NMDA Receptors in the Spinal Cord Exert Excitatory Influences on Spinal Motor Output in Rats

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ABSTRACT—The role of NMDA receptors in the regulation of spinal motor output was studied in rats. Muscle tension of the hind limbs of decerebrate animals and spinal reflex potentials in anesthetized animals were recorded. Intrathecal injection as well as systemic or intra-4th ventricular injection of (+)-5-methyl-10-11-dihydro-5H-dibenzo[a,d]cyclohepta-5-10-imine maleate (MK-801) reduced muscle tension. Systemic MK-801 did not alter monosynaptic reflexes either in intact or spinal rats, but attenuated polysynaptic reflexes in spinal rats. Thus spinal NMDA receptors participate in spinal motor output in the presence of some specific factors such as descending facilitation and preceding segmental depolarization, which remove the Mg$^{2+}$ blocking.

Keywords: NMDA receptor, Decerebrate rigidity, Spinal reflex

At most glutamatergic synapses in the central nervous system, both non-N-methyl-D-aspartate (non-NMDA) and NMDA receptors mediate fast excitatory synaptic transmission. At the resting potential, however, non-NMDA receptors largely contribute to the excitatory postsynaptic potentials (EPSPs) due to the Mg$^{2+}$ blocking of NMDA receptors (1). Neurons normally receive various neuronal signals some of which might affect the membrane potential. These signals include brief depolarization by the stimulation of non-NMDA receptors or long-lasting depolarization by the modulation of ionic currents through G-protein-coupled receptors (2), leading to removal of the Mg$^{2+}$ blocking. Furthermore, numerous factors can modify the strength of neuronal signals, which may alter how NMDA receptors contribute to synaptic transmission.

Neurons in the spinal cord are influenced by the supraspinal level, and enhanced descending excitatory influences to lower spinal levels have been demonstrated to be of importance in the motor system in decerebrate animals (3). Here we demonstrate that contribution of spinal NMDA receptors to motor output from the spinal cord is highlighted especially in decerebrate animals. In this study, muscle tension of the hind feet and reflex potentials propagating along the ventral roots, both of which reflect activities of spinal motoneurons, were employed to explore the role of NMDA receptors in the mediation of output signals from the spinal cord.

Experiments were performed in accordance with the Guiding Principles for the Care and Use of Laboratory Animals approved by The Japanese Pharmacological Society and also in accordance with Sankyo’s Code of Ethical Research. Male Wistar rats (Charles River Japan, Inc., Tokyo) weighing 300 – 390 g were used for all the experiments. Preparation and measurement of decerebrate rigidity were performed according to the method of Ono et al. (4). Briefly, rats were each fixed on a stereotaxic apparatus under anesthesia with ether. Bilateral lesions of the midbrain (AP 0, V −3, L ±1.5, according to the rat brain atlas of Pellegrino et al. (5)) were obtained by injection of radio frequency currents (500 kHz, about 25 mA with the lesion generator RGF-4; Radionics, Inc., Burlington, MA, USA) for 3 min keeping the tissue temperature at 75 – 85°C (RF-lesion rigidity). After lesioning, anesthesia was discontinued. For the measurement of muscle tension, a motor-driven mechanical device was used to dorsiflex the hind feet of a rat placed in a holder. The tension developed during a stroke of the dorsiflexion of 4 mm in length over a 6-s period was recorded. This dorsiflexion, applied every 60 s, generated the tension including active tension, which is separable from the passive tension remaining after an overdose of pentobarbital for the sacrifice of the animal at the end of the experiments.

For the experiments of spinal reflex potentials, rats were
anesthetized with urethane (1 g/kg, i.p.) and α-chloralose (25 mg/kg, i.p.), and they were artificially ventilated. For the spinal preparations, the spinal cord was cut at the level of vertebra Th8. Laminectomy was performed in the lumbosacral region. Dorsal and ventral roots below L4 were cut bilaterally and dorsal and ventral roots of L4 and L5 were isolated. A skin pouch was formed at the site of the dissection so that the exposed tissues could be covered with liquid paraffin, which was kept at 36 ± 0.5°C. Rectal temperature was maintained at 36 ± 0.5°C by a heating pad. Spinal reflex potentials were recorded from L5 ventral roots in response to electrical stimulation (0.2 Hz, 0.05 ms, supramaximal) of L5 segmental dorsal roots. Bipolar silver-silver chloride wire electrodes were used both for stimulation and recording. The reflex potentials were amplified, displayed on an oscilloscope and recorded.

(+)-5-Methyl-10-11-dihydro-5H-dibenzo[a,d]cyclohept-5-10-imine maleate (MK-801) was synthesized in our laboratories. For intrathecal injection, MK-801 was injected in a volume of 10 μl of saline via a polyethylene tube (SP6; I.D. 0.20 mm, O.D. 0.50 mm; Natsume, Tokyo), which was inserted into the subarachnoid space through the intervertebral foramen between L3 and L4 according to the method described by Satoh et al. (6), followed by an injection of 10 μl of saline to clear the catheter. For intra-4th ventricular injection, MK-801 was injected in a volume of 5 μl of saline via a polyethylene tube (SP10; I.D. 0.28 mm, O.D. 0.61 mm; Natsume), which was inserted into the 4th ventricle through a hole drilled in the skull, followed by an injection of 5 μl of saline to clear the catheter.

All data were normalized to the averaged data obtained before administration, and expressed as means ± S.E.M. Paired Student’s t-test (two-tailed) was used to compare means with pretreatment level. Differences at \( P<0.05 \) and \( P<0.01 \) were considered significant.

Intravenous (0.1 and 0.3 mg/kg), intra-4th ventricular (10 and 20 μg) or intrathecal (10, 20 and 40 μg) injection of MK-801, a selective non-competitive blocker of NMDA receptors, revealed dose-dependent attenuation of muscle tension in decerebrate rats. Figure 1 shows time-course graphs for the decrease in muscle tension following MK-801 injection with three different routes. Maximal decrease in muscle tension was obtained around 10 min following intravenous injection. With local injections into the 4th ventricle or the subarachnoid space, an additional 10 min was needed to reach a maximal effect, which may reflect a gradual increase in concentration due to passive diffusion into the tissue. In Table 1, the maximal effects (% inhibition) for each injection route are summarized.

Both in intact and spinal rats, intravenous MK-801 (0.3 and 1.0 mg/kg) did not affect monosynaptic reflexes (MSR, Fig. 2). MK-801 inhibited polysynaptic reflexes (PSR) only in spinal rats (Fig. 2B). Dose-dependent decrease in PSR was maintained up to 60 min after administration (% inhibition at 15 min and 60 min: 20.0 ± 5.2% and 32.2 ± 4.2%, respectively, with 0.3 mg/kg, \( n=4 \), \( P<0.05 \); 38.4 ± 8.9% and 38.4 ± 6.7%, respectively, with 1.0 mg/kg, \( n=5 \), \( P<0.01 \)). Surprisingly, effects of MK-801 on PSR in intact rats were excitatory (Fig. 2A). This increase was obtained within 5 min and was abolished within 30 min. PSR was increased by 34.6 ± 4.2% with 1.0 mg/kg
In this study, we have demonstrated that NMDA receptors in the spinal cord mediate the spinal motor output in decerebrate rigidity and spinal segmental reflexes. Depolarization of the membrane potential is essential for NMDA receptors to be activated by glutamate (1). Neurons receive various neuronal signals that elicit depolarization. Those signals may include brief depolarization by the stimulation of non-NMDA receptors or long-lasting depolarization by the modulation of ionic currents through G-protein-coupled receptors (2). Moreover, types of neuronal signals or relative contribution of those signals may differ between physiological and pathophysiological situations.

The descending neurotransmitter systems originating in the supraspinal structures are known to affect the excitability of the cells in the spinal cord. The descending noradrenergic system originating from the locus coeruleus is facilitatory in the mediation of spinal motor output (3, 7, 8). Increased noradrenergic activity in the spinal cord contributes to the generation of RF-lesion rigidity (3). Furthermore, similarity in the profile of RF-lesion rigidity with that of intercollicular decerebrate rigidity (γ-rigidity) (4) suggests that γ-motoneurons receive enhanced tonic segmental excitatory input due to increased γ-loop activities. This may elicit temporal summation of EPSPs mediated by non-NMDA receptors. Thus in decerebrate animals, both tonic descending and segmental excitation contributes to the activation of spinal NMDA receptors.

In segmental spinal reflexes recorded in spinal preparations, NMDA receptors participate in polysynaptic transmission. Prominent inhibition of PSR with MK-801 in our spinal preparations is plausible considering that removal of the Mg²⁺ blocking by depolarization elicited by monosynaptically released glutamate provides NMDA receptors

### Table 1. Maximal % inhibition of muscle tension by MK-801

| MK-801  | % inhibition | n  |
|---------|--------------|----|
| 0.1 mg/kg i.v. | 54.3 ± 6.2** | 6 |
| 0.3 mg/kg i.v. | 80.5 ± 6.2** | 6 |
| 10 μg i.c.v.   | 50.1 ± 13.4* | 5 |
| 20 μg i.c.v.   | 72.0 ± 9.4** | 4 |
| 10 μg i.t.     | 28.6 ± 17.7  | 4 |
| 20 μg i.t.     | 59.4 ± 14.3* | 4 |
| 40 μg i.t.     | 87.9 ± 4.6** | 4 |

i.v.: intravenous, i.c.v.: intra-4th ventricular, i.t.: intrathecal. Each value is expressed as the mean ± S.E.M. *P<0.05, **P<0.01 vs pretreatment level.

(n = 6, P<0.01).

In this study, we have demonstrated that NMDA receptors in the spinal cord mediate the spinal motor output in decerebrate rigidity and spinal segmental reflexes.

Fig. 2. Spinalization reveals excitatory contribution of spinal NMDA receptors to the spinal segmental reflexes. A trace shows monosynaptic and polysynaptic reflex potentials (MSR and PSR). MK-801 (closed circle: 0.3 mg/kg, open circle: 1.0 mg/kg) or saline (open square) was intravenously injected at time zero in intact (A) and spinal (B) rats. Each point represents the reflex amplitude (mean ± S.E.M. of 4–6 experiments) calculated as a percentage of the averaged control value obtained during the 10 min period prior to the injection.
that may be functional when polysynaptic signals arrive at motoneurons. This result supports the results by Farkas and Ono (9) demonstrated in rats spinalized at the level of vertebra C1.

Our results with local application indicate that NMDA receptors in the brain stem also contribute to the generation of decerebrate rigidity. Moreover, systemic MK-801 increased in PSR in intact rats in spite of inhibition of PSR obtained in spinal rats. Action of MK-801 at the upper brain region may overcome the action on the spinal cord and appears to lead to a net increase in PSR by MK-801 in intact rats. Although glutamate receptors in the upper brain regions play an important role in the generation of motor disorders such as Parkinson’s disease (10), further studies must be made to evaluate the role of supraspinal NMDA receptors in the regulation of spinal motor output.

In conclusion, spinal NMDA receptors mediate spinal motor output in decerebrate rigidity and spinal segmental PSR. Enhancement of descending facilitation by decerebration or preceding depolarization by monosynaptically released glutamate appears to be essential for the recruitment of functional NMDA receptors in the spinal cord.

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