ABILITY OF THE OPIATE κ-AGONIST BREMAZOCINE TO FACILITATE THE FLEXOR REFLEX OF THE RIGHT HIND LIMB IN SPINAL RATS

V. L. Tsibul'skii, A. G. Kobylyanskii, and G. Ya. Bakalkin

UDC 616.832-089.85-092.9-07:616.8-009.818-02:615.212.7:547.943

KEY WORDS: asymmetry; flexor reflex; electromyogram; bremazocine.

Opiates and opioid peptides modify motor reflexes in intact and spinal animals, by interacting with spinal motoneurons and interneurons [6, 8, 10]. These data indicate the existence of additional functions of opioid peptides, unconnected with pain perception, in the spinal cord, and most probably involved in the regulation of spinal reflexes. It was shown previously that opioids can induced postural asymmetry of the hind limbs in anesthesized rats with a divided spinal cord [3, 7]. These data were obtained by visual recording of asymmetry, which was carried out after abduction of the hind limbs. It remained unclear whether postural asymmetry develops as a result of flexion of one limb or extension of the other.

The aim of this investigation was to answer this question by recording electromyographic activity of symmetrical flexors and extensors of the thigh of rats in which asymmetry was induced by the opiate κ-agonist bremazocine.

EXPERIMENTAL METHOD

The spinal cord of male Wistar rats weighing 140-180 g was exposed under ether anesthesia and physiological saline (10 μl per animal per minute) or bremazocine (100 ng per animal) was injected into the subarachnoid space below the level of division of the spinal cord at T3-T4. One hour after the injection and division of the spinal cord, under deep ether anesthesia (absence of the corneal reflex) both hind limbs of the rat, lying freely on the table, were extended and lowered and the difference (in mm) between the projections of the left and right lower limbs on the animal's axis of symmetry was measured. This procedure was repeated four or five times in succession and the mean value of asymmetry was calculated. Asymmetry of 3 mm or more was considered to be significant. The electromyogram (EMG) of symmetrical groups of flexors and extensors of the thigh of both hind limbs was recorded simultaneously in four channels, by means of bipolar electrodes consisting of injection needles, 0.4 mm in diameter, inserted with an interelectrode distance of 3 mm, on the EEG-4214 encephalograph (Nihon Kohden, Japan). The signal from the output of the encephalograph led to the electromyograph (filter 100-1000 Hz) and from it to an integrator (S75-76 and S76-22, Colborn Instruments). Recording of the EMG began during measurement of postural asymmetry under anesthesia, after which the ether mask was removed and recording continued for 10-20 min after restoration of the corneal reflex (Table 1, "After anesthesia"). Spontaneous and evoked electromyographic activity was recorded. In the latter case, an identical painful stimulus was applied with steel needles at symmetrical points of the limbs, trunk, and tail, or dish electrodes were applied to the animal's limbs and square pulses with a duration of 1 msec and a frequency of 100 Hz were passed through them. The strength of the current varied from 2 to 5 mA and the duration of stimulation from 0.1 to 3 sec. It was considered that asymmetry takes place if the electromyographic activity of symmetrical muscles differed by not less than 1.5 times (in most cases, during the development of asymmetry it differed by 2-3 times or more). Statistical analysis was carried out, by means of Fisher's and the chi-square tests.

EXPERIMENTAL RESULTS

In the control group none of the rats developed postural asymmetry in excess of 2 mm (Table 1). After injection of bremazocine 80% of the rats developed postural asymmetry of

Central Research Laboratory, Ministry of Health of the USSR, Moscow. (Presented by Academician of the Academy of Medical Sciences of the USSR V. N. Smirnov.) Translated from Byulleten' Éksperimental'noi Biologii i Meditsiny, Vol. 105, No. 1, pp. 6-9, January, 1988. Original article submitted June 19, 1987.

 0007-4888/88/0001-0006$12.50

TABLE 1. Changes in EMG Activity of Flexors and Extensors of the Thigh in Spinal Rats under the Influence of the Opiate κ-Agonist Bremazocine

Substance	No. of rats	Animals under anesthesia			Animals 10-20 min after anesthesia	
		postural asymmetry	spontaneous activity of extensors	evoked activity of flexors	spontaneous activity of extensors	evoked activity of flexors
Physiological saline	10	0 (10) 0	0 (9 + 0) 1	1 (8 + 1) 0	3 (3 + 1) 3	1 (6 + 2) 1
Bremazocine	39	3 (8) 28	6 (29 + 0) 4	3 (17 + 3) 16	11 (15 + 3) 10	4 (15 + 2) 18

Legend. Number of rats in which postural asymmetry or muscular activity was not observed + number of rats with EMG of symmetrical amplitude shown in parentheses. On left of parentheses – number of rats with a shorter projection of the left limb or with predominance of EMG activity of the left limb; on right of parentheses – number of rats with a shorter projection of the right limb or predominance of EMG activity of the right limb.

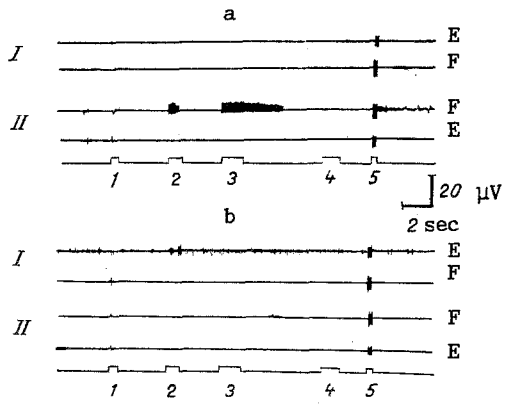

Fig. 1. EMG of left (I) and right (II) hind limbs of a rat. a) Rat under ether anesthesia 1 h after injection of 100 ng of bremazocine: spontaneous activity absent, responses to pricks applied symmetrically to digit (1), sole of foot (2), and leg (3) of right and left hind limbs can be seen, pricks of the tail (4) ineffective, electrodermal stimulation of the foot (5) evokes a response; b) control rat after anesthesia: spontaneous activity of left extensor can be seen, no responses to nociceptive stimulation. E) Extensor, F) flexor.

the hind limbs amounting to between 3 and 9 mm ($p < 0.001$). Under these circumstances, in 90% of cases the projection of the right limb on the axis of symmetry was less than the projection of the left, and it differed significantly from the random distribution: in 50% of cases it was less on the left and in 50% of cases less on the right ($p < 0.001$; Table 1).

Recording the EMG in these same rats showed that under deep ether anesthesia spontaneous activity of the flexors in the control group was completely absent, and bursts of spontaneous extensor activity were observed in only one of the 10 rats. In rats of the experimental group spontaneous activity was found more frequently, but not significantly so, than in the control: spontaneous extensor activity was recorded in 27% of rats and flexor activity (right) in 8%.

The extensors did not respond to nociceptive stimulation. Conversely, flexors of anesthesized animals responded with bursts of EMG activity to a painful stimulus applied at different points of the same limb, but not of the opposite limb. The percentage of rats with response of the thigh flexors in the experimental group was significantly greater (by 2.8 times; $p < 0.05$) than in the control. In the case of pricks, the more proximally the prick was applied, and the greater the force used, the greater the amplitude and duration of the

bursts of EMG activity (Fig. 1). Responses of the flexors to electrodermal stimulation obeyed the same rules: the lowest threshold (2.5-3.0 mA, duration of stimulation from 0.5 to 1 sec) was found when the stimulus was applied in the region of the ankle; with an increase in strength of the current and in the duration of stimulation the response of the flexors increased in amplitude and duration. Comparison of the EMG responses on the right and left in rats of the experimental group showed that the number of animals with a response of the right flexors was 5.3 times greater than the number of animals with a response of the left, a result which differs significantly ($p < 0.03$) from the random distribution of 50% right and 50% left. In about 90% of rats of the experimental and control groups no response of the left flexor was recorded, despite an increase in strength of the current to 5 mA and in the duration of stimulation to 3 sec.

To answer the question whether postural asymmetry develops as a result of flexion of one limb and/or extension of the other, we compared the results obtained by measurement of asymmetry in the same animals visually and by means of the EMG. This comparison could not be made actually during measurement of postural asymmetry: no changes whatever could be found in muscular activity during and after extension of the hind limbs. Activity was evidently low in amplitude and short in duration, but was sufficient to lead to postural asymmetry with spontaneous extensor and flexor activity and their activity evoked by nociceptive stimulation showed that the side of the "flexed" limb during visual assessment of asymmetry (or more precisely, the limb for which the projection along the animal's axis of symmetry was smaller) coincided with the side of greater evoked activity of the flexors in 71% of cases (i.e., the probability of coincidence was 0.71) and this coincidence was significantly ($p < 0.005$, chi-square test) greater than the probability of random coincidence, which was 0.41. The probabilities of random and experimentally determined coincidence of the data did not differ in the case of spontaneous and evoked extensor activity and spontaneous flexor activity. Consequently, postural asymmetry may develop as a result of the response of the flexors of one hind limb (in the case of bremazocine – the right) to tactile and nociceptive stimulation (flexor reflex) and to stretching of the flexors (myotatic reflex) during extension of the hind limbs. The patterns and relationships revealed under anesthesia also continued to be observed after anesthesia (Table 1). Incidentally, spontaneous activity of extensors could disappear and reappear, and it was observed more often on one particular side (Fig. 1). In cases when the extensors of both limbs exhibited equally high activity, it was never observed simultaneously, but alternated between right and left.

The phenomenon of asymmetry after spinalization of animals was familiar in the past to Sherrington: "Such inequality or dissimilarity of the spinal reflexes right and left does not necessarily afford any evidence that the spinal lesion is asymmetrical. Intercurrent circumstances suffice to impress slightly different reflex habits on the two limbs, ... " [5]. If bremazocine is regarded as one such "intercurrent circumstance," the most interesting feature is that it nearly always facilitates the flexor reflex of the right limbs. The effects of bremazocine cannot be due to selective inhibition of the flexor reflex of the left limb, for in the control group, just as in the experimental group, responses of the flexors of the left limb were infrequent. This inhibition of the flexor reflex in animals of the control group was probably connected with release of endogenous opioids after spinalization of the animals. The opiate antagonist naloxone is known to facilitate reflexes in spinal animals and to prevent their inhibition by morphine [6, 8]. Specific opioid agonists, including those of κ-type, when injected intrathecally in doses of 10-100 μg per rat, inhibit reflex responses to nociceptive stimulation [10]. In the present experiments bremazocine in a dose of 100 ng per animal (i.e., 100-1000 times smaller than that used in [10]) facilitates the flexor reflex, but only of the right limb. This can be understood on the basis of the mechanism of autoregulation of the opioid system which has been postulated in the spinal cord (specific in relation to the type of opioid receptors on the left and right), by analogy with the known mechanism of regulation of activity of the nigrostriatal dopaminergic system through presynaptic autoreceptors. In fact, opioid receptors have been found on the presynapses of many neurons, including κ-receptors on afferents of locus ceruleus neurons [9].

To conclude, it can be stated that asymmetry of spontaneous EMG activity of flexors and extensors in rats, and evoked EMG activity in cats also, in spinal animals has been found after injection of extracts of halves of the brain and extract of the brain of a rat with a unilateral lesion, respectively [1, 4]. During continuous stretching of the limbs, moreover, asymmetry of EMG activity of flexors was found in spinal rats after unilateral injec-

tion of colchicine into the motor cortex before spinalization [2]. A detailed comparison of the effects of bremazocine and of the hypothetical endogenous postural asymmetry factors [1, 4] is not yet possible because of the significant differences between the methods and models used.

LITERATURE CITED

1. T. V. Avaliani, N. M. Yakovlev, A. A. Smetankin, and O. V. Bogdanov, Fiziol. Zh. SSSR, No. 4, 472 (1985).
2. E. I. Varlinskaya, G. P. Obukhova, and G. A. Vartanyan, Byull. Éksp. Biol. Med., No. 3, 259 (1984).
3. A. G. Kobylyanskii, Neuropeptides: Their Role in Physiology and Pathology [in Russian], Tomsk (1985), p. 83.
4. G. N. Kryzhanovskii, V. K. Lutsenko, and M. Yu. Karganov, Byull. Éksp. Biol. Med., No. 1, 14 (1982).
5. C. Sherrington, The Integrative Action of the Nervous System, New Edition, Cambridge (1947), p. 250.
6. D. M. Catley, R. W. Clarke, and J. E. Pascoe, J. Physiol. (London), 339, 61 (1983).
7. E. I. Chazov, G. Ya. Bakalkin, K. N. Yarygin, et al., Experientia, 37, 887 (1981).
8. A. W. Duggan and R. A. North, Pharmacol. Rev., 35, 219 (1984).
9. L. McFadzean, G. Henderson and R. G. Hill, Neuropeptides, 5, 233 (1984).
10. C. Schmauss and T. L. Yaksh, J. Pharmacol. Exp. Ther., 228, 1 (1984).