9. E. G. Shaskan and S. H. Snyder, J. Pharmacol. Exp. Ther., 175, 404 (1970).
10. K. Sundaran and S. Mahajan, Physiol. Chem. Phys., 13, 523 (1981).
11. D. Wong, J. Horng, and R. Fuller, Biochem. Pharmacol., 22, 311 (1973).

ROLE OF THE HYPOTHALAMIC OPIOID SYSTEM IN MECHANISMS OF MORPHINE ANALGESIA

G. I. D'yachkova and L. V. Kalyuzhnyi

UDC 615.212.7:547.943].015.4:612.826.4.015.2:[547.95:547.943

KEY WORDS: morphine; analgesia; hypothalamus; nalorphine; met-enkephalin.

Descending inhibitory (antinociceptive) mechanisms of the brain stem, in particular, the periventricular structures of the brain — central gray matter, nuclei raphe, and hypothalamus [1], are considered to participate in the pain-relieving action of morphine. However, most workers link the effect of opiates with an action on structures of the mesencephalon and nuclei raphe [10]. It has recently been shown that synthesis of endorphine [8] and enkephalins [6] takes place in the paraventricular region of the hypothalamus, where a high density of opiate receptors [5] and of neurons sensitive to microiontophoretic application of endogenous opiates has been found [4]. In addition, electrical stimulation of this brain region evoked a phenomenon of analgesia [1, 2]. These data indicate a definite role of the paraventricular region of the hypothalamus in the mechanism of the analgesic action of morphine.

The object of this investigation was to compare changes in morphine analgesia during blockade of opiate receptors of the periventricular region of the hypothalamus and in the nuclei raphe by met-enkephalin and nalorphine.

EXPERIMENTAL METHOD

Analgesia was determined in 13 waking rabbits of both sexes, weighing 2.5-3.0 kg, by measuring the latent period (LP) of the withdrawal of the the tail in response to a nociceptive temperature stimulus (the tail-flick test). At the same time the evoked potential (EP) in the sensomotor cortex in response to nociceptive electrodermal stimulation (EDS) of the contralateral hind limb of the animal, evoking a behavioral avoidance response in the unrestrained rabbit, was analyzed. It was shown previously [3] that changes in the amplitude of the second positive wave of the sensomotor cortical EP correlate with changes in pain sensitivity determined by the tail-flick test and with parameters of autonomic responses during injection of morphine and acupuncture stimulation. EP were analyzed on the NTA-1024 amplitude-phase analyzer (Orion), on the basis of 10 realizations. Parameters of EDS were: a single square pulse (1 msec, 9-10 mA). For intracerebral injections, guide cannulas were implanted bilaterally into the cranial bones 3-5 days before the experiment began, in accordance with coordinates from [7], into the paraventricular region of the hypothalamus, and into the region of the large raphe nucleus. Met-enkephalin (100 μg) and nalorphine (100 μg) were injected in a volume of 1 μl by means of a microsyringe in the course of 90 sec. Isotonic NaCl solution in the same volume was injected as the control. The region of injection of the drugs was subsequently examined histologically. Morphine in a dose of 5 mg/kg was injected intravenously.

The numerical data were subjected to statistical analysis by Student's t test.

EXPERIMENTAL RESULTS

Before injection of morphine LP according to the tail-flick test on the rabbits averaged 21.1 ± 7.0 msec (Fig. 1). EP in the sensomotor cortex in response to nociceptive EDS was

Laboratory of Physiology of Pain, P. K. Anokhin Institute of Normal Physiology, Academy of Medical Sciences of the USSR, Moscow. (Presented by Academician of the Academy of Medical Sciences of the USSR V. V. Zakusov.) Translated from Byulleten' Eksperimental'noi Biologii i Meditsiny, Vol. 96, No. 8, pp. 54-56, August, 1983. Original article submitted January 31, 1983.

 0007-4888/83/9608-1094$07.50

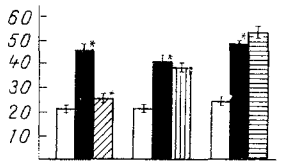

Fig. 1. Effect of nalorphine on LP of tail-flick test in rabbits during temperature stimulation against the background of morphine analgesia. Unshaded columns — original LP; black columns — LP 20 min after intravenous injection of morphine; obliquely shaded column — LP 10 min after injection of nalorphine into hypothalamus; vertically shaded column — LP 10 min after injection of nalorphine into region of large raphe nucleus; horizontally shaded column — LP 10 min after injection of 0.9% NaCl into hypothalamus. Ordinate, time (in sec). *P < 0.05.

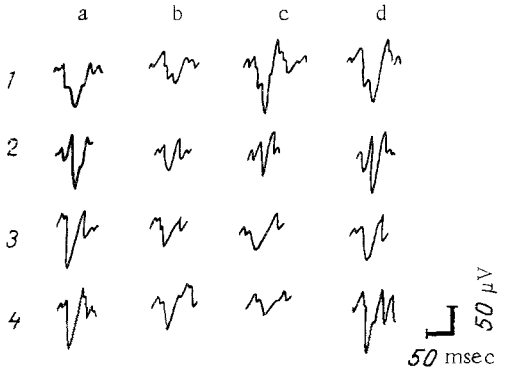

Fig. 2. Effect of nalorphine and met-enkephalin on sensomotor cortical EP of rabbits against the background of morphine analgesia. a) Background; b) 20 min after injection of morphine; c) 10 min after injection of nalorphine into hypothalamus (1), into region of large raphe nucleus (2), of 0.9% NaCl into hypothalamus (3), and of met-enkephalin into hypothalamus (4); d: 1, 2, 3) 60 min after previous injection, 4) 10 min after injection of nalorphine into hypothalamus.

characterized by a primary response with LP of 10-12 msec and amplitude 15-30 μV and by a second positive wave with LP of 20-40 msec and a mean amplitude of 70-90 μV (Fig. 2a).

Morphine considerably increased LP in the tail-flick test to 44.9 ± 8.6 sec, i.e., on average by 112.0 ± 10.3%. The amplitude of the second positive wave of the sensomotor cortical EP in response to nociceptive EDS fell to 38.2 ± 3.4 μV, i.e., by 47.2 ± 6.3% relative to the initial level. It must be pointed out that met-enkephalin, if injected into the hypothalamus, caused an even greater decrease in the amplitude of this EP component — to 27.9 ± 5.8 μV, i.e., by 61.5 ± 4.1% below the initial level. Nalorphine, injected into the hypothalamus, completely abolished the effect of met-enkephalin (Fig. 2: c, 4, d, 4).

LP in the tail-flick test 10 min after isolated injection of nalorphine into the hypothalamus against the background of morphine analgesia was reduced on average to 25.1 ± 10.5 sec, which differed significantly from its value after injection of morphine but did not differ significantly from this parameter in intact animals (Fig. 1). In response to nociceptive EDS after microinjection of nalorphine, the amplitude of the second positive wave of the sensomotor cortical EP increased to 96.2 ± 4.7 μV, which differed from the value of EP before microinjection of the peptide but did not differ from the original value before injection of morphine (Fig. 2c, 1).

No statistically significant changes in LP in the tail-flick test or in the sensomotor cortical EP in response to nociceptive EDS were observed 10 min after injection of nalorphine

into the region of the large raphe nucleus (Fig. 1; Fig. 2c, 2). However, 30 min after microinjection of nalorphine, LP in the tail-flick test on the experimental animals was reduced on average to 24.3 ± 9.0 sec, which was 58.8 ± 3.1% of its value before injection of the peptide. The amplitude of the second positive wave of the sensomotor cortical EP was increased to 73.0 ± 5.9 μV, or 193.1 ± 7.1% of its value before injection of nalorphine.

Microinjections of physiological NaCl solution into the paraventricular region of the hypothalamus and into the region of the large raphe nucleus of the control animals caused no changes in LP according to the tail-flick test or in the sensomotor cortical EP in response to EDS (Fig. 1; Fig. 2c, 3).

The results thus indicate that nalorphine, injected into the paraventricular region of the hypothalamus, completely blocks morphine analgesia, as shown both by the behavioral indicator of the tail-flick test and the electrophysiological indicator of a change in EP. A similar but weaker effect, and with a much longer LP was produced by nalorphine when injected into the raphe nucleus. Characteristically nalorphine, unlike its effect when injected into the hypothalamus, when injected into the reticular formation, thalamus, and septum did not change morphine analgesia [11]. This is evidence that morphine analgesia is mediated mainly through "opioid structures" of the hypothalamus. The weaker effect of nalorphine when injected into the raphe nucleus on morphine analgesia may evidently be mediated through the morphological connections of this region with the hypothalamus, as has been postulated with regard to effects of opiates injected into the central gray matter of the brain [9].

LITERATURE CITED

1. A. V. Val'dman and Yu. D. Ignatov, The Central Mechanisms of Pain [in Russian], Leningrad (1976).
2. E. V. Golanov and L. V. Kalyuzhnyi, Dokl. Akad. Nauk SSSR, 242, 469 (1978).
3. V. V. Yasnetsov, Farmakol. Toksikol., No. 2, 44 (1982).
4. V. V. Yasnetsov, V. A. Pravdivtsev, M. I. Titov, et al., Dokl. Akad. Nauk SSSR, 260, 1502 (1981).
5. S. Atwen and M. Kuhar, Brain Res., 129, 1 (1977).
6. J. C. Beauvillain, G. Frama, and J. Poulain, Cell Tissue Res., 224, 1 (1982).
7. E. Fifkova and J. Marsala, Stereotaxic Atlases for the Cat, Rabbit and Rat, Prague (1965).
8. J. Fukota, L. Nakai, and H. Imura, Brain Res., 201, 492 (1980).
9. I. Hosobuchi, J. Rossier, F. Bloom, et al., in: Advances in Pain Research and Therapy, Vol. 3, New York (1979), p. 515.
10. D. J. Mayer, in: Advances in Pain Research and Therapy, Vol. 3, New York (1979), p. 385.
11. Tsou Kong, Acta Physiol. Sinica, 12, 338 (1963).