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Short communication

Preferential enhancement of inhibitory synaptic transmission by CS-722 in the ventral horn neurons of neonatal rat spinal cord

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Abstract

We studied the modulatory effect of (*R*)-4-chloro-2-(2-hydroxy-3-morpholinopropyl)-5-phenyl-4-isoxazolin-3-one hydrochloride (CS-722), a centrally acting muscle relaxant, on synaptic transmission in the ventral horn neurons of neonatal rat lumbar spinal cord in slices using whole cell recording techniques. Pharmacologically isolated excitatory or inhibitory postsynaptic currents (EPSCs and IPSCs) were evoked by stimulating neighboring neurons. CS-722 preferentially enhanced IPSCs with little effects on EPSCs. Moreover, CS-722 reduced paired pulse facilitation of γ-aminobutyric acid (GABA)-mediated IPSCs, suggesting its action on the presynaptic terminal. Preferential facilitatory effects on inhibitory synaptic transmission seem to be one of the mechanisms underlying muscle relaxation of this compound.

Keywords: EPSC (excitatory postsynaptic current); IPSC (inhibitory postsynaptic current); Paired pulse facilitation; Spinal cord; Muscle relaxant; (Slice)

1. Introduction

(*R*)-4-Chloro-2-(2-hydroxy-3-morpholinopropyl)-5-phenyl-4-isoxazolin-3-one hydrochloride (CS-722, Fig. 1A) is a newly synthesized centrally acting muscle relaxant, and has a muscle relaxant activity and depressant effects on the spinal reflex without interfering arousal response in electroencephalograph (Tanabe et al., 1992a,b). These properties of this compound would promise smooth rehabilitation to patients with spasticity following stroke, head injury and spinal cord injury, because excessive depression of the higher central nervous system by centrally acting muscle relaxants in use sometimes induces drowsiness and sedation which prevent rehabilitation.

We have already shown that depression of spinal interneuronal activities is thought to be one of the mechanisms underlying muscle relaxation of this compound (Tanabe et al., 1992b). In the present study, effects of CS-722 on pharmacologically isolated excitatory and inhibitory postsynaptic currents (EPSCs and IPSCs) were examined in ventral horn neurons of the spinal cord using acute slice preparations. Preferential enhancement of inhibitory synaptic transmission by CS-722 was observed, and its action on the presynaptic terminal was suggested.

2. Materials and methods

2.1. Preparations

Wistar rats, 4–10 days old, were decapitated with scissors under ether anesthesia and the lumbar spinal cord was quickly isolated. Slices 120–150 μm in thickness were prepared using a vibrating slicer as described previously (Edwards et al., 1989; Takahashi, 1990). After 1 h incubation at 37°C, a slice was transferred to the chamber mounted on a microscope stage and superfused with standard Krebs solution (flow rate, about 3 ml/min) having the following ionic composition (mM): NaCl, 113; KCl, 3; NaH_2PO_4, 1; $NaHCO_3$, 25; D-glucose, 11; $CaCl_2$, 2; $MgCl_2$, 1; pH 7.4 after bubbling with 95% O_2 and 5% CO_2.

2.2. Electrical recording

Whole-cell recordings were made from ventral horn neurons visually identified under Nomarski optics as described previously (Edwards et al., 1989; Takahashi, 1990).

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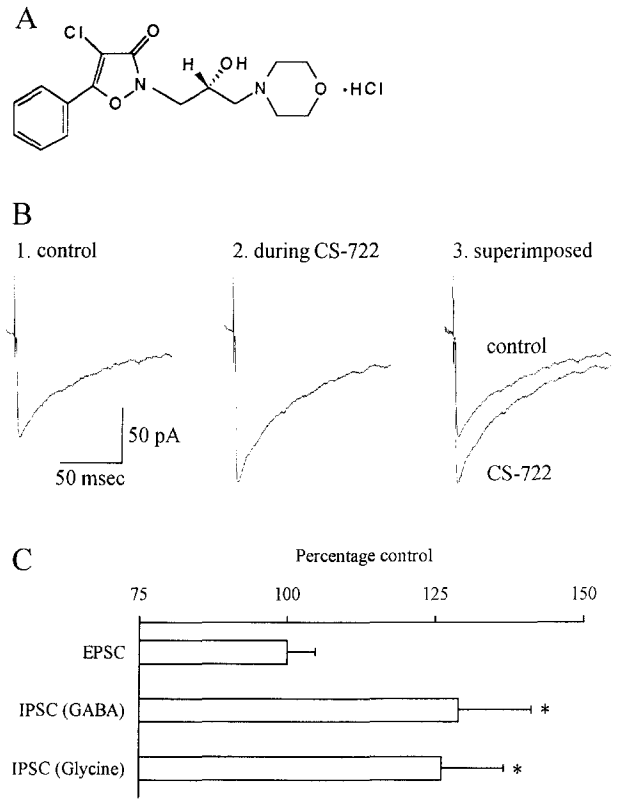

Fig. 1. CS-722 preferentially enhanced inhibitory synaptic transmission. *A*: the chemical structure of CS-722. *B*: enhancement of γ-aminobutyric acid (GABA)-mediated IPSCs by CS-722. *B*.1–3: sample records of GABAergic IPSCs (average of 25 consecutive responses). *B*.1: control response. *B*.2: response obtained during CS-722 (100 μM) application. *B*.3: superimposition of control IPSCs and enhanced IPSCs during CS-722. GABAergic IPSCs were evoked by stimulating neighboring neurons via a glass pipette filled with 1 M NaCl under CNQX (5 μM) and glycine (0.5 μM) at a holding potential of −70 mV. *C*: summary graph of modulatory effects of CS-722 on excitatory and inhibitory synaptic transmission. The control amplitude of evoked synaptic currents was normalized to 100% for each experiment ($n = 9–10$). Enhancement of GABAergic and glycinergic IPSCs was statistically significant (* $P < 0.05$).

The patch pipette resistance was 3–5 MΩ when filled with an internal solution containing (mM): CsCl, 140; NaCl, 9; $MgCl_2$, 1; EGTA, 0.2; Hepes, 10; pH neutralized to 7.4 with CsOH. The access resistance was less than 20 MΩ, continuously monitored, and occasionally compensated. Synaptic currents were evoked by stimulating neighboring neurons (within 100 μm from the patch pipette) via a glass pipette filled with 1 M NaCl. A voltage pulse of 200 μs in duration at 0.1–1 Hz with suprathreshold intensity was applied between the electrode and platinum wire in the bath. EPSCs were recorded in the presence of (−)-bicuculline methiodide (10 μM, referred to simply as bicuculline in the text) and strychnine hydrochloride (0.5 μM, referred to simply as strychnine in the text). γ-Aminobutyric acid (GABA)-mediated IPSCs (referred to simply as GABAergic IPSCs in the text) and glycinergic IPSCs were recorded in the presence of 6-cyano-7-nitroquinoxaline-2,3-dione (CNQX, 5 μM) and strychnine (0.5 μM), and CNQX (5 μM) and bicuculline (10 μM), respectively. When GABAergic IPSCs were recorded, 2 mM $ATPNa_2$ was added into the recording pipette. The records were stored on a PCM tape recorder (10 kHz), filtered at 4 kHz, and digitized at 2–4 kHz for computer analysis. All experiments were carried out at room temperature (21–24°C), and the membrane potential was held at −70 mV. The data were analyzed by Student's *t*-test. The results were taken as significant when P values were less than 0.05.

2.3. Drugs

The drug CS-722 was synthesized in this laboratory. CNQX was purchased from Tocris Neuramin (Bristol, UK); (−)-bicuculline methiodide and strychnine hydrochloride from Sigma (St. Louis, MO, USA).

3. Results

3.1. Effects on EPSCs and IPSCs

CS-722 (100 μM) showed differential effects at excitatory and inhibitory synapses (Fig. 1B and Fig. 1C). In two out of nine neurons, the amplitude of EPSCs was enhanced (31.1 and 14.6% increase), while the average increase in the amplitude of nine neurons was 0.0 ± 4.8% (mean ± S.E.M., Fig. 1C). It suggests that excitatory synaptic transmission is quite resistant to CS-722, although there exists a small population of excitatory synapses that is sensitive to the compound. In contrast, CS-722 enhanced the amplitude of GABAergic IPSCs in six out of nine neurons (20–111.5% increase), and the average increase of nine neurons was 28.9 ± 12.3% ($P < 0.05$, Fig. 1C). Fig. 1B illustrates the typical effect of CS-722 on GABAergic IPSCs. Glycinergic IPSCs were also facilitated in six out of ten neurons (28.9–78.8% increase), and the average increase of ten neurons was 26.1 ± 10.5% ($P < 0.05$, Fig. 1C).

3.2. Effects on paired pulse facilitation

In the ventral horn of neonatal rat spinal cord, GABAergic synapses show frequency-dependent facilitation, that is sensitive to presynaptic manipulations that change the probability of release (Tanabe and Kaneko, 1996). As shown in Fig. 2A, two consecutive stimuli of identical strength with 20 ms interval resulted in enhancement of the second IPSC. With increase in the amplitude of the first IPSC by CS-722 (100 μM), facilitation was decreased (Fig. 2A.2). Fig. 2B shows the averaged effects of CS-722 on the first and second IPSC. CS-722 enhanced the first IPSC by 30.4 ± 8.3% ($n = 5$), but its effect on the second IPSC was rather inhibitory (1.6 ± 2.1% decrease). Preferential increase in the first response ($P < 0.05$) sug-

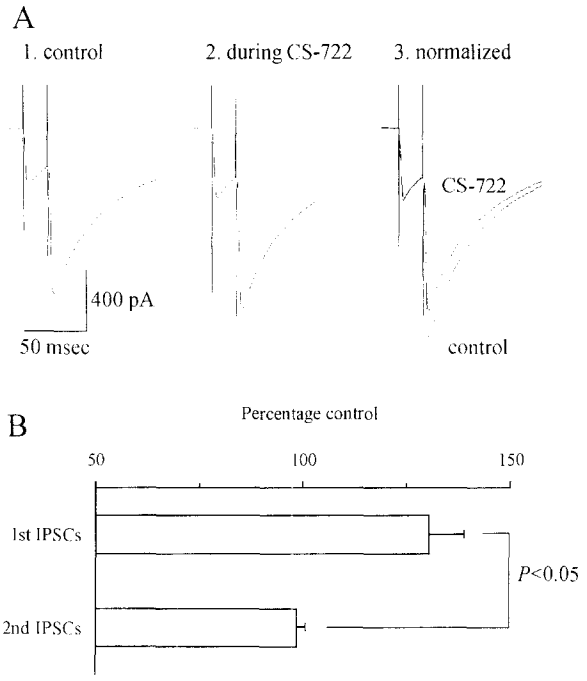

Fig. 2. CS-722 decreased paired pulse facilitation with increase in the mean amplitude of GABAergic IPSCs. *A*: sample records of IPSCs (average of nine consecutive responses) evoked by paired stimuli (20 ms interval) delivered at a frequency of 0.1 Hz. *A*.1: control response. *A*.2: response obtained during CS-722 (100 μM) application. *A*.3: the control first IPSC was scaled to the first IPSC during CS-722 application. *B*: summary graph of differential effects of CS-722 on IPSCs evoked by paired stimuli. Preferential enhancement of the first IPSC means decrease in paired pulse facilitation ($n = 5$, $P < 0.05$).

gests that CS-722 acts on the presynaptic terminal to change the probability of release in the ventral horn of the spinal cord.

4. Discussion

Whole-cell recordings were made from ventral horn neurons of neonatal rat lumbar spinal cord in slice, and effects of CS-722 on pharmacologically identified synaptic currents were studied. The present study revealed that CS-722 preferentially enhanced inhibitory synaptic transmission rather than excitatory transmission. Although enhancement of EPSCs was also observed in a small subset of the neurons, the overall effects of CS-722 on motor output are inhibitory and lead to muscle relaxation.

When two consecutive stimuli of identical strength with close intervals are applied to a synapse, the second synaptic response is enhanced because of an increase in transmitter release at the second stimulation, referred to as paired pulse facilitation (McNaughton, 1982). Paired pulse facilitation reflects the increased probability of release following the first stimulation and thereby is considered to be presynaptic in origin. GABAergic inhibitory synapses in the ventral horn of neonatal rat spinal cord have been found to show paired pulse facilitation that is sensitive to presynaptic manipulations (Tanabe and Kaneko, 1996). Decrease in paired pulse facilitation by CS-722, that was evident from preferential enhancement in the amplitude of the first IPSC, suggests that CS-722 acts on the presynaptic terminal to increase the probability of GABA release. Possibly, presynaptic action of CS-722 is a common mechanism underlying enhancement of EPSCs and especially glycinergic IPSCs, although postsynaptic modulatory effects remain to be studied. IPSCs recorded from neonatal rat spinal motoneurons are reported to have two components, strychnine-sensitive and bicuculline-sensitive, and occasionally these two components are observed in IPSCs evoked by a single neuron (Takahashi, 1992). This means that there is a population of the neurons that releases both glycine and GABA from the terminal. Immunocytochemical studies also show the colocalization of GABA and glycine in the nerve terminal in the spinal ventral horn (Shupliakov et al., 1993; Taal and Holstege, 1994). Such neurons could be stimulated in this study. Thus, CS-722 could increase the release probability of GABA and glycine which are colocalized at the same nerve terminal and thereby enhance GABAergic and glycinergic IPSCs.

In summary, CS-722 preferentially potentiated inhibitory synaptic transmission in the ventral horn of neonatal rat spinal cord, by an action on the presynaptic terminal that increased the probability of transmitter release. It seems that this preferential enhancement of the inhibitory transmission effectively reduces excessive motor output, and is one of the mechanisms underlying muscle relaxation of CS-722.

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