THE REINFORCING BUT NONANALGESIC ACTION OF OPIOID STIMULATION OF THE VENTRAL TEGMENTAL AREA

É. É. Zvartau, N. A. Patkina, and A. S. Morozova

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KEW WORDS: reinforcement; analgesia; opiate receptors; veneral tegmental area.

The discovery of heterogeneity of the antinociceptive and emotionally positive action of analgesics may serve as the basis for an oriented search for analgesics free from potential toxicomanic effects. Previously the writers obtained evidence of a difference in the analgesic and psychotropic effects of morphine in the course of development of tolerance and sensitivity to the action of naloxone [1, 4].

This paper gives the results of a comparative study of the antinociceptive and secondary reinforcing action of certain opioid agonists with different profiles of receptor selectivity on microinjection into the ventral tegmental area (VTA). We know that electrical stimulation of VTA can induce analgesia [10]. More recently VTA has been regarded as an important cerebral substrate for the reinforcing effect of morphine [5, 6, 12].

EXPERIMENTAL METHOD

Experiments were carried out on 63 male albino rats. The animals were anesthetized with pentobarbital and guiding cannulas implanted unilaterally into the region of the ventral tegmentum, using coordinates from the atlas [7]. From 3 to 6 days later, the antinocicpetive action of preparations was studied by the use of thermal (estimation based on the latent period of the tail withdrawal reflex), mechanical (application of clips to the base of the tail, assessment relative to the vocalization threshold), and electrical [3] stimuli 5-30 min after the microinjection.

Secondary reinforcing effects were assessed by the method in [2]. The apparatus consisted of a box measuring 50 × 25 × 30 cm, divided into two halves, one of which was painted black, the other white. The black half was further darkened by a perforated lid. The box was illuminated with a 25 W lamp with mat screen, suspended at a height of 0.5 m. The experiments were set up in an insulated room, with general artificial lighting and with masking of random noise by music. The time during which the animals stayed in each compartment of the box and their movements to and fro were recorded automatically.

For preliminary determination of initial preference the rats were placed one after the other in the right hand corner of the light half of the box, and the number of to and fro movements and the length of stay in each compartment were recorded for 15 min. This type of testing was repeated 3 times. Next, the animals remained under investigation for 3 days. For this purpose, a partition was placed between the two halves of the box. After a mock intracerebral injection, the animals were placed for 15 min in the preferred (dark) half of the box. After 2-3 h they were given an injection of the preparation or solvent (control) and the rats were quickly placed for 15 min in the unpreferred (light) half of the box. The next day the final testing was done in accordance with the same scheme as at the beginning of the experiment.

Morphine (10 μg), phenazocine (30-60 μg), bremazocine (20 μg), and phencyclidine (20 μg) were injected in a volume of up to 1 μl in the course of 1 min. After the end of the experiments the brain was investigated histologically. In this paper we give data for experiments with verified location of the chemical electrodes within the brain. The results were subjected to statistical analysis by parametric and nonparametric methods.

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TABLE 1. Changes in Responses to Thermal, Mechanical, and Electrical Nociceptive Stimuli after Injection of Preparations into VTA (M ± m)

Substance	Latent period of tail withdrawal reflex, sec				Threshold of vocalization in response to clipping of the tail, relative units				Intensity of emotional-painful response to electrical stimulation of tail			
	0 min	10 min	20 min	30 min	0 min	10 min	20 min	30 min	0 min	10 min	20 min	30 min
Morphine	13,7±0,8	15,1±0,9	14,7±0,9	13,7±1,5	2,06±0,08	2,17±0,10	2,24±0,12	2,43±0,15*	5,00	4,55	3,59	2,74
Bremazocine	13,4±1,7	12,4±1,5	12,5±1,0	13,5±1,4	2,31±0,21	2,12±0,24	2,25±0,25	2,43±0,35	5,00	4,80	4,80	4,20
Phencyclidine	11,5±2,7	10,6±1,9	11,3±1,9	11,5±2,0	2,90±0,33	2,40±0,24	2,40±0,24	2,80±0,20	5,00	5,00	5,00	5,00

Legend. Intensity of emotional-painful response shown as sum of probabilities of appearance in a group of animals to painful electrical stimulation which, in the control, evoked a generalized affective reaction with each of the five following components: biting the electrode, squeaking, turning, crying, chewing the electrodes. *$P < 0.05$.

TABLE 2. Secondary Reinforcing Effects of Morphine, Bremazocine, and Phencyclidine in Response to Microinjection into VTA (M ± m)

Experimental conditions	Dose	Number of animals	Change in duration of stay in unpreferred compartment after pharmacologic conditioning, sec	Number of animals with pharmacologic conditioning effect, %
Control (isotonic NaCl solution)	1 μl	8	+13,7±22,4	0
Morphine	10 μg	9	+524,3±66,1***	100*
Bremazocine	20 μg	7	+32,8±15,4	0
Phencyclidine	20 μg	6	+344,2±136,5**	75*

Legend. *P < 0.025 (Fisher's exact method), **P < 0.05 (Wilcoxon–Mann–Whitney test), ***P < 0.001 compared with control.

EXPERIMENTAL RESULTS

Microinjection of morphine into VTA was not accompanied by any antinociceptive effect during observation for 20 min (Table 1). At the 3rd minute the threshold of response to mechanical stimulation was raised and a similar tendency was noted for painful electrical stimulation. No analgesic effect was exhibited on any of the three models after injection of bremazocaine and phencyclidine. Signs of an opiate behavioral syndrome were not present in the rats, but motor activation and intensification of investigative behavior were observed. The animals' movements under these circumstances were toward the side opposite to the site of microinjection.

The secondary-reinforcing properties of morphine in response to application to VTA were very clear. All nine rats developed a strong conditioned preference reflex (Table 2). A weaker but significant effect was produced by phencyclidine. No animal receiving three intrategmental microinjections of bremazocine (20 μg) increased the length of its stay in the light half of the box.

The conditioning action of morphine in a separate series of experiments took place against the background of preliminary subcutaneous injection of naloxone in a dose of 1 mg/kg. In these experiments no animal formed a conditioned place preference reflex.

Microinjection of morphine into VTA is thus manifested by an emotionally positive, but not antinociceptive effect of the analgesic. The tendency for the nociceptive responses to diminish 30 min after microinjection must be attributed to diffusion of the drug from the site of its application [8, 13]. The reinforcing effect of morphine is specific, as is shown by its sensitivity to naloxone.

In its receptor profile, morphine is an unselective agonist mainly of μ-opioid receptors, whereas bremazocine has stronger affinity for κ-receptors [9]. Phencyclidine interacts with specific receptors, which are regarded as identical with σ-opiate receptors [11, 14].

It can be concluded on the basis of the results of this investigation that VTA is a trigger zone for the positive-reinforcing effect, but not for the antinociceptive effect of opiates, and that an important role in the realization of the reinforcing effect of opioid agonists is played by μ- and σ-opioid receptors.

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EFFECT OF THYROTROPHIN RELEASING HORMONE ON OPIATE RECEPTORS OF THE RAT BRAIN

A. M. Balashov and M. R. Shchurin

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An important role in the regulation of neurotransmitter processes is played by biologically active substances known as neuromodulators, which affect the release and uptake of transmitters and the sensitivity of receptors to them. The most important contribution to modulation of the "classical" monoamine mediator systems is made by neuropeptides, including endogenous opioids [2]. Meanwhile, much less attention was been paid to the study of regulation of the functions of opiate and other peptidergic systems of the CNS, which possess modulator activity. Information on this problem in the literature is not yet sufficient to allow definite conclusions to be drawn with respect either to the mechanisms of this regulation or to the nature of the factors responsible for it.

Investigations have shown the mutual influence of peptidergic and neurohumoral systems: Close connections have been found between β-endorphin, on the one hand, and blood plasma levels of various hormones, on the other hand [7, 12]. It has been demonstrated that disturbances of the functional relations between the neuroendocrine and neuromodulator systems may lie at the basis of the development of psychotic states [6].

It is understood that modulator systems can be regulated at any level and, in particular, at the level of reception of peptide ligands. Salsolinol has been shown to cause changes in binding activity of opiate receptors *in vitro* relative to enkephalins, and to exhibit, under these circumstances, ability to interact antagonistically with receptor structures [5]. It has also been shown that different types of opiate receptors can be regulated by guanyl nucleotides and by certain metallic cations [11].

It has recently been shown that the hypothalamic hormone thyrotrophin releasing hormone (TRH) has the properties of a morphine antagonist, blocking its inhibitory action on respiration and, to a lesser degree, its analgesic action [3]. This suggests that the antagonistic effects of TRH are mediated through its interaction with opiate receptors.

The aim of this investigation was to test this hypothesis experimentally.

Laboratory of Neurochemistry of Alcoholism and Drug Addictions, V. P. Serbskii All-Union Research Institute of General and Forensic Psychiatry, 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. 102, No. 8, pp. 174-176, August, 1936. Original article submitted December 26, 1985.

