ACTION OF NALOXONE ON EMOTIONALLY POSITIVE AND ANTINOCICEPTIVE EFFECTS OF HYPOTHALAMIC STIMULATION IN RATS

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Naloxone (5 mg/kg, subcutaneously) had no significant effect on the electrical self-stimulation response in rats with electrodes in the lateral hypothalamus. An analgesic effect of stimulation of the "reward" zones was found in three animals, in the form of raising of the threshold of pain vocalization during electrical stimulation of the tail. The antinociceptive action was abolished by naloxone. Morphine (3 mg/kg) activated the self-stimulation response, whereas naloxone antagonized this action. The role of opiate receptors in self-stimulation effects and in central analgesia is discussed.

KEY WORDS: naloxone; self-stimulation; opiate receptors; morphine; antinociceptive system.

Naloxone is a "pure" morphine antagonist whose effect is produced through binding with opiate receptors in the brain [9, 14]. Morphine, the agonist of the opiate receptors, is known to be a powerful analgesic and euphoria-inducing agent [1]. The action of morphine in inducing euphoria during development of dependence has been studied in detail experimentally on models of the self-stimulation response [4]. Monoaminergic mechanisms have been shown to participate in the self-stimulation (SS)-facilitating effect of morphine [5]. The object of this investigation was to study the role of opiate receptors in the mechanism of emotionally positive and antinociceptive effects of hypothalamic stimulation.

METHODS

Experiments were carried out on 14 noninbred male albino rats with monopolar electrodes implanted in the lateral hypothalamus. The SS response was studied in a Skinner's box with one lever, pressure on which led to electrical stimulation (ES) of the brain by a "volley" of 30 monophasic pulses, 1 msec in duration, with a frequency of 100 Hz, and an amplitude of 60-300 μA. Stimulation from the output of the isolating unit of an ESU-2 stimulation was applied through a 100 kΩ resistor, connected in series with the load. The number of times the animal pressed the lever, the total duration of ES, horizontal and vertical activity, and the number of grooming acts were recorded. The duration of the SS session was 5-10 min.

Nociceptive stimulation was applied to six rats by electrical stimulation of the base of the tail from the second output of the isolating unit of the stimulator, using the same parameters of ES but without the 100 kΩ resistor in the circuit. The range of voltages used during the investigation of the nociceptive response was 30-80 V. The order of the experiment to determine the antincociceptive effect of central stimulation was as follows: 1) determination of the threshold of pain vocalization (four to five tests), 2) administration of naloxone or isotonic saline, 3) repeated determination of the pain threshold after 15-20 min, 4) ES of the hypothalamus for 5 min with volleys each of 30 pulses every second for 5 min, 5) determination of the threshold of the nociceptive response against the background of ES of the hypothalamus between 5 and 7 min after it began.

Isotonic saline and naloxone in a dose of 5 mg/kg (the naloxone was generously provided for the investigation by Endo Laboratories, Inc.) were injected subcutaneously in a volume of 0.1-0.2 ml/100 g body weight, and morphine was injected intraperitoneally in a dose of 3 mg/kg. The location of the electrodes within the lateral hypothalamic region was verified histologically.

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TABLE 1. Effect of Naloxone and Morphine and a Combination of Both on Response to SS

Substance and dose	Number of experiments	Change in number of self-stimulations (M ± m) compared with control, taken as 100%
Isotonic NaCl solution (0.1 ml/100 g)	16	98,3±7,8
Naloxone (5 mg/kg)	23	95,9±5,0
Morphine (3 mg/kg)	10	158,6±5,6*
Morphine (3 mg/kg) + naloxone (5 mg/kg)	5	97,8±6,3

*P < 0.001.

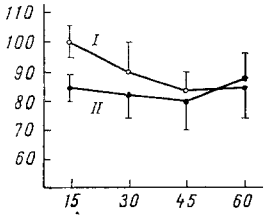

Fig. 1

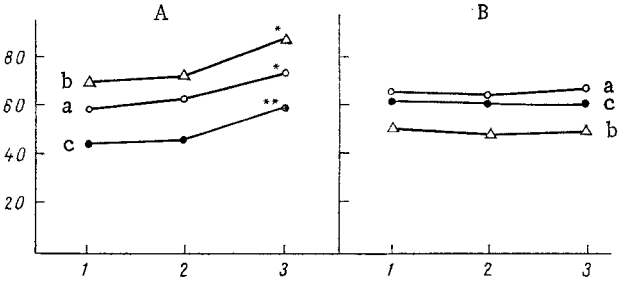

Fig. 2

Fig. 1. Changes in number of self-stimulations with time after injection of isotonic saline (I) and naloxone (II). Ordinate, number of self-stimulations (in % of control level, taken as 100%); abscissa, time (in min). Mean values and errors of means for group of five rats shown.

Fig. 2. Changes in threshold of pain vocalization after isotonic saline (A) and naloxone (B). a, b, c) Experimental animals Nos. 68, 70, and 73; *P < 0.22; **P < 0.01. Ordinate, intensity of nociceptive electrical stimulation of tail (in V); abscissa: 1) control determinations of threshold for squeaking; 2) the same, 20 min after injection of isotonic saline or naloxone; 3) the same after electrical stimulation of hypothalamus for 5 min.

RESULTS AND DISCUSSION

Data showing the effect of antagonist (naloxone) and agonist (morphine) of opiate receptors on the principal indicator of the SS reaction (the number of self-stimulations) are given in Table 1. Naloxone, injected in a dose of 5 mg/kg subcutaneously 20 min before SS, did not cause statistically significant changes in this parameter. To test the possible effect of the drug over a wider range of times, in experiments on five rats the action of naloxone and isotonic saline was compared at intervals in the course of 1 h after injection (Fig. 1). A slight tendency was found, although it was not statistically significant (by Student's test $t = 2.3$; by Wilcoxon's two-sample test $T = 3$, $P > 0.05$) for the response to SS to be inhibited 15 min after injection of naloxone. One- and two-factor dispersion analysis revealed no significant effect of naloxone compared with isotonic saline and with the time factor (index of strength of influences η_i^2 for random variations was 0.898, F' for factorial variation was 1.9, $P > 0.05$ [6]). Meanwhile, in individual experiments, SS fell to 70-80% of the control, but this effect was unstable and not reproducible. Correlation was not found between individual variants of the response to naloxone and features of SS such as the strength of the current, the structure of behavior with respect to individual behavioral components, the initial expressiveness of SS (Spearman's rank correlation coefficient $\rho = -0.2$, $P > 0.05$), the original horizontal and vertical activity in the pauses between pressing on the lever (values of ρ 0.27 and -0.52 respectively, $P > 0.05$), and the trend of the changes in SS after injection of isotonic saline.

TABLE 2. Individual Characteristics of Response to SS with or without Accompanying Analgesic Effect

Animal No.	Strength of current, μA	Analgesic effect	Number of self-stimulations in 5 min	Character of pressing on lever	Behavior in pauses	Response to naloxone*
68	44	+	279—314	With paw, frequent licking of lever	Moderate grooming	91,7±5,8
70	120	+	164—277	Regular pressing with paw, small motor component (turning head)	» »	91,0±15,0
73	100	+	206—250	With paw, biting lever	Active grooming, moderate horizontal activity	120,6±23,6
69	80	—	263—299	With paw, episodes of biting and chewing	Active investigative reaction, grooming	75,5±6,5
71	60	—	177—342	Attacking lever and biting, pressing with paw, ejaculation	Moderate horizontal activity, grooming	108,0±23,0
72	100	—	70—80	Careful and short presses with paw	Running away into corner of box after pressing	94,0±7,3

*Number of presses compared with control (100%).

SS was increased after injection of morphine (3 mg/kg, intraperitoneally, 50-60 min before the test). Naloxone, injected after morphine, abolished its facilitatory action (Table 1).

The effect of ES of the "reward" zones on the nociceptive response was investigated in experiments on six rats. Compulsory ES, in volleys of 30 shocks one every second, was applied using a current of the same strength as that used to study SS. The behavior of all the animals during ES was characterized by an orienting reaction with sniffing, looking, and standing up on the hind limbs. Three rats showed definite analgesia with elevation of the pain vocalization threshold by 16-27% (Fig. 2A). Naloxone completely abolished the antinociceptive effect of hypothalamic stimulation (Fig. 2B). Comparison of the different characteristics of the animals with and without a central analgesic effect (Table 2) revealed no definite correlates of antinociceptive action based on the threshold of SS, the number and character of the operant responses, behavior during the pauses, or the response to naloxone.

These experiments thus confirmed observations on the analgesic effect of ES of the hypothalamus [8, 10, 15]. The fact that on the basis of behavioral indices it was impossible to differentiate any "points" ES of which was accompanied by an analgesic action or not indicates the relatively independent topographic organization of "reward" elements and of the antinociceptive system (ANCS) in the zone of stimulation, in harmony with the concept of the fragmentary organization of behavior [3]. A "focus" of ANCS is known to be located in the periaqueductal and periventricular gray matter [2, 12]. The fact that ANCS is activated through the emotiogenic hypothalamic zones confirms the view of ANCS as a component of the functional system of affective behavior [2].

Naloxone, a drug with selective antimorphine action [9], completely abolished the analgesic effect of ES of the hypothalamus. Blocking of opiate receptors by naloxone is known to reduce the analgesic action of ES of the midbrain and raphe nuclei [7, 13]. Alil et al. [7] explained incomplete antagonism of naloxone, in particular, by the fact that, despite blockade of the receptors of endogenous morphine-like substances, partial analgesia was produced because of probable electrical activation of the postsynaptic elements of ANCS. In this context the present results showing complete abolition of the analgesic effect by naloxone may be indirect evidence that the analgesic effect of ES of the hypothalamus is realized through the mesenchephalic substrate of ANCS and mediated by activation of peptidergic projections and involvement of mechanisms connected with endogenous morphine-like substances.

The response to SS proved to be resistant to the action of naloxone. However, the "reward" effect was considerably strengthened by injection of morphine, the agonist of the opiate receptors. In turn, naloxone antagonized this activating action. Consequently, whereas mechanisms associated with the formation and action of endogenous ligands of opiate receptors do not play an essential role in the realization of the positively reinforcing effects of ES of the lateral hypothalamus, activation of SS by morphine is a process with a peptidergic component. Our observations confirm Goldstein's opinion [11] that the endorphin system

is normally in a stable state and blockade by naloxone can be clearly revealed if the system is first activated by a suitable stimulus.

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NEUROCHEMICAL MECHANISMS OF THE ACTION OF EUPHYLLINE IN CEREBROVASCULAR INSUFFICIENCY

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In cerebrovascular insufficiency the ammonia concentration in the brain tissue shows a marked increase, glutamate dehydrogenase (GD) activity was reduced, and there is no significant change in the levels of glutamine and protein amino groups. Euphylline, which promotes normalization of the disturbed cerebral circulation, removes the excess of ammonia by restoring GD activity in the reductive amination reaction. The effect of euphylline is most clearly demonstrated in the presence of acute cerebral ischemia, evidence of the effect of the drug on the neurochemical mechanisms of compensatory regulation of the cerebral blood flow.

KEY WORDS: Euphylline; cerebral circulation; nitrogen metabolism; glutamate dehydrogenase.

A leading role in the therapeutic effect of euphylline in cerebrovascular disturbances is ascribed to the part played by the vascular component in its action [1, 10, 12]. The writers established the ammonia-neutralizing action of euphylline [2, 6], which is in harmony with views regarding the role of neurochemical mechanisms in compensatory regulation of the cerebral blood flow [4, 5]. This was the basis for the study of the effect of euphylline on the system of ammonia formation and removal under conditions of a reduced blood flow.

METHODS

Experiments were carried out on noninbred adult rats. The dynamics of changes in the indices of nitrogen metabolism and in the blood supply to the rats' brain was studied after unilateral occlusion of the common carotid artery. The local cortical blood flow was deter-

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