9 ± 2% immediately after the balloon inflation and was 2.0 ± 1.3% at the end of 9 min of aortic occlusion. After approximately 10 min of reperfusion, SCBF overshot baseline to 143 ± 22.7% and finally, after 30 min of reperfusion, decreased to a baseline level (103.1 ± 18.4%). There were no significant differences among the groups throughout the experiment. (table 2)

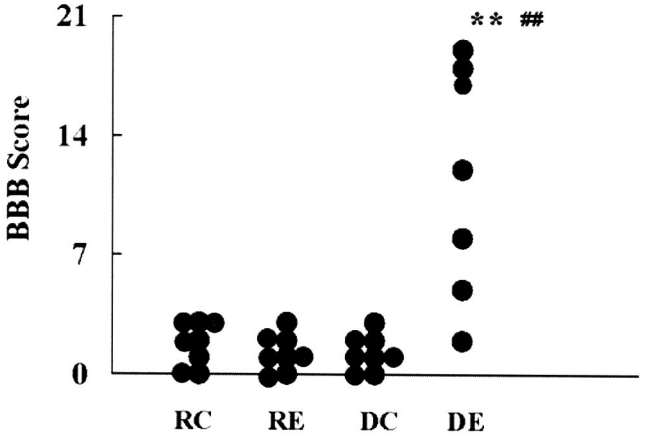

Fig. 2. Basso-Beattie-Bresnahan (BBB) score assessed at 7 days in animals after 9 min of spinal cord ischemia. The most pronounced neuroprotection in animals occurred with pretreatment of epidural electrical stimulation 24 h before spinal cord ischemia. ## P < 0.01 compared with delayed control (DC) group. ** P < 0.01 compared with rapid preconditioning (RE) group. DE = delayed preconditioning; RC = rapid control.

Study 3

Table 3 shows representative behavioral analysis data from the groups of rats treated with or without epidural electrical stimuli. Behavioral data were analyzed using quantal analysis resulting in P50 values as shown in figure 5. In behavioral analysis assessed 7 days after spinal cord ischemia (fig. 5A), the P50 of the RE group (7.21 ± 0.35 min) was not significantly different from that of the RC group (7.64 ± 0.43 min). Electrical treatment 24 h before aortic occlusion significantly prolonged the P50 to 9.07 ± 0.44 min at 7 days after spinal cord ischemia, a shift of approximately 15.0% compared with the DC group (fig. 5B).

Discussion

The current study using a rat aortic occlusion model investigated whether epidural electrical stimulation can induce the ischemic tolerance in spinal cord ischemia. Our results showed that pretreatment with electrical stimulation at 24 h but not 30 min before ischemia protected the spinal cord against ischemic injury by aortic occlusion and that this stimulation prolonged P50 by approximately 15.0% compared with the control group. Because there was no difference in the spinal cord blood flow measured by laser flowmeter during aortic occlusion between the DE and DC groups, the neuroprotective effect in the DE group was not caused by an increase in collateral flow in the spinal cord.

The mechanism responsible for the electrical preconditioning–mediated neuroprotection in the rat spinal cord ischemic model is not clearly understood. Studies of spinal cord ischemic have shown that ischemic pre-

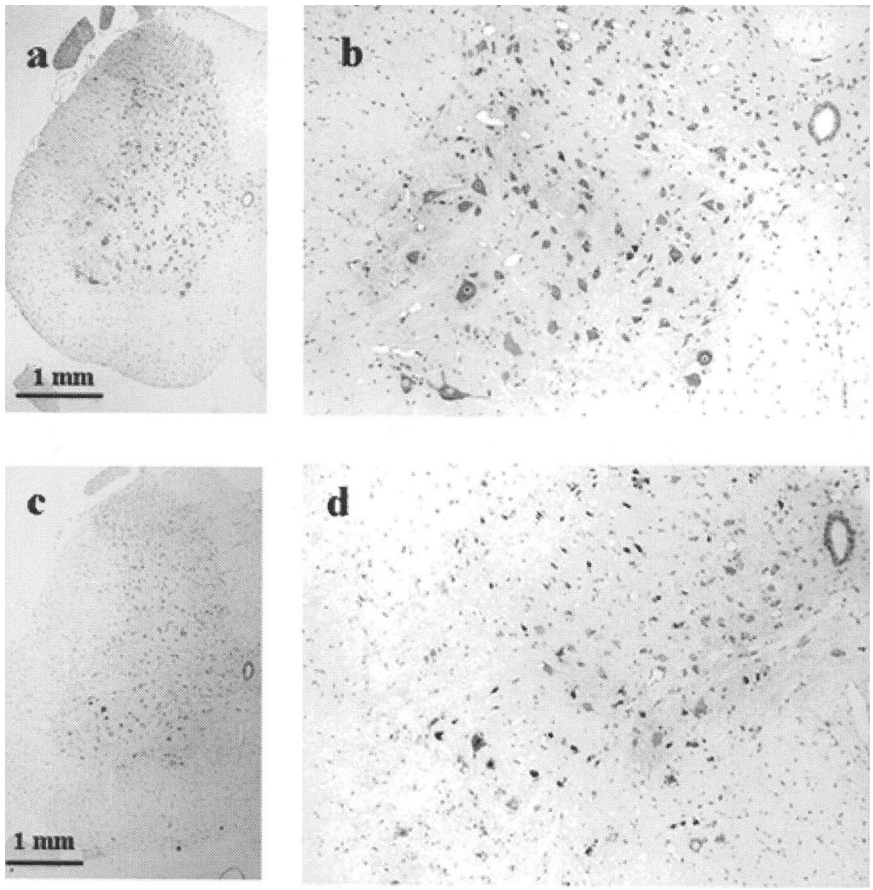

Fig. 3. Light microphotograph of transverse sections. (*A* and *B*) Transverse section of spinal cord taken from the lumbar spinal segment from an animal in the delayed preconditioning group with normal motor function (Basso-Beattie-Bresnahan score: 19) at 7 days of reperfusion. Normal appearance of spinal motor neurons and medium-sized interneurons can be seen. (*C* and *D*) Transverse section of spinal cord taken from the lumbar spinal segment from a rat in the delayed control group with paraplegia (Basso-Beattie-Bresnahan score: 1) at 7 days of reperfusion. The presence of extensive necrosis between laminae V and IX in the lumbosacral segments can be seen.

conditioning can induce ischemic tolerance in rats,[2] rabbits,[3] and dogs.[4] In studies[5,21] using a rat spinal cord ischemic model, endogenous spinal cord protection against ischemia–reperfusion injury can be induced by acute and delayed mechanisms that have been respectively referred to as rapid and delayed ischemic preconditioning. One possibility for rapid ischemic preconditioning is to be associated with local release of adenosine and activation of spinal adenosine A1 receptor. The current study does not provide evidence for acute ischemic tolerance by electrical epidural stimulation. An increase in adenosine release and adenylate cyclase activity, 30 min after electroconvulsive shock in the rat, was suggested by Gleiter *et al.*[22] Although the neuroprotective effects of adenosine and resulting activation of A1 receptor have been shown in *in vivo* cerebral ischemia models,[23,24] it was mentioned that neuraxial adenosine A1 agonist provided partial protection and adenosine A1 antagonist was effective in partially blocking the development of acute ischemic tolerance induced by ischemic preconditioning.[5] This suggests the existence of mechanisms other than the adenosine neuromodulatory system that may contribute to the mediation of acute ischemic tolerance. Therefore, it can be speculated that activation of adenosine receptor, even if adenosine release was increased after epidural electrical stimulation, may not be sufficient to produce a neuroprotective effect against spinal cord ischemia in our rat model.

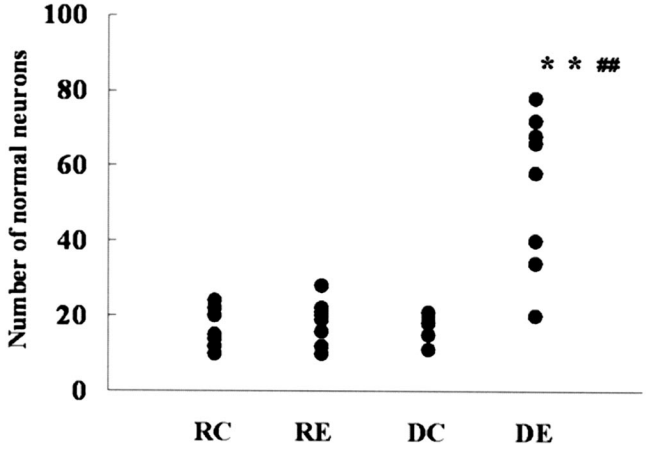

Fig. 4. Number of normal spinal neurons between laminae V and IX at 7 days after injurious spinal cord ischemia. Each symbol represents data for each animal. ## Significant difference from the delayed control (DC) group. ** $P < 0.01$ compared with the rapid preconditioning (RE) group. DE = delayed preconditioning; RC = rapid control.

Delayed ischemic preconditioning seemed to be induced by *de novo* protein synthesis, *e.g.*, heat shock protein (hsp) 70–72,[5,25,26] glia-derived neurotrophic fac-

Table 2. Changes in Spinal Cord Blood Flow before, during, and after Aortic Occlusion

	RC Group	RE Group	DC Group	DE Group
n	6	6	6	6
Before aortic occlusion (baseline)	100	100	100	100
1 min after aortic occlusion, %	5.9 ± 2.0†	6.1 ± 2.9†	6.9 ± 3.2†	6.5 ± 2.2†
9 min after aortic occlusion, %	2.0 ± 1.3†	3.6 ± 2.2†	2.0 ± 1.1†	2.1 ± 2.0†
10 min of reperfusion, %	143.0 ± 22.7*	159.0 ± 27.2*	155.6 ± 19.1*	145.6 ± 18.2*
30 min of reperfusion, %	103.1 ± 18.4	110.9 ± 20.0	102.0 ± 12.6	112.0 ± 16.2

Data are presented as mean ± SD.

* $P < 0.05$ compared with baseline. † $P < 0.05$ compared with baseline.

DC = delayed control; DE = delayed preconditioning; RC = rapid control; RE = rapid preconditioning.

tor,[27,28] metallothioneins, and so on.[29] Yenari *et al.*[25] found that hsp72 overexpression in rat brain protects neurons from transient focal ischemia and kainate-induced injury. More recently, Rajdev *et al.*[26] showed that constitutive overexpression of the rat hsp70 gene in mouse brain provided marked protection against ischemic infarction in a model of permanent focal cerebral ischemia. With respect to trophic factors, Sakurai *et al.*[28] demonstrated that glia-derived neurotrophic factor overexpression on motor neurons by adenovirus-mediated gene delivery in rabbit spinal cord can reduce the occurrence of motor neuronal death after transient spinal cord ischemia. In some studies on the effect of electrical stimulation to the central nervous system, the results, comparable to ischemic preconditioning, suggested that hsp[30] or trophic factors[31,32] were induced in the rat brain after electrical convulsion shock. Taken together with those data, hsp or trophic factors or both are likely to be induced in the spinal cord by electrical epidural stimulation and contributed to this ischemic tolerance in the current study.

Table 3. Comparison of the Effect of Epidural Electrical Stimuli and Sham on Neurologic Outcome after Spinal Cord Ischemia in Rats

Duration of Ischemia, min	Behavioral Outcome for Treatment Groups							
	RC Group		RE Group		DC Group		DE Group	
	N	P	N	P	N	P	N	P
4	1	0	1	0	1	0	1	0
5	2	0	2	0	2	0	2	0
6	3	0	3	0	2	0	2	0
7	3	2	2	2	3	0	3	0
8	2	1	1	3	1	3	3	1
9	0	2	0	2	1	5	3	1
10	0	2	0	2	0	3	0	2
11	0	2	0	2	0	1	0	1

The results above are behavioral readings for each group of rats. They are given as the number of rats either normal (N) or paraplegic (P) for each treatment group.

DC = delayed control; DE = delayed preconditioning; RC = rapid control; RE = rapid preconditioning.

In the current study, we demonstrated that a single epidural electrical stimulation 24 h before spinal cord ischemia induced a significant ischemic tolerance against spinal cord ischemia and prolonged the P50 by approximately 15% compared with the control (DC group). It is unknown whether a 24-h interval between epidural electrical stimulation and spinal cord ischemia would be the optimal therapeutic window. In measuring changes in messenger RNA (mRNA) concentrations of brain-derived neurotrophic factor (BDNF), a single electrical convulsive shock increased an abundance of BDNF mRNA 6 h after the shock, which returned to baseline at 24 h after the shock.[31] In addition, Alter *et al.* [33] found that electroconvulsive shock is associated with a brain BDNF protein up-regulation as determined by enzyme-linked immunosorbent assay measurement. A peak up-regulation of BDNF protein was seen at 15 h after electroconvulsive shock. According to those data, if epidural electrical stimulation was applied 6–15 h before exposure to the injurious ischemia, a more potent tolerance against ischemic spinal cord injury could be achieved, resulting in prolonging the P50 to a greater extent than shown in the current study.

Although a single epidural electrical stimulation was administered to the spinal cord in this study, repeated electrical stimulation might be capable of producing a more potent ischemic tolerance. Recently, it was shown that repeated electrical convulsion shock enhances both gene and protein expression for BDNF.[34,35] In contrast, Zetterstrom *et al.*[31] demonstrated that repeated electrical convulsion shock produced a longer-lasting type of BDNF than a single stimulation. With respect to hsp70, it was reported that both repeated and single electrical convulsion shocks were similarly effective for the induction of hsp70 mRNA.[30] Because it remains controversial as to whether repeated electrical stimulation to the central nervous system enhances synthesis of trophic factors, hsp, and so on, it would be worthwhile to compare the potency of ischemic tolerance between single and repeated epidural electrical stimulations in the spinal cord.

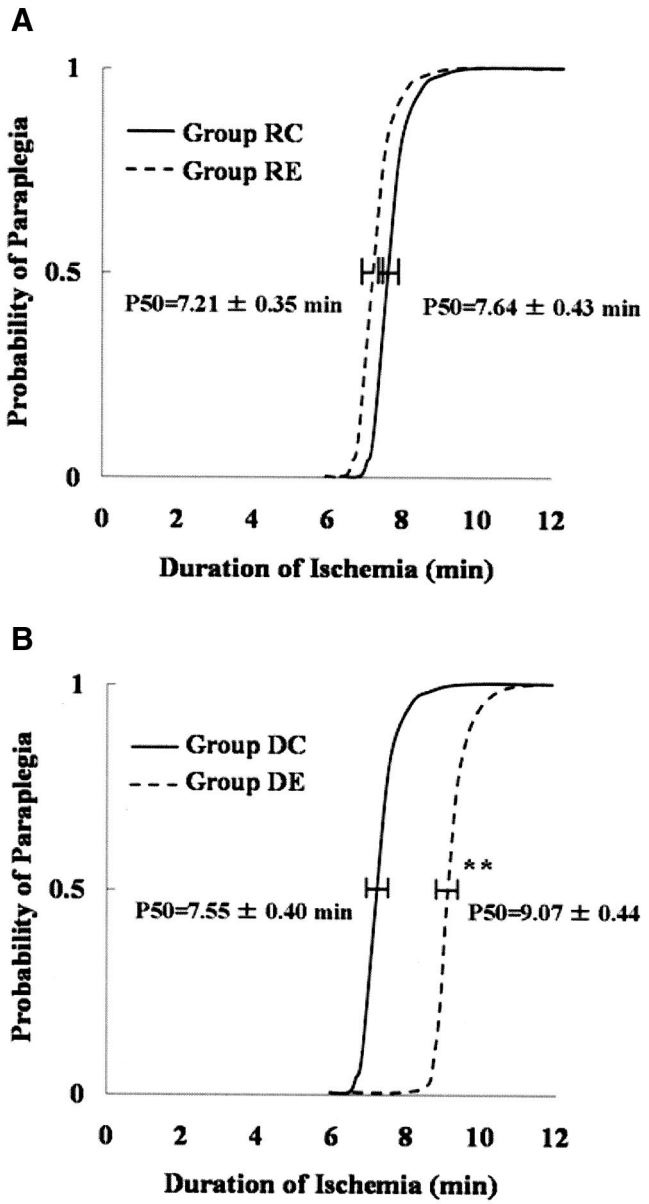

Fig. 5. Percentage of rats that are paraplegic as a function of the duration of ischemia (aortic occlusion) in minutes measured 7 days after spinal cord ischemia. (*A*) Results of study for rapid preconditioning. The interval of aortic occlusion associated with 50% probability of resultant paraplegia (P50) of the rapid preconditioning (RE) group (7.21 ± 0.35 min) was not significantly different from that of the rapid control (RC) group (7.64 ± 0.43 min). (*B*) Results of study for delayed preconditioning. Compared with the curve of the delayed control (DC) group (*dashed line*), the curve labeled *DE* (delayed preconditioning; *solid line*) shows a significant increase in P50 (9.07 ± 0.44 *vs.* 7.55 ± 0.40 min; $P < 0.01$). *Horizontal bars* represent SEs at the P50 required to produce paraplegia.* Significant difference in P50 from the DC group.

In conclusion, we demonstrated that epidural electrical stimulation could induce ischemic tolerance against ischemic spinal cord injury in the rodent model. Although the optimal setting for this electrical preconditioning should be determined in future studies, it is suggested that epidural electrical stimulation may be a useful approach to provide spinal protection in patients undergoing aortic cross clamping.

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