Effects of GABAergic Drugs on the Recovery of Reflex Potentials after Spinal Cord Ischemia in Cats

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ABSTRACT—Effects of GABAergic drugs on the recovery of reflex potentials after spinal cord ischemia were examined in anesthetized spinal cats. Monosynaptic reflex (MSR) and polysynaptic reflex (PSR) potentials, elicited by electrical stimulation of the tibial nerve in spinal cats, were recorded from the lumbo-sacral ventral root. The spinal reflex potentials were immediately depressed by occlusion of the thoracic aorta and the bilateral mammary arteries for 10 min. The potentials recovered gradually to the control level within 90 min after reperfusion. Pretreatment with bicuculline (0.3 mg/kg, i.v.), a GABA antagonist, or semicarbazide (200 mg/kg, i.v.), an inhibitor of GABA synthesis, accelerated the recovery of PSR potentials after the removal of the arterial occlusion. In contrast, pretreatment with aminooxyacetic acid (10 mg/kg, i.v.), an inhibitor of GABA degradation, retarded the recovery of PSR potentials, and this effect was overcome by the addition of the opioid antagonist naloxone (10 mg/kg, i.v.). These results suggest that the GABAergic system retards the recovery of PSR potentials after a brief spinal cord ischemia, which can be antagonized by naloxone.

Keywords: Ischemia, Spinal cord, Naloxone, GABAergic drug

When traumatic forces such as contusion, compression and distraction are applied to the spinal cord, resultant spinal cord ischemia may produce irreversible injuries of neurons (1, 2). Therefore, the search for a drug that can preserve spinal cord function during spinal cord ischemia may be of clinical importance. Several studies have demonstrated that a large increase in γ-aminobutyric acid (GABA) release was observed during brain or spinal cord ischemia (3–5). However, the pathophysiological significance of the increased GABA release in the ischemic and reperfused neural tissues remains unclarified. Therefore, the first aim of the present study was to examine the effects of GABAergic drugs on changes in reflex potentials during spinal cord ischemia and reperfusion.

Several experimental studies have shown that the opioid antagonist naloxone accelerates neurological recovery after traumatic or ischemic spinal cord injury (6–10). We have previously reported that not only naloxone but also levallorphan promoted the recovery of the polysynaptic reflex (PSR) potentials after spinal cord ischemia in spinal cats (11). However, the underlying mechanism(s) of the beneficial effect of naloxone on the neurological recovery remain unclarified. It has been reported that naloxone blocked GABA-mediated synaptic inhibition in the central nervous system (3, 12). Therefore, it is possible that naloxone might improve the neurological recovery after the spinal cord ischemia by influencing the GABAergic system. The second aim of the present study was to examine the influence of naloxone on the GABA-mediated changes in neurological recovery after brief spinal cord ischemia.

MATERIALS AND METHODS

Animal preparations

All experiments were performed under the regulations of the Animal Research Committee of the School of Medicine, Chiba University. Adult cats of both sexes weighing 1.8–3.1 kg were anesthetized with urethane (600 mg/kg, i.p.) and α-chloralose (40 mg/kg, i.p.), and supplemental doses of anesthetics were given when needed during experiments. The animal was spinalized at the C1 level and then artificially ventilated with room air through an endotracheal tube at a rate that maintained the end tidal CO2 between 3.5% and 4%. The right femoral vein and the left femoral artery were cannulated.
Experimental protocol and drugs

To produce lumbo-sacral spinal cord ischemia, the aorta and the bilateral internal mammary arteries were occluded using bulldog clamps for 10 min. An occlusion of 10 min was repeated at an interval of more than 110 min. We have previously reported that the recovery process of the reflex potentials was reproducible when the occlusion was repeated two or three times under this experimental condition (11). In other words, there were no significant differences in the recovery of the amplitude of MSR potentials and the area of PSR potentials among the first, second and third occlusion. In the first series of experiments, one of the following drugs was administered intravenously before the second occlusion: 1) bicuculline (0.3 mg/kg), a selective GABA-receptor antagonist, was intravenously administered 10 min prior to the second occlusion; 2) semicarbazide (200 mg/kg), a glutamic acid decarboxylase (GAD) inhibitor, was intravenously administered 180 min before the second occlusion; 3) aminooxyacetic acid (AOAA) (10 mg/kg), a GABA transaminase (GABA-T) inhibitor, was intravenously administered 150 min before the second occlusion. It was reported that treatment with semicarbazide for 3 hr depleted the GABA level (13, 14), whereas treatment with AOAA for 150 min significantly increased the GABA level in the central nervous system (14).

The second series of experiments were performed to examine whether naloxone can modify the effects of GABAergic drugs on the recovery of spinal reflex potentials. AOAA (10 mg/kg) was administered before the second occlusion, and then naloxone (10 mg/kg) was injected 10 min before the third occlusion. In some experiments, bicuculline (0.3 mg/kg) was administered prior to the second occlusion, and then naloxone (10 mg/kg) was added before the third occlusion. In part of the experiments, AOAA or bicuculline was administered before the second occlusion and saline was administered before the third occlusion.

The spinal cord reflex potentials were recorded prior to spinal cord ischemia (control) and various times after the reperfusion. Recovery of the reflex potentials was analyzed by measuring the amplitude and the area of MSR and PSR potentials, which were averaged from 5 consecutive sweeps. The reflex potentials before the first control occlusion served as the control.

Statistics

All values are expressed as means±S.E.M. Statistical analyses were performed by Student’s t-test, and P values of less than 0.05 were considered significant.

RESULTS

Effects of GABAergic drugs on the recovery of spinal reflex potentials after spinal cord ischemia

The spinal cord reflex potentials, recorded from the lumbo-sacral ventral roots of spinal cats, consisted of MSR and subsequent PSR potentials (Fig. 1). These potentials were completely depressed within 2–3 min after the occlusion of the thoracic aorta and the internal mammary arteries. After the removal of the occlusion, the potentials reappeared gradually and returned to the control level within 90 min, as shown in Fig. 1. It was confirmed that the time course changes in reflex potentials during reperfusion were reproducible when the spinal cord ischemia of 10 min was repeated at an interval of 110 min or more.

Bicuculline at a dose of 0.3 mg/kg per se did not significantly affect MSR and PSR potentials during the pre-ischemic period. Pretreatment with bicuculline significantly accelerated the recovery of PSR potentials after the second spinal cord ischemia, as shown in Fig. 1. At 90 min after reperfusion, the PSR potentials were larger than those of the preocclusion level (145±4% of the control level, P<0.05, n=9). However, bicuculline did not significantly affect the recovery of MSR potentials. Semicarbazide also accelerated the recovery of PSR potentials, but not MSR potentials, as observed with bicuculline (Fig. 2). Pretreatment with semicarbazide (200 mg/kg, i.v.) slightly increased the PSR potentials (121±16%, n=6). Semicarbazide accelerated the recovery of PSR potentials after the second spinal cord ische-
The PSR potentials were significantly larger than that of the preocclusion level (168±19% of the control level, P <0.05, n=6) at 90 min after reperfusion. In contrast, AOAA retarded the recovery of PSR potentials after spinal cord ischemia, as shown in Fig. 3. Pretreatment with AOAA (10 mg/kg, i.v.) per se did not significantly affect the PSR potentials (96±5%, n=9). AOAA retarded the recovery of the PSR potentials after spinal cord ischemia without any significant influence on MSR potentials. At 90 min, the PSR potentials were about 42±3% of the control level, which was significantly different from the recovery after the first spinal cord ischemia (P<0.01). Although we observed the recovery of PSR potentials up to 110 min after reperfusion in some experiments, further improvement of PSR potentials could not be observed.

**Influence of naloxone on the GABAergic drugs-modified recovery of reflex potentials after spinal cord ischemia**

In the following experiments, influences of naloxone on the GABAergic drugs-modified recovery of reflex potentials were examined. Administration of naloxone (10 mg/kg, i.v.) prior to the second spinal cord ischemia significantly accelerated the recovery of the reflex potentials, as shown in Fig. 4 (170±13% of the control at 90 min, n=5). As already mentioned, AOAA (10 mg/kg, i.v.) retarded the recovery of the PSR potentials after the second spinal cord ischemia. At 90 min after the second occlusion following AOAA administration, the recovery of the PSR potentials was 42±3% of the control level in 9 cats. In 3 of 9 cats, the third occlusion was produced without any additional drug treatment at 110 min after the second occlusion. At 90 min after reperfusion following the third occlusion, the recovery of the PSR potential was still depressed and was 61±4% of the control level. When naloxone (10 mg/kg, i.v.) was added prior to the third occlusion in 5 cats, it reversed the inhibition of PSR potential recovery by AOAA and accelerated the recovery of the potentials (Fig. 4). The recovery of PSR potentials at 90 min was 170±19% of the control, which was significantly greater than that after the post-AOAA 3rd occlusion.
In animals that were treated with bicuculline (0.3 mg/kg, i.v.) prior to the second occlusion, the recovery of PSR potentials was accelerated (145±4% of the control level at 90 min, n=9). At 110 min after the second occlusion, the spinal cord ischemia was repeated without additional treatment in 3 animals, and the PSR potential was 154±8% of the control level before the 1st occlusion. The recovery of PSR potentials was not significantly different from that after the 2nd post-bicuculline ischemia. In 5 cats treated with bicuculline, naloxone (10 mg/kg, i.v.) was administered before the third spinal cord ischemia. In this experimental condition, naloxone failed to accelerate the recovery of reflex potential further after spinal cord ischemia, as shown in Fig. 4. The acceleration of the PSR potential recovery after the combined treatment with bicuculline and naloxone (162±28%, n=5) was not significantly different from that after the third post-bicuculline ischemia (154±8%).

**DISCUSSION**

Several reports demonstrated that the release of GABA was increased during cerebral ischemia (3, 4). It was also reported that acute spinal ischemia followed by four days reperfusion produced a decrease in the free GABA level of the spinal cord, potentially resulting from an increased GABA release (5). Previously we also observed that amino acid neurotransmitters including GABA were reduced in the spinal cord of cats with experimental hind-limb rigidity of spinal origin (15). During acute ischemia of the central nervous system, excessive release of amino acid neurotransmitters may be produced by neuronal depolarization and/or inhibition of the energy-dependent reuptake (3). In addition, the GABA level in the ischemic neural tissue may be potentially increased due to activation of the synthesis enzyme (GAD) and inhibition of the degradation enzyme (GABA-T) in the ischemic environment (16). Whatever the mechanism involved, it is conceivable that the GABA level in the ischemic spinal cord is increased. However, the pathophysiological significance of the increased GABA level in the acute ischemic condition is not fully understood. In the present study, influences of GABAergic drugs on the recovery of reflex potentials after spinal cord ischemia were examined.

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**Fig. 2.** Effect of semicarbazide on the recovery of the reflex potentials after spinal cord ischemia in spinal cats. Upper and middle panels indicate actual records of reflex potentials obtained before (control) and 15, 30 and 90 min after the 1st control spinal cord ischemia and the 2nd post-semicarbazide ischemia, respectively. The lower graph summarizes the recovery of polysynaptic reflex (PSR) potentials after the 1st control (closed circles) and the 2nd post-semicarbazide spinal cord ischemia (open circles). The recovery is expressed as % of the area of the preocclusion PSR potentials before the 1st control occlusion. Values are expressed as means±S.E. of 6 experiments. *P<0.05 vs the recovery after the 1st control ischemia.
Control

Pre-occl.  Reperfusion
15 min  30 min  90 min

AOAA

200 µV

5 msec

Fig. 3. Effect of aminooxyacetic acid (AOAA) on the recovery of the reflex potentials after spinal cord ischemia in spinal cats. Upper and middle panels indicate representative records of reflex potentials obtained before (control) and 15, 30 and 90 min after the 1st control spinal cord ischemia and the 2nd post-AOAA ischemia, respectively. The lower graph summarizes the recovery of polysynaptic reflex (PSR) potentials after the 1st control (closed circles) and the 2nd post-AOAA spinal cord ischemia (open circles) in 9 experiments. The recovery is expressed as % of the area of the PSR potentials before the 1st control occlusion. Values are expressed as means±S.E. *P<0.05 and **P<0.01 vs the recovery after the 1st control ischemia.

Fig. 4. Effects of naloxone alone and combined with GABAergic drugs on the recovery of polysynaptic reflex (PSR) potentials after spinal cord ischemia. The recovery is expressed as % area of preocclusion PSR potentials at 90 min after reperfusion. Values are expressed as means±S.E. of 5–9 experiments. *P<0.05 vs the recovery after the 1st control ischemia.
In the present study, we used three kinds of GABA-ergic drugs; i.e., bicuculline, semicarbazide and amino-oxyacetic acid. Semicarbazide, a glutamic acid decarboxylase inhibitor, is known to block GABA synthesis and deplete GABA in the central nervous system within several hours (13, 14). In contrast, AOAA, a GABA-T inhibitor, is reported to block GABA degradation and to elevate the GABA level in the central nervous system (14). The present study revealed that semicarbazide as well as the GABA\textsubscript{A} antagonist bicuculline accelerates the recovery of PSR potentials, whereas AOAA retards it after spinal cord ischemia. However, the recovery of MSR potentials was hardly affected by any of these GABAergic drugs. It was suggested that GABA is a neurotransmitter of interneurons, but probably not that of primary afferent neurons coupled to motoneurons. Therefore, a possible increase in GABA release might depress the PSR potentials by inhibiting afferent depolarization of interneurons in the dorsal horns without affecting monosynaptic contacts with motoneurons. GABA might open the chloride channels and inhibit the neuronal discharge. It is known that the spinal interneurons are more vulnerable to the ischemic insult than the motoneurons, and that spinal cord ischemia of an approximately 45-min duration produces rigidity of the hind limbs (17).

In this study, naloxone reversed the AOAA-induced inhibition of the recovery of PSR potentials after spinal cord ischemia. In addition, naloxone failed to accelerate further the recovery of PSR potentials when bicuculline had already improved the PSR potentials. Therefore, it is possible that naloxone might improve the recovery of PSR potentials, at least in part, by antagonizing GABA receptors. Indeed, it was demonstrated that naloxone interacts with GABA receptors in the brain (12). However, it was reported that dynorphin, an endogenous agonist for \kappa-opiate receptor, exacerbated neurological dysfunction after spinal cord injury (18). On the other hand, it was demonstrated that both dynorphin and levallorphan improved the motor dysfunction in the gerbil model of unilateral cerebral ischemia (19). Since levallorphan is a \kappa-opiate receptor agonist and a \mu-opiate receptor antagonist, they concluded that \kappa-opiate receptor stimulation may be beneficial for protection against ischemic neural tissues. In our previous study (11), morphine failed to affect the recovery of PSR potentials after a brief spinal cord ischemia, although naloxone and levallorphan promoted the recovery. Therefore, it is unlikely that naloxone improved the functional recovery by antagonizing opiate receptor, although we can not completely exclude this possibility from the present study.

In the present study, pretreatment with bicuculline, semicarbazide or naloxone accelerated the recovery of PSR potentials and maintained them above the preocclusion level after reperfusion. At the present time, it is not clear why the PSR potentials were maintained above the control level after the treatment with these drugs. One possible explanation may be that some excitatory neurotransmitter(s) might also be released during the spinal cord ischemia, and the potentiating effect on the PSR potentials might be unmasked after the blockade of the GABAergic system.

Many studies have indicated that naloxone improves neurologic recovery after spinal cord injuries produced by contusion or ischemia (6-10). Mechanisms of the beneficial effect of naloxone have been postulated to be the antagonism of neurotoxicity produced by excitatory amino acids (20) and the inhibition of transmembrane \textsuperscript{2+} influx (8, 21). However, GABAergic antagonisms, potentially observed in this study, might in part contribute to the acceleration of functional recovery.

It has been assumed that an excitatory amino acid, such as glutamate and aspartate, may play an important role in the mediation of postsischemic neuronal cell death (22, 23). On the other hand, GABA is supposed to be beneficial for the neuronal survival following ischemia by means of counteracting the excitotoxic action of glutamate and aspartate (24-26). The present experiments enabled us to observe only acute functional recovery after spinal cord ischemia. Further chronic studies are needed to determine whether antagonism of GABA receptors may cause delayed neuronal death more severely after longer spinal cord ischemia, although it accelerates the functional recovery after a brief spinal cord ischemia.

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