EFFECT OF CLOFELIN ON NEURONAL SPIKE ACTIVITY IN THE MIDBRAIN AND POSTERIOR HORN OF THE SPINAL CORD

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UDC 612.822.014.46:[615.225.
2.017:615.212].085.1

KEY WORDS: pain; midbrain; spinal cord; clofelin

The pain relieving action of clofelin and of other central adrenopositive drugs has been clearly demonstrated in recent years, and on that basis the concept of the adrenergic regulation of pain has been formulated [3-5,7,12,14]. Meanwhile some of its most important postulates require further detailed examination. Whereas the neurochemical and receptor aspects of adrenergic analgesia can be considered to be sufficiently well elaborated, the neurophysiological mechanisms of the analgesic effect of clofelin-like compounds have received much less study. Analysis of the action of adrenopositive drugs on the homeostatic regulation of nociception, and the discovery of the neuronal mechanisms of their effect and of their specificity in relation to influences of a nociceptive nature are particularly important.

The effect of clofelin was accordingly studied on nociceptive responses of neurons of the midbrain — a structural component of the endogenous painrelieving systems of the body, and of neurons of the spinal cord — the afferent input system controlling the spread of impulsation to effector cells and to structures at a higher level of the CNS.

EXPERIMENTAL METHOD

Experiments were carried out on 13 unanesthatized curarized cats, which were artificially ventilated and heated. The preparatory operation was performed under ether anesthesia. Neuronal spike activity in the posterior horn of the spinal cord at the mid-lumbar level and of neurons of the central gray matter of the midbrain was derived extracellularly by glass microelectrodes filled with 3.5 KCl and with a resistance of 3-5 MΩ. The electrodes were inserted into the brain by means of an MSh-80 step motor stereotaxically, using atlases of the cat spinal cord and brain. The EPM computer system (Research Institute of Experimental Medicine, Academy of Medical Sciences of the USSR) was used to amplify bioelectrical activity, and for visual monitoring and photographic recording. The skin of the hind limb was crushed ipsilaterally to the side of recording by means of dental forceps, and the pulp of the upper canine or molar tooth was stimulated by square pulses, 0.5 msec in duration, through implanted electrodes [2]. The intensity of mechanical and electrical stimulation was 20% higher than the threshold for appearance of emotional-affective manifestations of pain in the conscious animals.

The drugs were injected intravenously in the following doses: clofelin (clonidine, from "Boehringer," West Germany) 0.1-0.5 mg/kg; naloxone (Narcan, from "Endo," USA) 0.1 and 1 mg/kg; yohimbine ("Regis," USA) 5 mg/kg, and prazosin (Pratsiol, from "Orion," Finland) 1 mg/kg.

EXPERIMENTAL RESULTS

Neurons in the posterior horn of the spinal cord had a mixed type of spontaneous activity with a frequency of 8.4-14.6 spikes/sec. Tactile stimulation of the skin of the hind limb in the region of their receptive field increased the frequency of the spike discharge the 23.0-25.5 spikes/sec, whereas nociceptive crushing of the skin increased it to 27.0-62.5 spikes/sec.

Clofelin led to dose-dependent inhibition of the spontaneous activity of these neurons after 5 min. At the peak of action of clofelin in a dose of 0.5 mg/kg, 30 min after its injection, single irregular discharges with a frequency of

Department of Pharmacology, I. P. Pavlov First Leningrad Medical Institute. Laboratory of Physiology of Pain, I.S. Beritashvili Institute of Physiology, Academy of Sciences of the Georgian SSR, Tbilisi. (Presented by Academician of the Academy of Medical Sciences of the USSR A. V. Val'dman.) Translated from Byulleten' Éksperimental'noi Biologii i Meditsiny, Vol. 108, No. 8, pp. 203-205, August, 1989. Original article submitted July 6, 1988.

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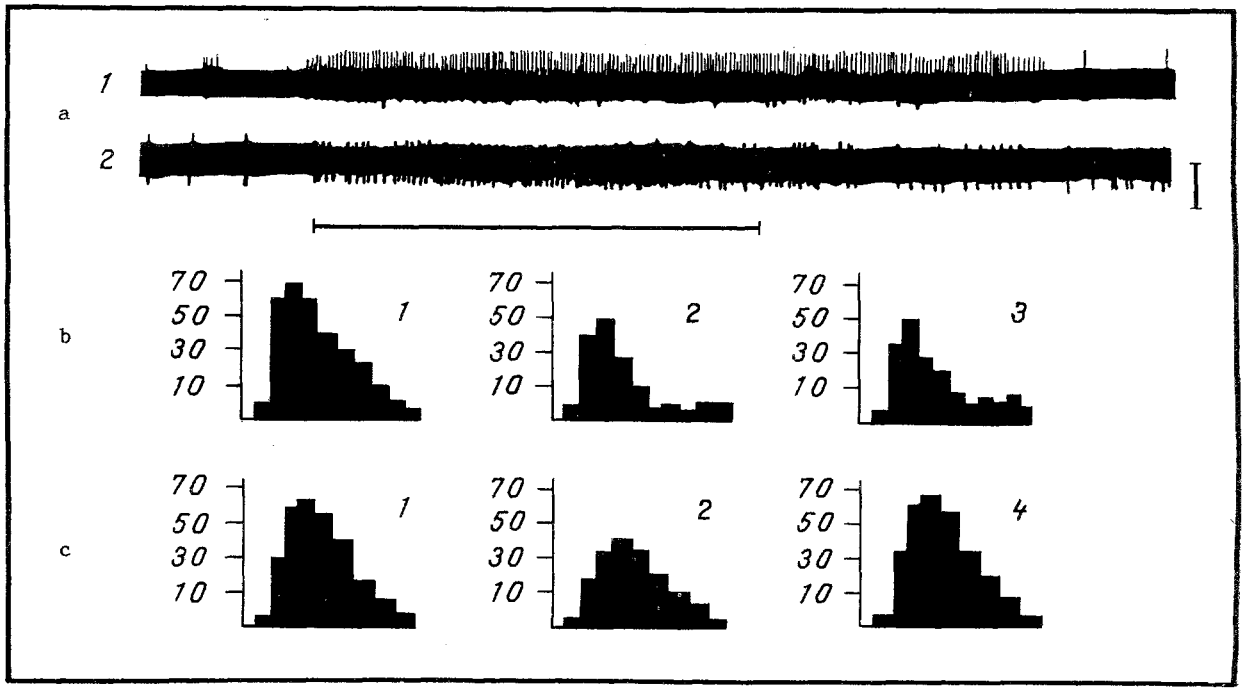

Fig. 1. Effect of clofelin on neuronal spike activity in posterior horn of spinal cord during nociceptive stimulation (a) and change in its effects under the influence of naloxone (b) and prazosin (c). 1) Control; 2) clofelin (0.5 mg/kg); 3) naloxone (1 mg/kg); 4) prazosin (1 mg/kg). Marker of stimulation (10 sec) shown below; calibration of amplitude 20 μV.

not more than 2 spikes/sec were recorded. Naloxone (1 mg/kg) and yohimbine did not change, whereas prazosin completely restored spontaneous neuronal activity when depressed by clofelin.

Clofelin did not change the responses of the spinal cord neurons to tactile stimulation of the receptive field, but led to dose-dependent inhibition of their responses to nociceptive stimulation (Fig. 1). In a dose of 0.1 mg/kg, for instance, clofelin definitely reduced the firing rate of the neurons in the poststimulus period. After its injection in a dose of 0.25 and, in particular, of 0.5 mg/kg the response of the neurons to nociceptive stimulation consisted of a low-frequency group of discharges, which appeared only during the period of stimulation. Naloxone did not change the depressant effect of clofelin. Yohimbine partially restored the response of the neurons to painful stimulation, but after injection of prazosin the responses of the neurons did not differ from those recorded in the control (Fig. 1).

Of 147 spontaneously active neurons in the midbrain (0.4-5.8 spikes/sec) 17 responded to stimulation of the pulp of different groups of teeth; 15 neurons showed a single type of activity, two a mixed type. Usually stimulation of a canine or molar tooth led to a neuronal response of bursting type, consisting of three or four discharges with a latent period of about 20 msec. In a dose of 0.1 mg/kg clofelin completely suppressed spike activity between 4 and 7 min after its intravenous injection. After 10-15 min spontaneous activity was restored. It is remarkable that naloxone gave a similar effect in a dose of 1 mg/kg.

Neuronal responses to nociceptive stimulation of the dental pulp also were completely inhibited by clofelin in a dose of 0.1 mg/kg (Fig. 2); midbrain neurons, moreover, were not activated by painful stimuli during 2 h of observation. When naloxone was injected against this background, neuronal responses to stimulation of the pulp of the molar and, especially, of the canine tooth were only partially restored.

The results showed that clofelin selectively inhibits nociceptive responses of neurons in the posterior horn of the spinal cord, but does not change their responses to nonpainful stimuli. One of the neurophysiological mechanisms of the effect of clofelin at the spinal cord level was thus established and its important role confirmed in the formation of adrenergic analgesia, discovered previously in experiments on conscious animals [4, 14]. It is remarkable that according

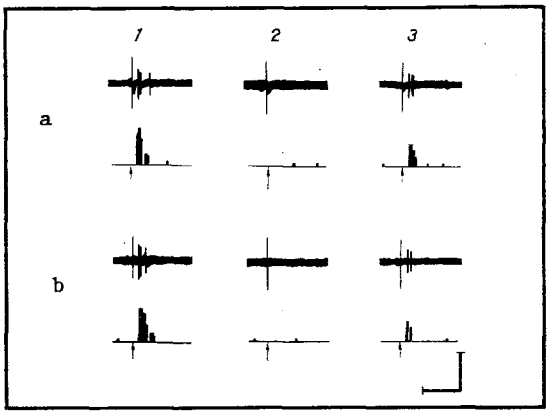

Fig. 2. Changes in neuronal responses in midbrain to nociceptive stimulation of pulp of canine (a) and molar (b) tooth, under the influence of clofelin and naloxone. 1) Control; 2) clofelin (0.1 mg/kg); 3) naloxone (1 mg/kg). Calibration, 10 spikes, 100 msec, width of channel 4 msec, number of samples 10.

to data in [4] clofelin, when injected parenterally, induced analgesia in various tests, whereas if given by intrathecal microinjections it inhibited nociceptive responses integrated mainly at the spinal cord level. Consequently, its analgesic effect is realized through interneurons responsible for the spread of nociceptive impulses within the spinal cord and, to a much lesser degree, for their transmission into the higher levels of the CNS.

The analgesic action of clofelin has been shown not to be connected with opioidergic mechanisms, but to be realized mainly through α_1-adrenoreceptors of the spinal cord, whose location in the region of the afferent input has been established by radioligand studies (for further details, see [14]). Our data, obtained for the first time on unanesthetized animals, are in agreement with experimental results obtained recent years, an anesthetized cats and rats. In spinal cats, clofelin selectively modified the excitability of primary afferents of C-fibers [6], and in rats this preparation, injection intrathecally, led to dose-dependent inhibition of neuronal responses in the posterior horn of the spinal cord, also only to stimulation of C-fibers; naloxone under these circumstances irreversibly abolished the effects of clofelin [13].

The functional role of midbrain neurons is not uniformly the same: some of them are regarded as truly "antinociceptive" neurons, participating directly in descending inhibition of nociceptive impulses at the spinal cord level [10, 11]. These neurons are activated by pain, on injection of opiates, and during stimulation analgesia. It has been shown that clofelin does not change stimulation analgesia induced by stimulation of the spinal cord in conscious animals [1]. Other neurons of the central gray matter perform a relay function, leading to generalization of nociceptive impulses in brain structures. Very probably one of the neurophysiological mechanisms of the analgesic action of clofelin is inhibition of relay neurons of the "analgesic systems" of the midbrain induced by it. It was shown previously [8, 9] that clofelin does not affect spontaneous or evoked activity of neurons of the dorsal nucleus raphe, and its analgesic effect is unchanged after destruction of the gigantocellular reticular nucleus. The fact that inhibition of spontaneous activity of the midbrain neurons recorded under the influence of clofelin and naloxone is of the same type shows that these neurons are under the control of both adrenergic and opioidergic neurotransmitter systems. As has been shown by intraventricular injection of clofelin, opiate systems may be one component of the analgesic systems which are triggered by clofelin through adrenergic, but not through opiate receptors of the brain [4]. The greater sensitivity of midbrain neurons than of neurons of the posterior horn of the spinal cord to clofelin is evidently the cause of its stronger analgesic effect in tests characterizing the mesencephalic level of pain integration [4]. A particular feature distinguishing the action of clofelin from that of morphine and its analogs must be emphasized. The effect of opiates and opioids is determined by their action on suprasegmental and segmental levels of pain integration and it is oriented toward monitoring of the ascending nociceptive flow and its descending regulation. The adrenergic analgesia induced by clofelin is based on its depressant action on nociception processes mainly at the brain level, and in larger doses, at the spinal cord level.

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ANALYSIS OF THE STRUCTURE OF SLEEP AND WAKING IN RATS WITH A PARKINSONIAN SYNDROME INDUCED BY 1-METHYL-4-PHENYL-1,2,3,6-TETRAHYDROPYRIDINE AND BY OXOTREMORINE

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UDC 616.858-092.9-092:
616.8-009.836

KEY WORDS: theta-rhythm; sleep—waking; parkinsonian syndrome; oxotremorine; MPTP

Disturbances of the structure of sleep and of its qualitative characteristics are often observed in patients with parkinsonism. In particular, in individual patients a decrease is observed in the relative contribution of fast sleep [7], delta-sleep [1], and reduction of "sleep spindles" and their restoration after treatment with levodopa [2, 12, 14], have been observed in individual patients. At the same time there is evidence that some clinical manifestations of the parkinsonian syndrome disappear during sleep in the relaxed state and are intensified during sustained wakefulness. However, the mechanism of the connection between the functional state and the symptom-forming process in parkinsonism still remains unclear.

The discovery of the neurotoxin MPTP (1-methyl-4-phenyl-1,2,3,6-tetrahydropyridine), which can damage nigrostriatal dopaminergic neurons and reproduce the most essential features of the parkinsonian syndrome [1, 9], has enabled the particular features of development of parkinsonism to be assessed from new standpoints.

The aim of this investigation was to study the structure of the sleep—waking cycle in an experimental parkinsonian syndrome induced by systemic administration of MPTP or of oxotremorine.

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(Presented by Academician of the Academy of Medical Sciences of the USSR A. V. Val'dman.) Translated from Bylleten' Éksperimental'noi Biologii i Meditsiny, Vol. 108, No. 8, pp. 206-209, August, 1989. Original article submitted December 25, 1988.

0007-4888/89/0008-1147$12.50