Sites of Action of CS-722, a Newly Synthesized Centrally Acting Muscle Relaxant

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ABSTRACT—Sites of the muscle relaxant action of CS-722 were investigated in rats. Doses injected intracerebroventicularly (i.c.v.) or intrathecally (i.t.) were determined according to measurements of CS-722 concentration in the brain stem and the spinal cord after systemic administration. When administered i.c.v. (50 and 100 µg) or i.t. (200 and 400 µg), CS-722 reduced the radio frequency decerebrate rigidity, although the effect after i.c.v. injection was transient. These results indicate that CS-722 exerts its muscle relaxant action by affecting both the supraspinal structure and the spinal cord. Spinal reflexes were not affected by CS-722 injected i.c.v. It seems that muscle relaxation is not always accompanied by impairment of the spinal reflex.

Keywords: Centrally acting muscle relaxant, CS-722, Local application, Decerebrate rigidity, Spinal reflex

(R)-4-Chloro-2-(2-hydroxy-3-morpholinopropyl)-5-phenyl-4-isoxazolin-3-one hydrochloride (CS-722, Fig. 1) is a newly synthesized centrally acting muscle relaxant that is expected to be advantageous for the treatment of spasticity and rehabilitation of patients with brain and the spinal cord disorders, such as stroke, head injury and spinal cord injury, because of its low possibility for eliciting adverse effects such as drowsiness (1, 2). CS-722 preferentially depressed the polysynaptic reflex (PSR) in intact and spinal rats, but it was less effective in spinal preparations (1, 3). These results suggested that the supraspinal structure was a possible site for the depressant effect of CS-722 on the spinal reflex. In addition to the depression of spinal reflex pathways (4), CS-722 might depress the spinal reflex at the spinal cord by inhibiting the descending facilitatory effect which is tonically active in intact preparations (5). A determination of sites responsible for depression of the spinal reflex, however, would not indicate the sites for muscle relaxation. Though an observation of drug effects on the spinal reflex is helpful for analyzing the mechanism of centrally acting muscle relaxants (6), muscle relaxation would not always accompany an impairment of the spinal reflex.

In this study, to clarify the site responsible for the muscle relaxant action of CS-722, effects of locally administered CS-722 on decerebrate rigidity were investigated, and CS-722 concentrations in the brain stem and the spinal cord were measured to ascertain whether doses injected locally were reasonable ones to employ. Supraspinal involvement in the depressant action of CS-722 on the spinal reflex was also examined, and the difference between the effects on decerebrate rigidity and those on the spinal reflex was discussed.

MATERIALS AND METHODS

Animals

Male Wistar rats (Charles River Japan Inc., Atsugi) weighing 300–400 g were used in all experiments.

Decerebrate rigidity

Preparation and measurement of decerebrate rigidity (by means of radio frequency lesions of the midbrain) were performed according to the method of Ono et al. (7). Rats were anesthetized with ether and fixed on a
stereotaxic apparatus. Radio frequency lesions were produced with a lesion generator (RFG-4; Radionics, Inc., Burlington, MA, USA) by maintaining tissue temperature at 75–85°C for 180 sec (500 kHz, about 25 mA). The coordinates of the lesions were AP 0, V -3, L ±1.5 (8). After lesion production, ether anesthesia was discontinued.

The decerebrated rat was placed in a holder. A motor-driven mechanical device was used to dorsiflex the hind feet. The tension developed during dorsiflexion was measured by means of strain gauges attached to the mechanical device and was recorded on a recorder. A stroke of the dorsiflexion of 4 mm in length during 6 sec was applied once per min. At the end of every experiment, the rat was sacrificed by ether inhalation. The tension obtained during flexion in the sacrificed rat was defined as the passive tension. Each active tension during dorsiflexion was obtained by subtracting the passive tension from the each recorded tension.

Spinal reflexes

Rats were anesthetized with urethane (1 g/kg, i.p.) and α-chloralose (25 mg/kg, i.p.) and were artificially ventilated. Laminectomy was performed in the lumbosacral region. Dorsal and ventral roots below L4 were cut bilaterally, and dorsal and ventral roots of segments 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.

The dorsal and ventral roots of segment L5 were respectively placed on bipolar silver-silver chloride wire electrodes for stimulation (0.2 Hz, 0.05 msec, supramaximal) and recording. The reflex potentials were amplified, displayed on an oscilloscope, and recorded as an average of eight consecutive reflexes. The amplitudes of monosynaptic reflex (MSR) and polysynaptic reflex (PSR) potentials were measured.

Determination of CS-722 content

CS-722 content of the tissue was investigated in different rats from those used in the experiments described above. Under ether anesthesia, a polyethylene catheter was inserted into the left femoral vein. At least 2 hr after the discontinuation of anesthesia, CS-722 was administered intravenously (i.v.). Five minutes after administration, the animal was killed by an overdose of pentobarbital, and the brain stem beneath the 4th ventricle and the whole spinal cord were removed.

Intra-4th ventricular (i.c.v.) or intrathecal (i.t.) injection was performed in rats which were anesthetized with urethane (1 g/kg, i.p.) and α-chloralose (25 mg/kg, i.p.) after ether anesthesia. Five minutes after i.c.v. or 10 min after i.t. injection, tissue samples were obtained.

Quantitative determination of CS-722 was carried out by a specific HPLC method. Brain and spinal proteins were removed by precipitation with methanol. Chromatography was performed on a reverse phase ODS column with UV detection at 272 nm using an internal standard method. Recovery was more than 95%.

Administration of CS-722

When administered systemically, CS-722 was dissolved in saline and administered in a volume of 1 ml/kg. For i.t. injection, CS-722 was injected in a volume of 10 μl of saline (pH 6.0) via a polyethylene tube (SP8; I.D. 0.20 mm, O.D. 0.50 mm; Natsume, Tokyo), which was inserted into the subarachnoid space through the foramen intervertebrale between L3 and L4 according to the method described by Satoh et al. (9), followed by an injection of 10 μl of saline to clear the catheter. For i.c.v. injection, CS-722 was injected in a volume of 5 μl of saline (pH 6.0) 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.

Statistics

Bonferroni’s multiple t-test (two-tailed, 10) was employed to examine the statistical difference between the control and drug-treated groups.

RESULTS

Concentration of CS-722 in the tissue after systemic or local administration

Concentrations of CS-722 in the brain stem and the spinal cord were determined 5 min after i.c.v. and 10 min after i.t. injection.

Table 1. CS-722 concentration in the tissue and depression of decerebrate rigidity
after i.t. injection, respectively, because muscle tonus was effectively reduced at the corresponding times in a preliminary study. CS-722 at the doses of 50, 100 and 200 pg were injected i.c.v. Intrathecal doses were 200 and 400 μg. Concentrations of CS-722 in the tissue are summarized in Table 1, with comparison to the tissue concentration 5 min after systemic administration (i.v.) of a slightly effective (10 mg/kg) and an effective dose (30 mg/kg) on elevated muscle tonus. After i.v. injection, decerebrate rigidity was maximally reduced within 5 min (data not shown). Table 1 indicates that the concentration of CS-722 in the brain stem after 100 pg, i.c.v. and that in the spinal cord after 400 pg, i.t. are comparable to those after 30 mg/kg, i.v.

After i.t. and i.c.v. injections, small amounts of CS-722 were detected in the brain stem and spinal cord, respectively (Table 1).

Effects of local applications of CS-722 on decerebrate rigidity and the spinal reflexes

CS-722 (50 and 100 μg) injected i.c.v. reduced decerebrate rigidity, but the rigidity returned to the predrug level within 10 min after injections of both doses (Fig. 2a). Intrathecal CS-722 (200 and 400 μg), however, showed a slow time course of suppression. It took 5 to 10 after i.t. injection, respectively, because muscle tonus was effectively reduced at the corresponding times in a preliminary study. CS-722 at the doses of 50, 100 and 200 μg were injected i.c.v. Intrathecal doses were 200 and 400 μg. Concentrations of CS-722 in the tissue are summarized in Table 1, with comparison to the tissue concentration 5 min after systemic administration (i.v.) of a slightly effective (10 mg/kg) and an effective dose (30 mg/kg) on elevated muscle tonus. After i.v. injection, decerebrate rigidity was maximally reduced within 5 min (data not shown). Table 1 indicates that the concentration of CS-722 in the brain stem after 100 μg, i.c.v. and that in the spinal cord after 400 μg, i.t. are comparable to those after 30 mg/kg, i.v.

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![Fig. 2](image-url)

**Fig. 2.** Effects of (a) intra-4th ventricularly or (b) intrathecally administered CS-722 on decerebrate rigidity. Abscissae: time after the drug injection. Ordinates: relative active tension (%). Each point represents the mean ± S.E.M. of 4–6 separate experiments calculated as a percentage of the value at time 0. *P < 0.05, **P < 0.01: statistical significance between saline (□)- and drug (○: 50 μg in (a), 200 μg in (b), □: 100 μg in (a), 400 μg in (b))-treated groups according to Bonferroni's multiple t-test (2 comparisons in 3 groups).

![Fig. 3](image-url)

**Fig. 3.** Effects of intra-4th ventricularly administered CS-722 (100 μg) on monosynaptic reflex (MSR) and polysynaptic reflex (PSR). Abscissae: time after the drug injection. Ordinates: relative response amplitude (%). Each point represents the mean ± S.E.M. of 3–4 separate experiments calculated as a percentage of the value at time 0. □: saline, ○: CS-722.
min to achieve the maximal suppression. Muscle tonus was gradually restored, although considerable remission of rigidity was still evident 30 min following the injection of 400 μg of the compound (Fig. 2b).

The spinal reflex was not depressed by i.c.v. injection of CS-722 (Fig. 3). Local application of saline (pH 6.0) in a corresponding volume did not affect the rigidity and the spinal reflex (Figs. 2 and 3).

The depression of decerebrate rigidity is summarized in Table 1 along with the concentration of CS-722 in the tissue.

DISCUSSION

Centrally acting muscle relaxants reduce hypertonia by acting on the spinal cord as well as on the supraspinal structure (11). It is reported that tizanidine depresses decerebrate rigidity by acting on the brain stem (12). Baclofen, diazepam, and tolperisone reduce decerebrate rigidity when administered intrathecally as well as intraventricularly, then both the spinal cord and the supraspinal structure are responsible for the muscle relaxation caused by baclofen, diazepam, and tolperisone (personal communication from Dr. T. Nakamura). In this study, the effects of locally administered CS-722 on decerebrate rigidity and the spinal reflex were investigated to clarify the sites that contribute to the muscle relaxant action of CS-722.

In the experiments using local administration, the problem to be solved is whether the doses injected are the appropriate ones. In this study, CS-722 concentrations in the brain stem and the spinal cord following i.c.v., i.t., and i.v. injections were compared. Because distribution of CS-722 would be uneven, especially after local administration such as by the i.t. route, and because the HPLC assay measures the mean concentration, underestimated values would be obtained. Results obtained here indicate that doses up to 100 μg, i.c.v. and 400 μg, i.t. are appropriate for examining the possible sites of action of CS-722.

CS-722 (50 and 100 μg, i.c.v.) reduced decerebrate rigidity, although the effect was short-lasting (Fig. 2a). The short-lived response can be explained by assuming vigorous vascular absorption through the venous plexus and the choroid plexus of the brain. Then, local CS-722 may be rapidly depleted.

The mean concentration of CS-722 in the spinal cord after 200 μg, i.t. of CS-722 was as much as that following 10 mg/kg, i.v., which only had a slight effect on the rigidity (Fig. 2b and Table 1). However, unexpectedly, 200 μg of CS-722 injected i.t. depressed decerebrate rigidity. Considering the measured mean concentration after i.t. to be a underestimated value, the local concentration of CS-722 must have been high enough to depress decerebrate rigidity. Long-lasting reduction of decerebrate rigidity was obtained after intrathecal administration of CS-722 (Fig. 2b). This suggests that the spinal cord is a site of the muscle relaxant action of CS-722. Delay in the vascular absorption could be responsible for the long-lasting action after intrathecal administration (14). Because the concentration of CS-722 in the brain stem after 100 μg, i.c.v. and that in the spinal cord after 400 μg, i.t. can be mimicked by 30 mg/kg, i.v., CS-722 injected systemically reduces muscle tonus by acting on both the supraspinal structure and the spinal cord.

From the previous observation on the PSR that CS-722 was less effective in spinal preparations than in intact ones (1, 3), the possible involvement of the supraspinal structure, in addition to the spinal cord, in the depressant activity of CS-722 on the spinal reflex has been suggested. As shown in Fig. 3, however, 100 μg of CS-722 injected i.c.v., which is enough to depress decerebrate rigidity, did not affect the spinal reflex. Higher sensitivity to systemic CS-722 of the spinal reflex in intact preparations rather than in spinal preparations, therefore, might be explained by an additional inhibitory effect in the spinal cord on the descending facilitatory effect which is tonically active in intact preparations. Failure of intraventricular CS-722 to reduce the spinal reflex in spite of apparent muscle relaxation (Figs. 2a and 3) points out clearly that the muscle relaxation can be achieved independently of impairment of the spinal reflex. The correlation between the muscle relaxation and reduction of the spinal reflex caused by spinal action of CS-722 is not completely clear, but inhibition of spinal interneurons, which was suggested by the decrease in the PSR, may contribute to decreased muscle tonus.

In summary, it has been shown that the spinal cord as well as the supraspinal structure are involved in the muscle relaxant activity of CS-722. Systemic CS-722 depresses the spinal reflex presumably by acting on the spinal cord, which partly explains its muscle relaxant action.

REFERENCES

1 Tanabe, M., Kaneko, T., Tonohiro, T. and Iwata, N.: The pharmacological properties of CS-722, a newly synthesized centrally acting muscle relaxant. Neuropharmacology 31, 1059–1066 (1992)
2 Kaneko, T., Tonohiro, T., Tanabe, M., Nagano, M., Sakai, J. and Iwata, N.: Separation of general depressant effects from antispastic effect of centrally acting muscle relaxants. Japan. J. Pharmacol. 55, Supp. I, 279P (1991)
3 Tanabe, M., Tonohiro, T., Kaneko, T., Iwata, N., Sakai, J. and Nagano, M.: Pharmacological study on (R)-4-chloro-2-(2-hydroxy-3-morpholinopropyl)-5-phenyl-4-isoxazolin-3-one hydrochloride (RS-722), a new centrally acting muscle relaxant. Japan. J. Pharmacol. 55, Supp. I, 279P (1991)
4 Tanabe, M., Kaneko, T., Tonohiro, T. and Iwata, N.: Mechanisms of spinal reflex depressant effects of CS-722, a newly synthesized centrally acting muscle relaxant, in spinal rats. Neuropharmacology 31, 649–954 (1992)

5 Hino, M., Ono, H. and Fukuda, H.: Supraspinal tonic influence on spinal reflexes and involvement in the effect of chlorpromazine. Gen. Pharmacol. 15, 155–158 (1984)

6 Ono, H., Fukuda, H. and Kudo, Y.: Mechanisms of depressant action of baclofen on the spinal reflex in the rat. Neuropharmacology 18, 647–653 (1979)

7 Ono, H., Nakamura, T., Ito, H., Oka, J. and Fukuda, H.: Rigidity in rats due to radio frequency decerebration and effects of chlorpromazine and mephenesin. Gen. Pharmacol. 18, 57–59 (1987)

8 Pellegrino, L.J., Pellegrino, A.S. and Cushman, A.J.: A Stereotaxic Atlas of the Rat Brain, 2nd edn., Plenum Press, New York (1979)

9 Satoh, M., Yasui, M., Fujibayashi, K. and Takagi, H.: Bestatin potentiates analgesic effect of intrathecally administered dynorphin in rats. IRCS Med. Sci. 11, 965–966 (1983)

10 Wallenstein, S., Zucker, C.L. and Fleiss, J.L.: Some statistical methods useful in circulation research. Circ. Res. 47, 1–9 (1980)

11 Kaneko, T., Ono, H. and Fukuda, H.: Simultaneous evaluation of drug effects on both the spinal cord and the descending pathways in rats. Arch. Int. Pharmacodyn. Ther. 287, 203–210 (1987)

12 Nakamura, T., Ono, H. and Fukuda, H.: Effects of alpha2-agonists and alpha,-antagonists on the radio frequency-lesioned intercollicular decerebrate rigidity. Japan. J. Pharmacol. 40, Supp. 62P (1986)

13 Nakamura, T., Ono, H., Fukuda, H., Tanabe, M., Kozuka, M. and Iwata, N.: Local cerebral glucose utilization in rats with decerebrate rigidity and effects of centrally acting muscle relaxants, diazepam and tizanidine. Biol. Pharm. Bull. 16, 33–35 (1993)

14 Giasi, R.M., D’Agostino, E. and Covino, B. G.: Absorption of lidocaine following subarachnoid and epidural administration. Anesth. Analg. 58, 360–363 (1979)