SUPPRESSIVE EFFECT OF CIRAMADOL ON THE BRADYKININ-INDUCED ACTIVITIES OF THE LAMINA V-TYPE CELLS OF THE RAT SPINAL DORSAL HORN

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The effects of a new analgesic drug, ciramadol, on single neuronal activities of lamina V-type cells of the spinal dorsal horn were investigated in spinal rats when applied microelectrophoretically to the cells or administered systemically. In the majority of lamina V-type cells examined, ciramadol inhibited the activities induced by intra-arterial injection of bradykinin (painful stimulation) but not those induced by mechanical noxious and innocuous stimuli. These results directly evidence that ciramadol acts on the lamina V-type cells of the spinal dorsal horn to block the bradykinin-induced (nociceptive) activities there.

Keywords — ciramadol; bradykinin-induced; unit activity; lamina V-type cell; spinal dorsal horn; microelectrophoretic application

INTRODUCTION

Ciramadol, a benzylamine derivative (1-*cis*-2-(dimethyl-amino-3'-hydroxy-phenylmethyl)-cyclohexanol), has an analgesic efficacy in humans.¹⁻⁴⁾ In our previous study,⁵⁾ an analgesic effect of ciramadol was evaluated by the bradykinin-induced flexor reflex test (rat),⁶⁾ but not by the tail-pinch test (mouse and rat) and hot-plate test (mouse) without marked motor impairment. The analgesic effect was considered to be mediated by specific opioid receptors as the effect was antagonized by naloxone in a small dose of 0.1 mg/kg, *i.v.* Furthermore, the main site of analgesic action of ciramadol was suggested to exist at the spinal cord.

In the bradykinin-induced flexor reflex test, the analgesic effects are assessed on the basis of changes in motor response and the effects on motor performance are usually taken into consideration. Such a practice can be excluded by using changes in neuronal activities of the afferent system involved in the sensation of pain as an index for evaluation of drug action. In the present experiments, the effects of ciramadol were investigated on the bradykinin-induced

nociceptive responses of the lamina V-type neurons of the rat spinal dorsal horn which are known to be involved in transmission of pain sensation. Particularly, a microelectrophoresis method was used to estimate direct action of ciramadol on the lamina V-type neurons.

MATERIALS AND METHODS

Male Sprague-Dawley rats (250–400 g) were employed. The animal was anesthetized with pentobarbital sodium (40 mg/kg, *i.p.*). A cannula made from polyethylene tubing of about 0.4 mm in diameter was retrogradely introduced in the right superficial femoral artery and fixed in place, so that the blood flow in the right femoral artery was not disturbed. The rat was placed on a stereotaxic apparatus. The vertebral column was immobilized with metal clamps. The spinal cord was exposed by laminectomy at the levels of T12–L2 for inserting electrodes and at the level of T8 where the spinal cord was transected. After removal of the dura mater over the T12–L2, the exposed spinal cord was covered with warm (36–38°C) mineral oil. The animal was immobilized with galla-

mine triethiodide and artificially respired. All the wound edges of skin and muscle were treated with lidocaine jelly (2%) throughout the experiments.

Single neuronal activities of the lamina V-type cells were extracellularly recorded from the right dorsal horn of the L1 segment using a tungsten microelectrode (5–8 μm in diameter) by means of conventional methods. For microelectrophoresis, a seven-barrelled multipipette (the tip diameter of 6–10 μm) was attached to a bent tungsten microelectrode for recording side by side with an epoxy glue in such a way that the latter protruded beyond the former by 10–30 μm . Solutions for phoresis were: ciramadol HCl (50 mM), naloxone HCl (a gift from Endo Labs., Garden City, U.S.A.: 50mM), mono-Na-L-glutamate (1 M) and NaCl (3 M). The pHs of these solutions were about 5. Glutamate was passed as an anion, while two other drugs were passed as cations. The NaCl-barrel was used for automatic balancing of current at the tip of the pipette. Lamina V-type cells were distinguished according to the recording site (500–900 μm , 744 ± 35 μm ($n=15$), from the surface of the spinal cord) and just above lamina VI area in which many neurons responded to the proprioceptive stimulation like joint movement and a physiological property showing the maximal response to noxious stimuli. In addition to lamina V-type cells, several lamina IV-type cells which responded only to innocuous stimuli of the receptive field but not to any of noxious stimuli used were encountered. We selected only units which were excited by glutamate phoretically applied (less than 70 nA).

As painful (noxious) stimuli, bradykinin (Protein-Research-Foundation, Mino, Japan: 0.5–2 μg in 0.1 ml physiological saline) was injected into the right femoral artery through the implanted cannula at intervals of 10 min. The difference between the number of spikes during 60 s period after and before each bradykinin injection was regarded as bradykinin-induced activity. In several cases mechanical noxious stimulation was given to the receptive field by means

of a clip (1 kg pressure) for 30 s. Tactile stimulation of the center of receptive field for 15 s was used as innocuous stimuli.

In some experiments ciramadol was intravenously injected into spinal rats in order to confirm a spinal action of the drug.

RESULTS

Results were obtained from 30 lamina V-type and 7 lamina IV-type neurons.

Ciramadol microelectrophoretically applied (138 ± 14 nA, 3.54 ± 0.37 min: $n=13$) inhibited the bradykinin-induced activity to 10–70% of each control (pre-drug) value in 11 out of 13 lamina V-type cells tested, but increased that to 150% in one and had no influence on another cell. An influence of naloxone on the inhibitory effects of ciramadol was examined in 2 neurons. When naloxone was phoretically applied (100 nA) for 8 min from 2 min before ciramadol application, the effect of ciramadol was reversed in the both cases (Fig. 1).

Microelectrophoretic application of ciramadol hardly influenced the non-nociceptive response to tactile stimulation in the same two neurons as those in which the inhibition of the bradykinin-induced response by the drug was simultaneously observed (Fig. 2). Furthermore, in 5 out of 7 lamina IV-type cells tested phoresis of ciramadol (117 ± 34 nA, 3.50 ± 0.68 min: $n=7$) did not affect the response to innocuous stimuli but depressed that in the other 2 cells.

The nociceptive responses induced by mechanical stimulation of the receptive field were not depressed by ciramadol phoretically applied (126 ± 15 nA, 3.22 ± 0.5 min: $n=9$) in 5 out of 9 lamina V-type neurons tested but rather augmented in 1 out of the 5 neurons. In the other 4 neurons, however, those responses were inhibited.

Spontaneous firing rates were not influenced by ciramadol (133 ± 10.5 nA, 3.4 ± 0.3 min: $n=22$) in 17 out of 22 lamina V-type cells tested but declined to 45–65% of control values in the other 5 cells.

Intravenous administration of 4 mg/kg of

FIG. 1. *Inhibitory Effect of Phoretically Applied Ciramadol (200 nA, 6 min) on the Bradykinin-Induced Response and Antagonism by Naloxone (100 nA, 8 min) in a Lamina V-type Cell of the Rat Spinal Dorsal Horn*

Bradykinin (2 µg) was intra-arterially injected every 10 min.

FIG. 2. *Selective Inhibition of the Bradykinin-Induced Response by Ciramadol Phoretically Applied (200 nA, 8min) in a Lamina V-type Cell of the Rat Spinal Dorsal Horn*

The response to tactile stimulation was not markedly influenced by the drug. Intra-arterial injection of bradykinin (0.5 µg) and tactile stimulation were given with an interval of 2 min every 10 min.

FIG. 3. *Inhibitory Effect of Systemically Administered Ciramadol (4 mg/kg, i.v.) on the Bradykinin-Induced Response in a Lamina V-type Cell of the Spinal Dorsal Horn of a Spinal Rat*
Bradykinin (1 μ g) was intra-arterially injected every 10 min.

ciramadol to spinal rats inhibited the bradykinin-induced responses to 40–65% of each control value in 4 out of 8 lamina V-type cells but not in the other 4 cells. In two out of the latter's additional administration of 4 mg/kg of

the drug (8 mg/kg in total) depressed the bradykinin-induced responses. These inhibitory effects appeared 5–15 min after ciramadol administration and lasted for 50–90 min (Fig. 3). Spontaneous activities were decreased by sys-

temic ciramadol in most of the neurons in which the bradykinin-induced responses were depressed by the drug.

DISCUSSION

In the present experiments Sprague-Dawley rats and intra-arterial injection of bradykinin were employed as the experimental animal and painful stimulation. Such experimental conditions were the same as those used in the previous study in which the analgesic effect of ciramadol was examined by the bradykinin-induced flexor reflex test.⁵⁾ The present study provided direct evidence that ciramadol blocks the bradykinin-induced (nociceptive) activities of the lamina V-type cells through a local action of the drug on the cells, as ciramadol microelectrophoretically applied to the lamina V-type neurons and systemically administered in spinal rats inhibited those activities. The blocking action of the drug at the spinal dorsal horn was mediated by specific opioid receptors as naloxone antagonized the action. In preliminary experiments on binding ability of ciramadol to mu, delta and kappa types of opioid receptors, ciramadol was found to inhibit the binding of mu and kappa ligands with relatively low doses (unpublished data). Therefore, the naloxone-antagonizable inhibition by ciramadol on the spinal dorsal horn may be mediated by mu and/or kappa types of opioid receptors. These findings suggest that the suppressive effect of ciramadol injected systemically or into the lumbar subarachnoid space on the bradykinin-induced flexor reflex in the previous experiments⁵⁾ was mainly attributed to the depression of nociceptive transmission at the lamina V-type cells of the spinal dorsal horn.

Ciramadol selectively inhibited the bradykinin-induced response of the lamina V-type neurons without influence on non-nociceptive response of these neurons to innocuous stimuli. Moreover, the drug did not influence the response to innocuous stimuli in most of lamina IV-type cells tested. This selective effect of ciramadol on nociceptive response is similar to that of morphine.⁷⁻¹⁰⁾ Such a selectivity is one

of the beneficial properties which specific analgesic drugs must have.

On the other hand, ciramadol did not depress the nociceptive response to mechanical noxious stimuli in the majority of lamina V-type cells tested. These results are not incompatible with the fact that the analgesic effect of ciramadol could not be evaluated by the tail-pinch test.⁵⁾ Morphine, however, suppresses nociceptive responses not only to intra-arterial injection of bradykinin¹⁰⁾ but also to mechanical noxious stimuli,^{11,12)} corresponding to the fact that morphine is effective in every analgesic test using animals. In this connection, Tyers¹³⁾ has proposed that relative involvement of subtypes of opioid receptors (mu and kappa) in opiate induced analgesia varies according to the type of noxious stimuli employed. It is of interest to know the reason why the profiles of analgesic effects of ciramadol and morphine are different. Such investigations are in progress in our laboratory.

REFERENCES

- 1) A. D. Cochrane, R. Bell, J. R. Sullivan and J. Shaw: Ciramadol. A new analgesic, *Med. J. Aust.*, **2**, 501–502 (1979).
- 2) M. J. Staquet: Analgesic effect of ciramadol in patients with chronic pain, *Curr. Med. Res. Opin.*, **6**, 475–477 (1980).
- 3) F. Camu: Double-blind comparison of the analgesic response to oral ciramadol (WY-15,705) and pentazocine in post-operative pain, *Eur. J. Clin. Pharmacol.*, **19**, 259–262 (1981).
- 4) R. J. Fragen and N. J. Caldwell: Comparison of three doses of oral ciramadol and placebo for the treatment of moderate to severe postoperative pain, *J. Clin. Pharmacol.*, **22**, 459–465 (1982).
- 5) A. Kubota, M. Satoh and H. Takagi: Sites of analgesic action of ciramadol, *Jpn. J. Pharmacol.*, **33**, 1085–1088 (1983).
- 6) M. Satoh, S. Kawajiri, K. Shishido, M. Yamamoto and H. Takagi: Bradykinin-induced flexor reflex of rat hind-limb for evaluating various analgesic drugs, *J. Pharm. Pharmacol.*, **31**, 184–186 (1979).
- 7) O. Calvillo, J. L. Henry and R. S. Neuman: Effects of morphine and naloxone on dorsal horn neurones in the cat, *Can. J. Physiol. Pharmacol.*, **52**, 1207–1211 (1974).
- 8) A. W. Duggan, J. G. Hall and P. M. Headley: Suppres-

- sion of transmission of nociceptive impulses by morphine: Selective effects of morphine administered in the region of the substantia gelatinosa, *Br. J. Pharmacol.*, **61**, 65–76 (1977).
- 9) J. Davies and A. Dray, Pharmacological and electrophysiological studies of morphine and enkephalin on rat supraspinal neurones and cat spinal neurones, *Br. J. Pharmacol.*, **63**, 87–96 (1978).
- 10) M. Satoh, S. Kawajiri, M. Yamamoto and H. Takagi: Selective action of enkephalins on lamina V neurons of the spinal dorsal horn: A microelectrophoretic study, "Integrative Control Functions of the Brain," Vol.1, ed. by M. Ito, Kodansha/Elsevier, Tokyo/Amsterdam, 1978, pp.114–116.
- 11) G. Belcher and R. W. Ryall: Differential excitatory and inhibitory effects of opiates on non-nociceptive and nociceptive neurones in the spinal cord of the cat, *Brain Res.*, **145**, 303–314 (1978).
- 12) K. Hanaoka, M. Ohtani, H. Toyooka, S. Dohi, K. Ghazi-Saidi, A. Taub and L.M. Kitahata: The relative contribution of direct and supraspinal descending effects upon spinal mechanisms of morphine analgesia, *J. Pharmacol. Exp. Ther.*, **207**, 476–484 (1978).
- 13) M. B. Tyers: A classification of opiate receptors that mediate antinociception in animals, *Br. J. Pharmacol.*, **69**, 503–512 (1980).