Effects of MK801 on evoked potentials, spinal cord blood flow and cord edema in acute spinal cord injury in rats

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Objectives: To determine whether MK801, an NMDA receptor antagonist, blocks glutamate excitotoxicity directly or via other mechanisms such as improving blood supply at the injury site in a rat model of spinal cord injury (SCI). In the present study, the effects of pre- and posttreatment with MK801 on axonal function, spinal cord blood flow (SCBF) and cord water content were studied after acute SCI in rats.

Methods: Somatosensory evoked potentials (SSEPs) and cerebellar evoked potentials (CEPs) were used to quantify electrophysiological function, and the hydrogen clearance technique and wet-dry weight measurements were used to measure SCBF and cord water content, respectively. Twenty rats received a 21 g clip compression injury of the cord at T1, and were then randomly and blindly allocated to either MK801 or saline groups. Each rat received an intravenous infusion of drug or saline four times during the experiment (16 min/infusion) with the first infusion (MK801 3 mg/kg) beginning 8 min pre-injury, and the other infusions (MK801 1.5 mg/kg) at 1 h intervals after injury. Control experiments on uninjured rats were performed in 10 rats using the same procedure as above except the clip compression injury of the cord was omitted.

Results: In the MK801 groups with or without SCI, the amplitude of the evoked potential peaks, especially the SSEPs, was significantly lower than in the saline group. There were no differences in SCBF or cord water content between the MK801 and saline groups.

Conclusion: Pre- and posttreatment with MK801 inhibits evoked potentials, but does not improve SCBF or cord edema after acute compression SCI in rats. For the first time it has been shown that MK801 produced a blockade of glutamate excitatory transmission in afferent pathways after SCI. Further work is required to determine whether this inhibition is reversible and related to neuroprotection and functional recovery after SCI.

Keywords: evoked potentials; MK801; NMDA; spinal cord blood flow; spinal cord injury; water content

Introduction

The excitatory amino acid (EAA) neurotransmitter glutamate is considered to play a pivotal role in the pathogenesis of neuronal death due to ischemia or trauma in the central nervous system.¹ There is increasing evidence that EAA-mediated processes contribute significantly to the progressive neuronal loss in these conditions. The mechanisms of EAA-mediated injury to neurons including major ionic shifts have been identified in *in vitro* and *in vivo* preparations.^{2–5} For example, glutamate not only caused early intracellular accumulation of sodium and chloride ions

resulting in acute cytotoxic edema, it also caused an early intracellular influx of extracellular calcium ions resulting in delayed damage of neurons.^{2,6} There are several subtypes of glutamate receptors and among these, the N-methyl-D-aspartic acid (NMDA), 1-amino-3-hydroxy-5-methyl-4-isoxazole-propionic acid (AMPA), and kainic acid (KA) receptors are especially important in inducing neurotoxicity.⁷ There is evidence that acute spinal cord injury (SCI) causes damage by two separate mechanisms: the initial mechanical disruption, and secondary damage due to a variety of vascular and biochemical processes,⁸ such as increased levels of intracellular Ca^{2+} ,^{9–12} liberation of free fatty acids, uncontrolled production of free radicals, phospholipid degradation and abnormalities in prostaglandin production.^{13–15} Since 1985, it has

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been known that there are NMDA and AMPA/KAR receptors in the spinal cord and that local damage is induced when sufficiently high concentrations of glutamate or its agonists are injected into the spinal subarachnoid space.^{16–19} Furthermore, SCI increases the tissue levels of glutamate and aspartate at the site of injuries.^{13,20}

Treatment with glutamate receptor antagonists can reduce neurotoxicity and provide neuroprotection following SCI. (+)5-methyl-10,11-dihydro-5H-dibenzo[a,b] cyclohepten-5,10-imine maleate (MK801), a glutamate receptor antagonist, produces a non-competitive use-dependent blockade of the NMDA receptor, which appears to mediate the lethal intracellular influx of calcium ions responsible for the neurotoxic effects of glutamate. However, treatment of experimental SCI in rats with MK801 has produced variable results. For example, MK801, administered systemically before or after SCI produced by the weight drop method, improved long-term neurologic recovery and reduced pathological changes and alterations in certain tissue cations,^{21,22} consistent with the hypothesis that NMDA receptors mediate certain pathophysiological changes and secondary cord damage after SCI. In contrast, MK801 did not improve neurologic function or spinal cord blood flow (SCBF) after a compressive injury.²³

To study the therapeutic value of MK801 in SCI in the present experiments, somatosensory evoked potentials (SSEPs) were used to monitor the dorsal columns, while cerebellar evoked potentials (CEPs) were used to assess conduction in the ventral spinal cord.²⁴ SSEPs and CEPs accurately reflect axonal function in the ascending tracts of spinal cord, and serial recordings after acute SCI provide information concerning the extent of the lesion and are an index of predicting functional outcome.^{24–26} SSEP measurement several hours after acute SCI has been used as a predictor of final behavioral recovery, which can take 4 weeks or more to become stable.^{27,28} Changes in evoked potential amplitude correlate with severity of the SCI and posttraumatic ischemia, and the latter is reversible by pharmacological intervention. Increase in SCBF at the site of SCI correlates with improvement in the function of acutely injured spinal axons,^{25,29} and glutamate signaling may play a role in modulation of blood flow and blood brain barrier permeability.³⁰ Therefore, we hypothesize that treatment with an NMDA antagonist after acute SCI would affect axonal function by blocking NMDA receptors and reducing neurotoxicity at the injury site, and that there would be an increase in blood flow and a decrease in edema in the injured spinal cord.^{31–33} To test these hypotheses, we observed the effects of pre- and posttreatment with MK801 on the axonal conduction, posttraumatic ischemia and cord edema by measuring evoked potentials, SCBF and cord water content, respectively, after acute compression SCI in rats.

Materials and methods

Animal preparation

Thirty adult male Wistar rats (mean weight 378 ± 22.4 g, range 340–430 g) were anesthetized by intraperitoneal injection of α -chloralose (75 mg/kg) and urethane (525 mg/kg), and then a tracheostomy was performed. Both femoral veins and the right femoral artery were cannulated. The left sciatic nerve was exposed under the biceps femoris and a bipolar stimulating electrode, consisting of two non-cutting stainless steel acupuncture needles (25 gauge) separated by 1 mm, was inserted directly into the sciatic nerve. A small piece of cork served to insulate the protruding ends on the other side of the nerve.

With the aid of an operating microscope, a small burr hole was placed over the right sensorimotor cortex, 3 mm caudal to the coronal suture and 1 mm lateral to the sagittal suture, and a second burr hole was made over the paramedian lobule of the left cerebellar hemisphere, 2 mm inferior and 2 mm lateral to the external occipital protuberance. A ball-shaped platinum recording electrode 0.8 mm in diameter was placed extradurally into each burr hole. Both recording electrodes were referenced to an Ag/AgCl disc electrode inserted between the tongue and hard palate. A platinum needle electrode was inserted in the upper thoracic paraspinal musculature for grounding.

Muscle relaxation was produced by intravenous injection of 0.7 mg pancuronium bromide followed by 0.4 mg every 30 min. Ventilation was maintained with a mixture of 1:2 nitrous oxide and oxygen by a Harvard rodent ventilator (Model 683, South Natick, MA, USA). A continuous intravenous infusion of a mixture of 0.9% sodium chloride, 5% albumin and 2% sodium bicarbonate was given at a rate of 7.0 ml/kg/h during the experiment to maintain blood pressure. Blood gases, mean arterial pressure (MAP), rectal temperature and haematocrit (HCT) were monitored throughout the experiment. Body temperature (BT) was maintained at 37–38°C with a heating pad, and blood gases were maintained in the physiological range (pH 7.35–7.45; PCO_2 35–45 mmHg; $PO_2 > 150$ mmHg).

A laminectomy was performed at T₁ and C₇ with the aid of an operating microscope, and the dura covering the dorsal aspect of the spinal cord was incised and retracted laterally. The injury was made with the extradural clip compression technique at T₁³⁴ (20 rats). The clip closure force was 21 g, and the duration of compression was 1 min. For the uninjured controls (10 rats), the same surgical preparation was applied except omission of clip compression to spinal cord.

Evoked potentials

The techniques for measuring the SSEPs and CEPs were similar to those reported previously from this laboratory.^{24,29,35} The SSEPs and CEPs were recorded

simultaneously on a Cadwell 8400 signal averager (Cadwell Laboratories Inc., Kennewich, WA, USA) with a sweep time of 90 msec. The bandpass filter was set for 30–3000 Hz, and the stimulation rate was 4.2 Hz at a current of 5 mamp and 50 μ s duration. Two hundred responses were averaged and replicated at least once for each record. The latency of the CEP and SSEP peaks was measured from the onset of stimuli to the peak of each wave, and the amplitude of the evoked potential waves was measured from the peak of one polarity to the immediately preceding peak of the opposite polarity. The amplitude of the initial wave was determined from the preceding baseline to the wave peak.

Measurement of spinal cord blood flow

SCBF was measured by the hydrogen clearance technique with a microcomputer-based recording system described in detail in previous reports from this laboratory.^{36,37} Two platinum-iridium microelectrodes with a 10 μ m tip diameter, straddled the midline dorsal vein (0.5 mm lateral to the midline), and were stereotactically inserted into the dorsal column of the spinal cord to a depth of 500 μ m at T₁. After a period for electrode stabilization, 5–7% hydrogen was administered through the ventilator until saturation was reached after which SCBF recording commenced with the hydrogen clearance curves recorded during the desaturation phase. Linear regression was used online to compare the presaturation polarographic baseline level with the desaturation baseline level. Flows with a drift in baseline measurement of more than 5% were discarded. The initial slope index method was used to calculate the SCBF from the logarithmically transformed desaturation curves.

Measurement of water content

At the end of the recording session (approximately 4.5 h after injury), the rats were sacrificed by bolus injection of sodium pentobarbital. Immediately after the injured and adjacent segments of the spinal cord (6.6–12.1 g) extending 1 mm beyond the border of the grossly haemorrhagic cord were removed, weighed on aluminium foil, dried at 105°C for 24 h, and reweighed. The percent water content was calculated as follows:

$$\text{water content (\%)} = \frac{\text{wet weight} - \text{dry weight}}{\text{wet weight}} \times 100$$

Experimental protocol

MK801 was dissolved in a vehicle solution of 0.9% saline. Thirty animals were randomly divided into four groups, the SCI with or without MK801 (10 rats/group) and uninjured controls with or without drug treatment (5 rats/group). The treatment group received MK801 at a dose of 3 mg/kg in the first infusion and 1.5 mg/kg in the following three infusions (total 7.5 mg/kg), while the control group received only

saline. All the animals were allocated randomly to one of the four groups before experiments, and the injuries were made without knowledge of the group to which an individual animal belonged.

After the initial surgical preparation including the laminectomy, CEPs, SSEPs and SCBF were recorded. Then 0.5 ml of MK801 solution or saline was infused intravenously over 16 min using a Harvard infusion/withdrawal pump. At the 8th minute of infusion, the injury was made at the T₁ segment. Five minutes after injury, CEPs and SSEPs were recorded again. The first posttreatment SCBF measurement started at the end of the first drug or saline infusion. After each of the subsequent three infusions of drug or saline, the SSEP, CEP and SCBF recordings were repeated at an interval of 1 h from the last infusion or recording. After completion of the last SCBF recording, the rats were sacrificed and the injured spinal cord was removed for assessment of water content.

Statistical analysis

The data were evaluated blindly and the treatment code was not broken until the conclusion of the statistical analysis. Comparisons at each time point between groups were made by univariate one-way analysis of variance (ANOVA), whereas comparisons within each group at different times were made by paired *t*-test. A stabilizing transformation (square root) of the evoked potential amplitude data was performed prior to parametric analysis. The results were expressed as the mean $\pm$ SEM (standard error of the mean), and the differences were considered significant at $P < 0.05$.

Results

SCI experiments

Physiological parameters The mean preinjury physiological parameters including MAP, HCT, pH, PCO₂, PO₂ and BT were not significantly different ($P > 0.05$) between treatment and control groups (Table 1). Furthermore, paired *t*-test showed that the pH, BT, PCO₂ and PO₂ remained constant throughout the experiment ($P > 0.05$). Although the pre- and post-injury haematocrit was similar between treatment and control groups ($P > 0.05$), the postinjury mean haematocrit of both groups was significantly lower than the preinjury values ($P < 0.01$, Table 1).

After spinal cord injury, the MAP decreased significantly in both groups (Table 1 and Figure 1), and then recovered partially 1 h later. However, one way ANOVA revealed that the post-injury MAP in the treatment group was significantly lower than in the control group at 5 min, 2 h and 3 h postinjury ($P < 0.05$).

Cerebellar evoked potentials Preinjury, the amplitude and latency of each CEP peak (N1, P1, N2 and P2)

Table 1 Physiological parameters*

Groups	MAP (mmHg)	HCT (%)	pH	BT (°C)	PO ₂ (mmHg)	PCO ₂ (mmHg)
MK-1h	113.0±3.1	38.2±1.1	7.37±0.096	37.4±0.10	241.7±9.3	40.0±0.5
Sal-1h	113.6±4.3	38.0±1.1	7.37±0.009	37.6±0.09	228.9±9.2	38.9±0.9
MK5m	47.0±1.4**	33.5±0.5**	7.39±0.059	37.1±0.14	223.3±8.8	36.9±0.5
Sal5m	87.1±3.7**	35.9±1.4**	7.39±0.011	37.2±0.16	226.2±8.7	37.3±0.7
MK1h	77.0±4.8**	32.9±1.0**	7.40±0.009	37.2±0.06	229.2±6.5	39.0±0.8
Sal1h	87.0±4.5**	33.3±1.0**	7.39±0.010	37.4±0.47	217.8±5.6	37.7±0.8
MK2h	71.7±4.0**	30.8±0.9**	7.40±0.009	37.2±0.09	222.6±9.2	38.6±0.1
Sal2h	87.7±5.5**	32.0±1.4**	7.40±0.008	37.4±0.18	224.3±5.3	37.0±0.4
MK3h	69.0±0.9**	29.0±0.5**	7.40±0.009	37.3±0.13	215.2±6.9	38.6±0.9
Sal3h	86.7±5.1**	30.8±1.2**	7.39±0.007	37.4±0.19	221.1±6.9	39.9±0.8
MK4h	71.8±5.1**	28.7±1.0**	7.40±0.077	37.1±0.10	212.2±5.5	39.8±0.9
Sal4h	81.8±4.6**	29.1±0.9**	7.39±0.008	37.3±0.16	218.4±4.9	40.6±0.6

*Values are means±standard error of the means. ** $P<0.01$. Comparison between pre- and postinjury within each group ($n=10$ per group). MK1h=1 h preinjury in MK801 group; Sal1h=1 h preinjury in saline group; MK5m=5 min postinjury in MK801 group; Sal5m=5 min postinjury in saline group; MK1h=1 h postinjury in MK801 group; Sal1h=1 h postinjury in saline group; MK2h=2 h postinjury in MK801 group; Sal2h=2 h postinjury in saline group; MK3h=3 h postinjury in MK801 group; Sal3h=3 h postinjury in saline group; MK4h=4 h postinjury in MK801 group; Sal4h=4 h postinjury in saline group

Figure 1 Mean systemic arterial blood pressure (MAP) recorded at different times. The preinjury MAP did not differ significantly between the two groups. Following SCI, there was a significant decrease in MAP in both groups. The decrease in the MK801 group was more significant than in the saline group at 5 min, 2 h and 3 h postinjury ($P<0.05$, $n=10$ per group, * = the difference between the saline and MK801 groups is statistically significant)

were not significantly different between the MK801 and control groups. As shown in Figure 2, the amplitude of the N1, P1 peaks decreased significantly after SCI ($P<0.01$, t -test). The late components of the CEPs, N2 and P2, disappeared by 5 min postinjury. At 1 h postinjury, the return of the N2 and P2 waves was observed in two and one rats respectively in the MK801 group, and in four and three rats respectively in the control group. Although the amplitude of N1

CEREBELLAR EVOKED POTENTIALS

Figure 2 Cerebellar evoked potential recordings from two rats, one from each of the saline and MK801 groups. Before SCI, the amplitude of the CEP peaks was similar between the two rats. Five minutes after SCI, it decreased significantly in both animals. From 1–4 h postinjury, the CEP amplitude partly recovered in the rat receiving saline, but remained low in the rat treated with MK801

and P1 was lower in the MK801 group than in the control group after SCI, one-way ANOVA revealed that only the reduction in N1 amplitude at 1 h postinjury was statistically significant ($P<0.05$, Figure 3).

Preinjury, the latency of each CEP peak did not differ significantly between the two groups, and there were no significant differences after injury ($P > 0.05$).

Figure 3 The amplitude of N1 peak of CEPs at different times. Preinjury, the N1 amplitude was similar in both groups. After SCI, there was a more significant attenuation of the amplitude of N1 peak in the MK801 group at 1 h postinjury in comparison with the saline group ($P < 0.05$, $n = 10$ per group, * = the difference between the saline and MK801 groups is statistically significant)

Figure 4 Somatosensory evoked potential recordings from two rats, one from each side of the saline and MK801 groups. Before SCI, the amplitude of each SSEP peak was similar between the two rats. Five minutes after SCI, it decreased significantly in both animals. From 1–4 h postinjury, the SSEP amplitude partly recovered in the rat receiving saline, but remained low in the rat treated with MK801

Somatosensory evoked potentials Preinjury, the amplitude and latency of each SSEP peak were not significantly different between the two groups (Figure 4). The amplitude of these peaks decreased significantly after SCI ($P < 0.01$, t -test), but one-way ANOVA revealed that the amplitude attenuation of these peaks (P1, N1 and P2) was greater in the MK801 group ($P < 0.01$, Figure 5A–C). Recovery of the amplitude was observed in the control group ($P < 0.05$) at 1, 2, 3 and 4 h postinjury in comparison with the value at

Figure 5 Effect of MK801 on the amplitude of P1 (A), N1 (B) and P2 (C) peaks of SSEPs. Preinjury, the amplitude of these peaks was similar in both groups. After SCI, there was a more significant decrease of P1, N1 and P2 amplitude in the MK801 group than in the saline group, particularly in N1 and P2 amplitude. ($P < 0.01$, $n = 10$ per group, * = the difference between the saline and MK801 groups is statistically significant)

5 min postinjury. In contrast, the amplitude of each peak remained low in the MK801 group after SCI ($P>0.05$). The t -test and one-way ANOVA indicated that the latency of each peak did not change significantly within each group or between the two groups ($P>0.05$).

Table 2 Mean values of SCBF and postinjury SCBF decrease (mean $\pm$ SEM) during the experiment. The SCBF decreased significantly after SCI ($n=10$ per group)

Groups#	SCBF	SCBF decrease
MK1h	71.8 $\pm$ 4.2	
Sal1h	76.3 $\pm$ 6.6	
MK5m	40.0 $\pm$ 5.2**	33.3 $\pm$ 3.4
Sal5m	44.0 $\pm$ 5.2**	33.4 $\pm$ 5.2
MK1h	37.7 $\pm$ 1.9**	33.9 $\pm$ 3.4
Sal1h	41.4 $\pm$ 4.6**	35.0 $\pm$ 4.9
MK2h	33.6 $\pm$ 2.7**	39.7 $\pm$ 3.0
Sal2h	44.0 $\pm$ 4.6**	35.3 $\pm$ 3.9
MK3h	35.9 $\pm$ 3.2**	37.5 $\pm$ 3.1
Sal3h	37.4 $\pm$ 3.9**	38.3 $\pm$ 3.2
MK4h	38.3 $\pm$ 3.3**	38.9 $\pm$ 4.6
Sal4h	37.5 $\pm$ 3.5**	38.9 $\pm$ 4.6

** $P<0.01$ comparison between pre- and postinjury within each group. SCBF: ml/min/100 g. #The group designations are described in Table 1

Spinal cord blood flow The pre- and postinjury SCBF was similar in the two groups (Table 2). Although there was a significant decrease in SCBF after SCI ($P<0.01$, t -test), the decrease was similar in the two groups. After SCI, the SCBF remained consistently low during the experiment in both groups ($P>0.05$).

Water content Although there was a slight reduction in the MK801 group, one-way ANOVA revealed that there was no significant difference in water content ($Df=1,17$; $F=0.559$; $P=0.4649$) between the MK801 group ($75.7\%\pm1.3$) and the control group ($77.8\%\pm2.5$).

Uninjured controls

Before and after infusion, the mean physiological parameters including MAP, HCT, PH, PCO₂, PO₂ and BT were not significantly different ($P>0.05$) between the MK801 and saline groups except that MAP in the MK801 group was slightly lower than in the control group at 13 min after infusion (95 vs 105 mmHg; $P<0.05$). Before infusion, the amplitude of each CEP or SSEP peak was not significantly different between MK801 and control groups, but there was a statistical reduction in N1 amplitude of CEP, and P1, N1 and P2 amplitude of SSEP in the MK801 group compared with the saline group following drug infusion (Figure 6A–D, $P<0.05$ or 0.01). In addition

Figure 6 Effect of MK801 on the amplitude of each peak of CEPs and SSEPs in uninjured controls. The amplitude of N1 (A) peak of CEP and P1 (B), N1 (C) and P2 (D) peaks of SSEP was similar between MK801 and saline groups before infusion. Intravenous injection of MK801 caused a significant amplitude reduction in these peaks in comparison with saline controls. (* $P<0.05$; ** $P<0.01$, $n=5$ per group)

Table 3 Effects of MK801 on recovery after *in vivo* SCI

	<i>Authors</i>	<i>Administration</i>	<i>Injury model</i>	<i>Observation time</i>	<i>Outcome measures</i>	<i>Results</i>
Acute studies	Li and Taylor (present study)	3 mg/kg iv, prior to and after SCI (1.5 mg/kg $\times$ 3)	rats, clip compression	4 h	multiple evoked potentials, spinal cord blood flow water content	reduced the amplitude of evoked potentials, did not affect spinal cord blood flow or water content at injured site
	Haghighi <i>et al</i> (1996) ⁴⁸	1 mg/kg, iv, 15 min prior to SCI	rats, weight compression	5 h	somatosensory evoked potential and morphology	improved evoked potential recovery, reduced hemorrhage and neurofilament damage
Chronic studies	Faden <i>et al</i> (1988) ²¹	0.2–5.0 mg/kg iv, 15 min after SCI	rats, weight-drop	30 days	behavior, neuroanatomy, water content, tissue cations	improved neurologic function, decreased histologic damage, no change in cord edema
	Gomez-Pinilla <i>et al</i> (1989) ²²	0.3 mg/kg, ip, 60 min before SCI	rats, weight drop	30 days	behavior, histology	improved neurologic function and decreased neuronal death
	Hao <i>et al</i> (1992) ⁴⁴	0.1–1.0 mg/kg, iv, 30 min before SCI	rats, laser	21 days	behavior, histology	decreased neurologic deficit and spinal cord necrosis
	Holtz <i>et al</i> (1991) ²³	10 mg/kg, iv, 60 min after SCI	rats, compression	4 days	spinal cord blood flow and behavior	no improvement in SCBF or behavior
	Yanase <i>et al</i> (1995) ³³	5 mg/kg, iv, 10 min after SCI	rabbits, compression	14 days	behavior, SCBF, water content	improved motor function and cord edema, but not SCBF

MK801 infusion did not have any significant effects on SCBF during the 4 h observation (50.0 ± 8.6 vs 46.7 ± 3.8 ml/min/100 g for the last measurement ($P < 0.05$, *t*-test).

Discussion

Several studies have shown a rapid elevation of the extracellular concentration of glutamate and aspartate in traumatic^{38,39} and ischemic⁴⁰ spinal cord lesions consistent with the hypothesis that these lesions are associated with a release of these excitatory amino acids at the lesion site. In the weight-drop model, Demediuk *et al* (1989) observed increased extracellular glutamate and aspartate as early as 5 min after SCI and found that the release of these amino acids was delayed, time-dependent and related to the severity of injury.¹³ Similarly, in a compression model, Farooque *et al* (1996, 1997) found that moderate and severe SCI induced a profound raise of extracellular EAA, and that moderate hypothermia (30–31°C) prolonged the EAA accumulation.^{41,42} The mechanisms of extracellular EAA accumulation after SCI may include both increasing release from vesicles, injured cells or blood and decreasing uptake by astrocytes and neurons.^{41,43} Intrathecal administration of NMDA, at a dose with no effects on uninjured animals, significantly worsens recovery after SCI.²¹ On the basis of these findings, several investigators analyzed NMDA receptor antagonists for the treatment of experimental SCI or

ischemia. MK801, a potent NMDA antagonist, has been one of the most frequently studied (Tables 3 and 4). Treatment with MK801, given 15 min after or 1 h before spinal cord contusive injury produced by the weight drop technique, significantly attenuated some of the neurochemical changes, limited the histologic damage, and improved neurologic recovery compared to saline-treated rats^{21,22,33} (Table 3). Similarly, administration of MK801, 10 min after compressive SCI at a dose of 5 mg/kg, significantly improved motor recovery in rabbits. In photochemically induced spinal cord lesions in rats, pretreatment with MK801 at a dose of 0.5–1 mg/kg also improved functional recovery during a 2-week posttraumatic observation period.⁴⁴ In contrast, Holtz and Gerdin found that MK801 at a dose of 10 mg/kg 60 min after injury (weight drop method) in rats, did not improve functional recovery during a 4-day observation period.²³ Thus, there is considerable evidence that NMDA receptors are involved in the secondary tissue damage following SCI. The inconsistent results of the effects of MK801 on SCI may be due to variations in the timing and dosage of drug administration (Table 3). In the present study, we chose to administer MK801 systemically with a relatively higher dose of 7.5 mg/mg, at which all the animals in MK801-treated group survived well during the 4 h period of observation. Furthermore, considering the 1 h plasma half-life time of MK801 in rats,⁴⁵ we treated the animals with MK801 repetitively before and after SCI,

Table 4 Review of neuroprotective effects of MK801 in experimental stroke

Ischemia	Models	Drug delivery	Outcome measures	Results	Ref
Focal cerebral ischemia	occlusion of middle cerebral artery (MCAO) induced by mechanical ⁴⁹⁻⁵² photochemical ⁵³ techniques or endothelin-1 ⁵⁴ in rats	pretreatment: 0.3–5.0 mg/kg, ip/ ^{46,49,50,52,54} posttreatment: 0.5 mg/kg iv, 30–60 min after ischemia ^{51,52} pre- + posttreatment: 5 mg/kg ip, 30 min before and 2.5 mg/kg at 8 and 16 h after ischemia ⁴⁶	infarction volumes or morphology by magnetic resonance imaging ⁵²	reduced damage volume of cortex, basal ganglia, ^{46,52} but not in striatum ⁵⁴ or pretreatment alone	46,49–54
	permanent right carotid artery occlusion (CAO) + 2 h right MCAO + left CAO in rats	2.5–5.0 mg/kg ip, 30 min prior to and 6–16 h after ischemia	infarct volume and cerebral blood flow (CBF)	reduced infarct volume and increased CBF	55
	MCAO induced by mechanical ^{56,57} or endothelin-1 ⁵⁸ in cats	pretreatment: 0.12–5.0 mg/kg iv ^{57,58} posttreatment: 0.5–5.0 mg/kg iv, 30–75 min after ischemia ^{56,57}	cortical damage volume	pre- and post-treatment reduced the damage in cerebral cortex	56–58
	MCAO in dogs	1 mg/kg iv, before occlusion	CBF	reduced CBF in all regions of the brain	59
	MCAO in Squirrel monkeys	1 mg/kg, iv, 20 min and again at 12 h after ischemia	behavioral deficit and ischemic infarction	did not reduce infarction or behavioral deficit	60
	5–10 min bilateral CAO in gerbils	pretreatment: 1–17 mg/kg ip/subcutaneous injection ^{61–65}	morphology in brain and internal Ca ⁶²	reduced neuronal loss at normothermia/hypothermia, but not at hyperthermia	61–65
		posttreatment: 1–10 mg/kg, ip, immediately 30 min after ischemia ^{62,64–66}	internal Ca and neuronal necrosis	did not reduce neuronal loss or internal Ca	62,64–66
		pre- + posttreatment: 2 mg/kg, ip, 30 min before and 150 min after ischemia	neuronal density in CA1	a small degree of neuroprotection	47
	bilateral CAO with hypotension ^{67–69} or vertebral artery occlusion in rats ^{70,71}	pretreatment: 0.1–10 mg/kg, iv/ip ^{67,71}	neuronal damage and microglial activation ⁷²	reduced microglial activation and neuronal death of CA1 ⁷² or did not prevent neuronal damage ^{67,71}	67,71,72
		posttreatment: 0.1–5.0 mg/kg, ip/iv, immediately 20 h after reperfusion ^{67,71}	neuronal damage and working memory ⁷⁰	no neuroprotection ^{67,68,71} or decreased cell loss within 5 h injection after ischemia ⁶⁹ or reduced the increased errors in ischemia ⁷⁰	67–71
Spinal cord ischemia		pre- + posttreatment: 2.5–5.0 mg/kg hippocampal damage ip, 1 h prior to and 8–16 h after occlusion		failed to attenuate hippocampal injury	71
	12 min aortic occlusion in rats	25 mM, intrathecal injection 2 h before occlusion	functional performance	increased the rate of recovery	73
	vascular occlusion by photo-sensitizing dye and laser beam in rats	0.1–0.5 mg/kg, iv, 10 min before occlusion	hypersensitivity and morphology	prevented hypersensitivity and morphological damage	74
	20–40 min occlusion of aorta in rabbits	1 mg/kg iv, 5 min prior to or immediately after occlusion	metabolic parameters and functions	increased the rate of recovery, but did not prevent paraplegia	75

and injected the drug slowly (16 min/infusion). MK801 delivered in this way has been shown to produce neuroprotection in experimental cerebral ischemia.^{46,47}

In the present study, we used a 21-g clip compression injury of the cord at T₁, which causes a spastic paraparesis²⁹ and produces a lesion involving the central and dorsal grey matter including the base of the dorsal columns.⁷⁶ In the present experiments, 5 min after SCI, the CEPs and SSEPs were still present in most of the rats, although the amplitude of the evoked potentials was markedly decreased (Figures 2 and 4), especially the CEPs. At 5 min after SCI, there was complete disappearance of the CEPs in two rats in the MK801 group and in one rat in the saline group. SSEPs were recorded in all rats in both groups 5 min after SCI, although the signals were of very low amplitude in three rats. These findings indicate that the CEPs were more sensitive to compression injury than the SSEPs. Analysis by one-way ANOVA and paired *t*-test showed that the latency of the CEPs and SSEPs was not significantly altered between pre- and postinjury in the MK801 and saline groups, which was similar to the results of Fehlings *et al.*²⁹

It is interesting to note that after SCI, the amplitude of the SSEP peaks in the MK801 group was markedly lower than that in the saline group, and the amplitude of the CEPs in the MK801 group was slightly lower than that of the saline group. Furthermore, the waveforms of both the CEPs and SSEPs differed markedly between the two groups after SCI. For example, in the SSEP recording, seven out of ten animals had only P1-N1 biphasic or P1 monophasic waveforms in the MK801 group, whereas multiphasic P1-N1-P2 or P1-N1-P2-N2 waveforms were present in nine out of ten animals in the saline group after SCI. These results indicate that MK801 caused significant inhibition of the evoked potentials (mainly SSEPs) during the first 4 h after SCI.

The mechanisms by which MK801 caused the inhibiting effects on the evoked potentials after SCI in the present study are not known. SSEPs are conducted primarily through the dorsal and dorsolateral funiculi in the rat but have components that are ventrally mediated. In contrast, the early components of the CEPs depend mainly on the integrity of the ventral spinal cord. Simultaneous recordings of both allow selective monitoring of the dorsal and ventral spinal cord.²⁴ In the present study, the attenuation of amplitude of the SSEPs and CEPs was caused by two major factors. First, the compressive injury of the spinal cord at T₁ blocked the signal transmission along afferent tracts from the peripheral to the central nervous system and induced the dysfunction of axonal conduction in the long tracts of the spinal cord. The second factor was blockade of NMDA receptors by MK801 in the somatosensory pathway, which reduced the evoked responses of neurons and/or axons at different sites in the afferent pathway including the injured spinal cord. This notion was strongly supported by the control experiments which indicate

that NMDA receptor antagonist MK801 produces remarkable inhibitory effects on the sensory signal transmission in animals without SCI (Figure 6).

Our results contrast to the observation reported by Haghighi *et al.*, who showed that Mk801 pretreatment improved the recovery of evoked potentials following a compressive SCI.⁴⁸ This discrepancy is probably due to the differences in the drug dose and application procedure, and the injury model (Table 3). Interestingly, these authors also found that MK801 reduced the size of hemorrhage in grey matters and protected the spinal cord white matter, though NMDA receptors probably do not present on the vessels of neural tissue^{77,78} and CNS white matter.^{79,80} However, our results are consistent with some investigations on the role of ionotropic glutamate receptor antagonists. For example, local injection of the non-NMDA receptor antagonist, 6,7-dinitro-quinoxaline-2,3-dione (DNQX), into the ventrobasal thalamic nucleus, resulted in a decrease in amplitude and an increase in latency of cortical SEPs.⁸¹ This indicates that non-NMDA receptors in the ventrobasal thalamic nucleus are involved in the transmission of cortical SEPs. In the pain model induced by formalin, treatment intrathecally with another NMDA antagonist, gamma-D-glutamyl glycine (DGG), produced profound inhibition of acute C-fibre evoked responses of the corresponding dorsal horn neurons.⁸² Chronic exposure to MK801 in embryos disrupts the somatotopic organization of cutaneous nerve projections in the chick spinal cord⁸³ and blocks the development of hyperalgesia following experimental nerve injury.⁸⁴ Also, intrathecal MK801 produced a dose-dependent reduction of tactile hyperesthesia in a peripheral neuropathic pain model in rats.⁸⁵ These findings are consistent with the hypothesis that blockade of glutamate neurotransmission by MK801 inhibits the flow of information along multisynaptic sensory pathways.⁸⁶

Another possible mechanism for the dampening effect of MK801 on the evoked potentials was the lowering of MAP since hypotension itself may induce reduction in the evoked potential amplitude.^{87,88} In the MK801 group, MAP was much lower than in the saline group after SCI, especially 5 min postinjury (47 and 87 mmHg respectively, Figure 1). A similar decline in MAP after the administration of MK801 has also been observed by other authors,^{21,33,46,89} though even in the presence of hypotension some found that MK801 improved functional recovery after SCI or reduced the cortical damage after focal cerebral ischemia. The mechanism of hypotension caused by SCI is due to interruption of sympathetic fibers and myocardial dysfunction.³⁶ The hypotension induced by systemic administration of MK801 probably contributed to the blockage of NMDA receptors in the cardiovascular regulating centers. Accumulating evidence suggests that synaptic activation of NMDA receptors in the nucleus of the tractus solitarius, rostral and caudal ventrolateral medulla and spinal

cord plays a key role in neural transmission of cardiovascular information in the central nervous system.⁹⁰ For example, local administration of MK801 in the area postrema or medial nucleus of the tractus solitarius effectively blocked NMDA-induced cardiovascular responses.⁹¹ Intrathecal injection of NMDA receptor agonists had independent effects on heart rate and blood pressure, and these effects were blocked by the NMDA receptor antagonist, 2-amino-5-phosphonovaleric acid (AP-5).^{92,93}

Acute SCI causes immediate mechanical damage to the microvasculature of the cord followed by a secondary injury to the vessels, such as vasospasm, the combination of which produces spinal cord ischemia which may be progressive.^{8,37} After acute cord clip compression injury in the rat, the decrease in regional SCBF at the injury site begins almost immediately and continues for at least 24 h. In the rat, there is a direct linear dose response relationship between the severity of injury as represented by clip force and the ischemic response.⁹⁴ In the present study, SCBF decreased from the pre-injury values of 71.8 and 76.3 ml/min/100 g tissue to 38.3 and 37.5 ml/min/100 g tissue in the two groups, respectively, after the 21 g clip cord compression injury during the 4 h period of observation (Table 2). However, there was no improvement in regional SCBF in the MK801 group. This result is similar to other studies published recently which showed no improvement in posttraumatic SCBF with MK801.^{23,33} In addition, after intrathecal administration of 3-(2-carboxypiperazin-4-yl) propyl-1-phosphoric acid (CPP), another glutamate antagonist, there was no change in SCBF compared with a saline control group in normal rats.⁹⁵ These findings are consistent with the observations that NMDA receptors have not been found in cerebral microvessels⁷⁷ and that NMDA agonists have no effect on cerebrovascular tone *in vitro*.

Spinal cord edema at the injury site develops immediately after experimental impact injury and continues for 24 h in rats.⁹⁷ In the present study, the water content of injured cord tissue was 75.7% in the MK801 group and 77.8% in saline group, with no difference between the two groups ($P > 0.05$) although both values are higher than the normal value (71.6%) for the rat spinal cord.⁹⁸ Our result is similar to the study by Faden *et al* with weight-drop injury, who also found MK801 did not reduce the water content in the cord after trauma.²¹ In contrast, there were also reports that MK801 attenuated the water content in the injured spinal cord in plate compression model of rabbits³³ and in neoplastic compression model of rats.⁹⁹ The mechanism underlying these discrepant results is probably related to different injury models of SCI and the various methods measuring water content.

The compounds directed to NMDA receptor have produced some promising results for the treatment of experimental stroke and trauma^{1,100} basing on the role of this receptor in these disorders. Unfortunately,

many of these potentially effective drugs have to be discontinued because of the unacceptable clinical side-effect.¹ In the present acute compressive SCI model, the inhibitory role of systemic MK801 in evoked potentials following SCI is probably related to the side effects of this agent since the same dose also produced a blocking effect in uninjured control animals. In the present study, however, the key issue is whether the inhibition of NMDA receptor antagonist after trauma is the harbinger of reduced excitotoxicity at the injury site. Following SCI, the increased extracellular glutamate may overactivate NMDA receptors and induce tissue damage via influx of Ca through these ion channels. Thus, the blocking effects of the systemic application of MK801 should also involve the NMDA receptors around injured spinal cord, though traumatic SCI might cause the downregulation of ionotropic glutamate receptors at injury site.¹⁰¹ Recently, our further investigation indicates that intramedullary injection of MK801 (30 nM) directly into injured cord statistically attenuated the amplitude of sensory evoked potentials in the same injury model,¹⁰² supporting the inhibitory role of this agent at injury site. Thus, we believe that treatment with NMDA receptor antagonist would improve the long-term conduction function of cord after the drug is washed out and would provide neuroprotection in a manner similar to Na channel blocker tetrodotoxin in the traumatic SCI.¹⁰³

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