Effects of Naloxone and Levallorphan on the Spinal Cord Reflex Potentials under the Spinal Ischemic Condition in Cats

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ABSTRACT — The spinal reflex potentials elicited by electrical stimulation of the tibial nerve were recorded from the lumbo-sacral ventral root in spinal cats. When the thoracic aorta and the bilateral internal mammary arteries were occluded for 10 min, the potentials were completely depressed. Reappearance of these potentials could be observed at about 10 min after removal of the occlusion and they gradually recovered. Intravenous injection of naloxone (1 or 10 mg/kg) or levallorphan (0.1 mg/kg) together with removal of occlusion significantly promoted the recovery of the polysynaptic reflex potential. Morphine (5 mg/kg) showed no particular effect on the recovery of potentials. Furthermore, pretreatment with morphine (5 mg/kg) did not influence the effects of these opioid antagonists. These results suggest that naloxone and levallorphan may preserve or potentiate the interneuronal activities of the lumbo-sacral spinal cord under the ischemic condition and that the effects may not be mediated through morphine-like opioid receptors.

Keywords: Spinal cord ischemia, Naloxone, Levallorphan, Polysynaptic reflex, Spinal interneuron

The pathophysiology of ischemic injuries in the central nervous system has been one of the major subjects of investigation in recent years. A number of animal experiments have shown that if a traumatic force such as contusion, compression, or distraction is applied to the spinal cord, secondary spinal cord ischemia occurs following mechanical injuries of the nervous tissues (1-5). This secondary ischemic condition of the spinal cord is thought to have clinical importance in the permanent neurological deficits produced by traumatic spinal cord injury. From such a point of view, drugs that may improve spinal cord blood flow after injury or drugs that may preserve spinal cord function under ischemic condition have been much expected to have therapeutic effects. In fact, there have been many studies on pharmacological intervention in spinal cord injury, especially after the report of Faden et al. (6), in which naloxone, an opioid antagonist, improved neurological function after experimental spinal cord injury in cats. Though several hypotheses have been proposed about the mechanism by which naloxone improves functional recovery, it has yet not been clarified.

Murayama and Smith (7) had produced hind limb rigidity in cats by clamping both the thoracic aorta and the bilateral internal mammary arteries for 47 min (ischemic spinal rigid cats). The technique is thought to induce the ischemic condition of the lumbo-sacral spinal cord highly and uniformly in each animal.

To increase our understanding of the mechanism of naloxone in spinal cord injury, the present study investigated the effects of two familiar opioid antagonists, naloxone and levallorphan, on the recovery of the spinal cord reflex potentials under the spinal ischemic condition produced by the above-described method.

MATERIALS AND METHODS

Recording of the spinal cord reflex potentials

Adult cats of both sexes, weighing between 1.7 and 4.1 kg, were anesthetized with ether and then intubated with an endotracheal tube. The spinal cord was transected at the Cl level, and the animals were artificially ventilated with room air at a rate that maintained end tidal CO₂ between 3.5 and 4%. The right femoral vein and artery were cannulated for the administration of drugs and monitoring of blood pressure, respectively. Body temperature was continuously monitored and maintained at approximately 37°C by means of a heating pad. Muscular relaxation was produced by intermittent intravenous injections of gallamine triethiodide,
and the subsequent experiment was done with local anesthetic applied around the wound margin. A laminectomy was performed on the lumbo-sacral area, the dura incised, and the spinal cord was immersed in a mineral oil pool. A bipolar silver electrode was placed on the severed L7 or S1 ventral root, and it was used for recording monosynaptic reflex potentials (MSR) and polysynaptic reflex potentials (PSR). The stimulating electrode was placed on the central part of the ipsilateral tibial nerve, and square-wave pulses of supramaximal intensity (eight to ten times the threshold level of MSR) of 0.2-msec duration were applied every five seconds. The temperature of the mineral oil pools in which the stimulating and the recording electrodes were placed was kept at approximately 37°C by means of a thermostatically controlled heating lamp. Evoked potentials were amplified and recorded by an oscilloscope (Nihon Kohden VC-9), and each recording was made with five consecutive sweeps superimposed on Polaroid film. To prevent the effect of unnecessary afferent impulses, the following nerves were transected: ventral roots from L6 to S3; femoral nerves; obturator nerves; muscle branches to the biceps femoris; semitendinosus; and semimembranosus, posterior femoral cutaneous nerves and posterior gluteal nerves of both sides: the common peroneal nerve, medial and lateral gastrocnemius nerves and saphenous nerve of the ipsilateral side, and the sciatic nerve of the contralateral side.

Occlusion protocol
To stop the blood flow to the lumbo-sacral spinal cord almost completely, the aorta and the bilateral internal mammary arteries were occluded with bulldog clamps for 10 min. After removal of the occlusion, the recovery process of the spinal cord reflex potentials, which had disappeared during the occlusion, was observed for the subsequent 110 min. Then, a second occlusion was made for 10 min. Therefore, the interval between both occlusions was just 2 hr. Immediately after removing the second occlusion, one of the drugs was injected intravenously, and its effect on the recovery process of the potentials was evaluated.

To establish an appropriate duration of ischemia, the occlusion time ranging from 5 to 20 min was checked beforehand, and 10 min was found to be the best for observation of the recovery in this particular experiment.

Drugs used
Naloxone hydrochloride (1 or 10 mg/kg), levallorphan tartrate (0.1 mg/kg), and morphine sulfate (5 mg/kg) were used. Each drug was dissolved in saline solution and administered through the femoral vein.

Preliminary experiments to assure pertinency of the method
Two ischemic procedures were repeated on the same animal, and the recovery patterns of the potentials during the first and the second reperfusion periods were compared with each other.

Measurement of the spinal cord reflex potentials
The spinal cord reflex potentials were recorded prior to spinal cord ischemia (control); at 30 sec after starting the occlusion; and at 1, 10, 15, 20, 30, 45, 60, 90, and 110 min after removal of the occlusion. To compare the recovery of the reflex potentials, the amplitude of MSR and the area of PSR of each recording were measured. The reflex potentials at 110 min after removal of the first occlusion was used as the control for the second occlusion.

Statistical analyses
The measurements were each expressed as a percentage compared with the control, and values given are the mean ± S.E.M. Student's t-test was used for statistical analyses, and a P value less than 0.05 was considered to indicate a statistically significant difference.

RESULTS
Changes of the reflex potentials with spinal cord ischemia
The spinal cord reflex potentials recorded from the lumbo-sacral ventral roots in spinal cats consisted of a monosynaptic reflex (MSR) and a subsequent polysynaptic reflex (PSR). When the thoracic aorta and the internal mammary arteries were occluded, a transient augmentation of MSR was observed between 30 and 60 sec after the occlusion in most animals, and all of the potentials were completely depressed within 2 or 3 min. Reappearance of the potentials could be observed: first, PSR at about 8 min, then MSR at about 14 min after removal of the occlusion; and then they gradually recovered to the normal level (Fig. 1, upper trace).

Preliminary experiments to assure pertinency of the method
The reflex potentials at 110 min after removal of the first occlusion were used as the control for the second occlusion. The recovery pattern of the potentials during the second reperfusion period was compared with that of the first one, and found to be almost the same (Fig. 1). There were no significant differences between the first and the second occlusion regarding the recovery of the amplitude of MSR or the area of PSR (Fig. 2). Additionally, histopathological examination revealed no
detectable change in the spinal cord after the experiments. Therefore, it was methodologically relevant to repeat the ischemic procedures on the same animal, and thereby the effects of a drug could be evaluated by injecting the drug after removal of the second occlusion. Subsequent experiments were carried out according to the occlusion protocol as described above.

Fig. 1. Changes of the reflex potentials with spinal cord ischemia in the spinal cat. Each trace shows the monosynaptic and polysynaptic reflex potentials recorded from the lumbo-sacral ventral root. Spinal cord ischemia was produced by simultaneous occlusion of the thoracic aorta and the bilateral internal mammary arteries for 10 min. Upper frames: the recovery course of the potentials after the first occlusion. Lower frames: the recovery course of the potentials after the second occlusion. The potentials at 110 min after removal of the first occlusion were used as the control for the second occlusion. In the case without drugs, the recovery patterns at both periods were almost the same.

Fig. 2. Effect of spinal cord ischemia on the spinal cord reflex potentials. Ordinate: percentage change of the amplitude of monosynaptic reflex potentials (MSR) and the area of polysynaptic reflex potentials (PSR); the pre-occlusion level is shown as 100%; values are means ± S.E.M. (n = 10). Abscissa: time of occlusion (0–10 min) and time after removal of occlusion (0–90 min). Filled triangle: MSR in the 1st occlusion; open triangle: MSR in the 2nd occlusion; filled circle: PSR in the 1st occlusion; open circle: PSR in the 2nd occlusion. There was no significant difference between the first and the second occlusion with regards to the recovery process of monosynaptic or polysynaptic reflex potentials.
Effects of drugs on the recovery pattern of the reflex potentials after spinal cord ischemia

As shown in Fig. 3, naloxone (10 mg/kg) injected intravenously after removal of the second occlusion hastened the reappearance and subsequent recovery of PSR remarkably and the area of PSR became larger than that of the control at 30 min.

Figure 4 summarizes the effects of naloxone (1 or 10 mg/kg), levallorphan (0.1 mg/kg) and morphine (5 mg/kg) on the recovery process of the amplitude of MSR. No drug had a significant effect except for naloxone (10 mg/kg) at 20 min during recovery.

In contrast, as shown in Fig. 5, naloxone (1 or 10 mg/kg) and levallorphan (0.1 mg/kg) significantly

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**Fig. 3.** Effect of naloxone (10 mg/kg, i.v.) on the spinal cord reflex potentials after spinal cord ischemia. Naloxone was administered together with removal of the second occlusion, resulting in remarkable promotion of the recovery of polysynaptic reflex potentials.

**Fig. 4.** Effects of naloxone (1 or 10 mg/kg, i.v.), levallorphan (0.1 mg/kg, i.v.), and morphine (5 mg/kg, i.v.) on the recovery process of the amplitude of MSR. No drug had a significant effect except for naloxone (10 mg/kg) at 20 min during recovery.

**Fig. 5.** In contrast, as shown in Fig. 5, naloxone (1 or 10 mg/kg) and levallorphan (0.1 mg/kg) significantly
promoted the recovery process of the area of PSR (P < 0.005); and at 45 min, the area of PSR became larger than the control level. The effects of 1 mg/kg of naloxone and 0.1 mg/kg of levallorphan were almost the same. Morphine did not cause any significant change. Furthermore, pretreatment with morphine (5 mg/kg) had little influence on the effects of 10 mg/kg of naloxone (Fig. 6) or 0.1 mg/kg of levallorphan (data not shown).

Effects of drugs on the reappearance time of the reflex potentials after spinal cord ischemia

Table 1 shows the mean time for the reappearance of the spinal cord reflex potentials after removal of the second occlusion. Without drug, the recovery of MSR was first noted at approximately 14 min after removal of occlusion, and that of PSR at approximately 8 min. Intravenous administration of naloxone (10 mg/kg) and levallorphan (0.1 mg/kg) significantly (P < 0.01) shortened the time for reappearance of PSR to 5.3 and 5.8 min, respectively. Morphine had no effect on the time for reappearance of PSR. No drug had any significant influence on the time for reappearance of MSR.

**Fig. 5.** Effects of naloxone (1 or 10 mg/kg, i.v.), levallorphan (0.1 mg/kg, i.v.), and morphine (5 mg/kg, i.v.) on the recovery process of polysynaptic reflex potentials after spinal cord ischemia. Ordinate: percentage changes of the area of polysynaptic reflex potentials; the pre-occlusion level is shown as 100%; values are means ± S.E.M. (n = 4–10). Abscissa: Time of occlusion (0–10 min) and time after removal of occlusion (0–45 min). Filled circle: control (no drug injection); open circle: naloxone, 10 mg/kg, i.v.; filled square: naloxone, 1 mg/kg, i.v.; open triangle: levallorphan, 0.1 mg/kg, i.v.; filled triangle: morphine, 5 mg/kg, i.v. Naloxone and levallorphan significantly promoted the recovery of polysynaptic reflex potentials. *P < 0.005.

**Fig. 6.** Effect of naloxone (10 mg/kg, i.v.) on the recovery process of polysynaptic reflex potentials after spinal cord ischemia with or without pretreatment of morphine (5 mg/kg, i.v.). Ordinate: percentage changes of the area of polysynaptic reflex potentials; the pre-occlusion level is shown as 100%; values are means ± S.E.M. (n = 3–5). Abscissa: time after removal of occlusion (0–45 min); open circle: naloxone, 10 mg/kg, i.v.; filled circle: morphine, 5 mg/kg, i.v. + naloxone, 10 mg/kg, i.v. Morphine was injected before the second occlusion. Pretreatment with morphine had no significant influence on the effects of naloxone.
Table 1. Time for reappearance of the spinal reflex potentials after removal of the second occlusion

| Group             | MSR    | PSR    |
|-------------------|--------|--------|
| Control (No drug) | (n = 10)| 14.28 ± 0.94 | 8.29 ± 0.62 |
| Naloxone 10 mg/kg, i.v. | (n = 5) | 12.60 ± 0.78 | 5.30 ± 0.72* |
| Levallorphan 0.1 mg/kg, i.v. | (n = 5) | 15.00 ± 1.20 | 5.80 ± 0.33* |
| Morphine 5 mg/kg, i.v. | (n = 4) | 14.50 ± 0.43 | 8.38 ± 0.21 |

Values are means ± S.E.M. in min. *Significantly faster recovery (P < 0.01) than that of the control group. MSR: monosynaptic reflex potentials. PSR: polysynaptic reflex potentials.

Table 2. Mean blood pressure (BP) at four time intervals in the second course

| Group             | Before occlusion | During occlusion | 5 min after reperfusion | 30 min after reperfusion |
|-------------------|------------------|------------------|-------------------------|-------------------------|
| Control (No drug) | (n = 6)          | 80.0 ± 7.4       | 11.7 ± 2.5              | 80.8 ± 13.6             | 76.7 ± 8.7 |
| Naloxone 1 mg/kg, i.v. | (n = 4)          | 80.8 ± 7.3       | 13.8 ± 2.1              | 73.8 ± 8.9              | 81.3 ± 3.7 |
| Naloxone 10 mg/kg, i.v. | (n = 5)         | 75.0 ± 3.2       | 14.0 ± 0.9              | 78.0 ± 14.8             | 76.0 ± 3.6 |
| Levallorphan 0.1 mg/kg, i.v. | (n = 5)        | 83.0 ± 4.8       | 12.5 ± 1.8              | 76.0 ± 7.4              | 84.0 ± 7.3 |
| Morphine 5 mg/kg, i.v. | (n = 5)         | 83.0 ± 7.7       | 10.0 ± 8.7              | 77.0 ± 5.2              | 78.0 ± 5.4 |

Values are means ± S.E.M. in mmHg.

Effects of drugs on mean blood pressure (BP)

Table 2 indicates the blood pressure (BP) measured at four time intervals in the second course. In all of the groups, the average BP before occlusion ranged between 75 to 83 mmHg and between 10 to 14 mmHg during occlusion. None of the drugs had any significant influence the BP during the reperfusion period.

DISCUSSION

Faden and colleagues (6) first reported that high doses of naloxone could improve neurological recovery after acute spinal cord injury in cats. Thereafter, in various experimental models, naloxone has been shown to ameliorate spinal cord blood flow, motor functions and electrophysiological activities after spinal cord trauma (8–10). Similarly, after ischemic injury of the spinal cord, naloxone also preserves motor functions and electrophysiological activities (11–13).

There are several hypotheses about the mechanism of naloxone, for instance, an action at the opioid receptors (14), an antagonism with excitatory amino acids (15), a blockade of calcium influx (16, 17), but the exact mechanism has not yet been determined. Up to the present, however, most investigators have attributed the beneficial effects of the drug to an improvement in spinal cord blood flow.

Handa et al. (18) reported that intraperitoneal injection of levallorphan (0.1 mg/kg) significantly improved motor function in the gerbil model of unilateral cerebral ischemia. However, to the best of our knowledge, there has been no report about levallorphan in spinal cord injury.

Ischemic spinal rigid cats can be produced by simultaneous occlusion of the thoracic aorta and the bilateral internal mammary arteries for 47 min. In these animals, interneurons are extensively destroyed while most of the motoneurons survive at the lumbo-sacral spinal cord level (7). Thus the spinal interneurons are more vulnerable than the motoneurons to the ischemic condition produced by this method.

In the present study, intravenous injection of nalox-
one (1 or 10 mg/kg) or levallorphan (0.1 mg/kg) significantly promoted the recovery of PSR, which mainly reflected the activities of interneurons susceptible to ischemia, without affecting the recovery of MSR which mainly reflected the activities of motoneurons. Therefore, as pointed out by many investigators, improvement in spinal blood flow could be considered as a possible effect of the drugs. In our experiments, these opioid antagonists as well as morphine demonstrated little influence on systemic blood pressure during the reperfusion periods. Moreover, the augmentation of PSR beyond the control level could not be attributed only to the local circulatory effects. Naloxone and levallorphan may not only preserve but also potentiate the spinal interneuronal activities under the ischemic condition.

Morphine (5 mg/kg, i.v.) itself showed no detectable effects on the recovery process of the potentials in our model. Besides, pretreatment with morphine (5 mg/kg) did not interact with the effects of naloxone (10 mg/kg). Morphine (5 mg/kg) also had no influence on the effects of levallorphan (0.1 mg/kg). Thus, it is unlikely that the promotive effects of naloxone and levallorphan on PSR observed in our model might be mediated through morphine-like opioid receptors.

Further studies are in progress to investigate the precise mechanism of these opioid antagonists.

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