LONG-TERM ENHANCEMENT OF MORPHINE ANALGESIA AFTER PERIODIC BLOCKING OF OPIATE RECEPTORS BY NALOXONE

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Compensatory hypersensitivity of various receptors after their long-term blocking by drugs is a sufficiently universal phenomenon [1, 4, 10] and, in particular, it is one characteristic of opiate receptors [6, 7, 12]. Meanwhile it is evident that differences in the analgesic and, evidently, other pharmacologic effects of the opiates are determined by the state of the opiate receptors – the target of action of this class of substances. Differences in the analgesic effects of morphine in rats with immobilization stress [2] and also after being kept under abnormal conditions [3], discovered by the writers previously, may perhaps be linked with the properties of opiate receptors – the dynamic stage in the regulation of endogenous opioid neurotransmission.

The aim of this investigation was to study changes in nociceptive responses and in morphine- and stress-induced analgesia in rats during exposure to factors influencing activity of opiate receptors, with the aid of their specific blocking agent, naloxone.

EXPERIMENTAL METHOD

Nociceptive responses of animals were assessed by the "tail withdrawal" method on the basis of measurement of the duration of the motor response of the rat's tail (latent period – LP) when immersed in hot water (60°C). Noninbred male rats weighing 240-260 g were used.

There were two series of experiments. In series I (36 rats), in order to assess correlation between the state of the opiate receptors and the analgesic effects of morphine, the time course of changes in LP was investigated in response to injection of morphine (6 mg/kg) at various times (30, 90, 180, and 300 min) after injection of naloxone (1 mg/kg). In the experiments of series II, naloxone (0.5 mg/kg) was injected into the experimental animals (24 rats, divided into three equal groups) three times a day (at 10 a.m. and 1:30 and 5 p.m.) for 3 days, whereas the control rats (24 rats, three groups) received the corresponding volumes of physiological saline. On the 4th day of the experiment, 18-20 h after the last injection of naloxone, changes in LP in the experimental and control rats observed after injection of morphine (2 and 6 mg/kg) and after immobilization of the animals for 2 h in constraining cages measuring 15 × 6 × 5.5 cm, were studied. Morphine analgesia (2 mg/kg) was estimated in the animals of both groups, also on the 8th day, 105 h after the last injection of naloxone. All drugs and physiological saline were injected subcutaneously into the animals at the base of the spine in a dose of 0.2 ml/100 g.

During the course of the experiment the animals' body weight was measured, and the thymus and adrenal glands were weighed on the 4th day, 24 h after the last injection of naloxone.

EXPERIMENTAL RESULTS

In the experiments of series I injection of naloxone caused a significant decrease in the intensity of morphine analgesia during the first 2 h of its action (Fig. 1). When morphine was injected 3 h after naloxone, significant enhancement of morphine analgesia was found, and it disappeared if morphine was injected 5 h after naloxone. The enhancement of

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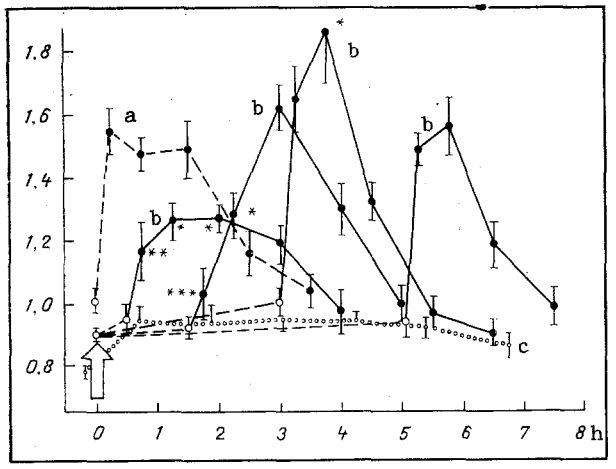

Fig. 1

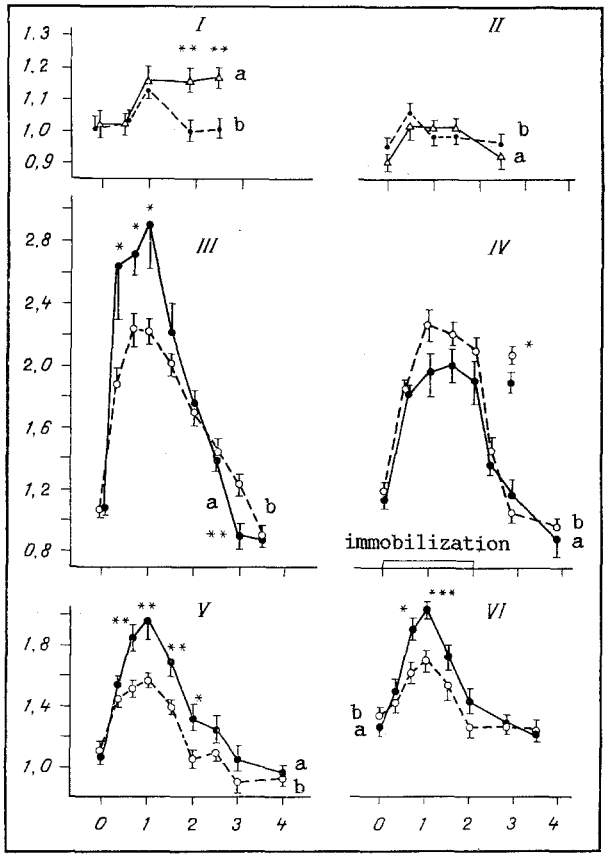

Fig. 2

Fig. 1. Time course of morphine analgesia when morphine was injected (6 mg/kg) at various times after naloxone in a dose of 1 mg/kg (arrow). a) Morphine injected alone; b) morphine injected after naloxone; c) naloxone injected alone. Here and in Fig. 2: abscissa, time (in h); ordinate, LP (in sec). *p < 0.05, **p < 0.01, ***p < 0.001: level of significance of differences.

Fig. 2. Nociceptive responses of rats to first (I) and seventh (II) injections of naloxone (0.5 mg/kg) and of physiological saline. I, II: a) Experiment, b) control; III, V, VI) time course of analgesia induced by morphine in doses of 6 mg/kg (III) and 2 mg/kg (V, VI), in experimental (a) and control (b) rats 20 h (III), 18 h (V), and 105 h (VI) after the last injection of naloxone; IV) dynamics of stress-induced analgesia observed after immobilization of rats of experimental (a) and control (b) groups (18 h after last injection of naloxone).

morphine analgesia discovered 3-4 h after injection of naloxone may be evidence of the development of a compensatory increase in sensitivity of the opiate receptors at this period after their blocking.

In the animals of series II, receiving naloxone, no significant changes were found in the background values of LP or body weight, measured before the first, fourth, and seventh injections of naloxone and on the 4th day of the experiment before injection of morphine. Meanwhile, the first injection of naloxone caused a significant rise of LP after 120 and 180 min of observation, whereas the seventh injection of naloxone was not accompanied by any significant changes in this parameter (Fig. 2: I, II).

It will be clear from Fig. 2, III, V that in animals periodically receiving naloxone, significant enhancement of morphine analgesia was observed 18-20 h after the last injection, by comparison with rats receiving periodic injections of physiological saline. With a dose of morphine of 2 mg/kg, the strength of the effect was doubled, but with a dose of 6 mg/kg the degree of this increase was less marked, and after 3 h the differences were reversed.

It will be clear from Fig. 2, VI that this enhancement of morphine analgesia persisted for a long time, but was a little reduced 105 h after the last dose of naloxone, compared with that observed when morphine was injected after 18 h.

As Fig. 2, IV shows, the time course of stress-induced analgesia observed after immobilization of the rats for 2 h differed only a little in the experiment and control, but the mean value of LP throughout this period of immobilization in rats receiving naloxone (32 determinations) was significantly lower than the corresponding parameter in the control.

Analysis of the morphological data for animals of the experimental and control groups revealed no significant differences in body weight (experiment 276.2 ± 6.9 g, control 278.1 ± 5.9 g), for the weight of the adrenals (24.7 ± 1.0 and 23.7 ± 0.9 mg) and thymus (515.5 ± 40.2 and 432.2 ± 34.0 mg), respectively.

These results are evidence that the analgesic effects of morphine are significantly depressed by acute blocking of opiate receptors by naloxone, after which a phase of potentiation of these effects is observed, which may be based on compensatory hypersensitivity of the opiate binding sites. These effects of morphine may be enhanced for a long time and by a considerable degree by the periodic use of naloxone, and this effect is evidently linked also with compensatory structural changes in the opiate receptors, including both changes in the number of binding sites and their affinity for opiates, and also, perhaps, in the relative numbers of receptors of different types.

The development of compensatory structural changes in opiate binding sites was demonstrated in radioreceptor studies during chronic blockage by naloxone or by naltrexone [6, 7]. For instance, after removal of a naloxone pellet (10 mg/kg on the 10th day) an increase in the number of binding sites of labeled ligands of μ-receptors in the midbrain, medulla, and pons was discovered after 1, 2, 8, and 16 days of the investigation, whereas in other central structures (hypothalamus, hippocampus, striatum, prefrontal cortex) they disappeared on the 2nd-4th day of observation [6]. A detailed analysis of the specific nature of these structural changes revealed that they involve μ- and δ-receptors, but do not change the affinity or density of binding sites of κ- and σ-receptors [12].

When these results are discussed it must be noted that periodic blocking of opiate receptors by naloxone has no significant effect on visually observed spontaneous behavior of the animals, their body weight, the weight of their adrenals and thymus, and also the background values of LP, possible evidence that endogenous opioid processes play an unimportant part in the vital activities of animals under "normal" conditions of existence and also, in particular, in the regulation of the basal level of the nociceptive responses of animals. Meanwhile periodic daily blocking of opiate receptors by naloxone (1 mg/kg, seven injections) in immobilized rats, while it had no significant effect on LP, prolonged their normalization after the end of exposure to stress [4]. Prolongation of analgesia was also found in the case of the combined use of morphine (6 mg/kg) and naloxone (1 mg/kg) in rats after the end of a 2-h period of immobilization [2]. Differences in the effect of naloxone itself on the animals' nociceptive responses are evidently linked with the particular features of opiate receptors in different functional states. Whereas in intact animals injection of this drug usually caused no significant changes in nociceptive responses, and in certain cases, led to analgesia [8], under conditions of electric shock [11], during experimental arthritis, and in animals tolerant to morphine [9], its use is accompanied by significant analgesia, or it prolongs the analgesia that is characteristic of these states.

Enhancement and prolongation of the analgesic effects of morphine were demonstrated previously when it was injected together with several other drugs of different classes: caffeine, neuroleptics, barbiturates, agonists of GABA receptors, and scopolamine [8]. This suggests that this enhancement of the analgesic effects of morphine may be based on interaction between opiate and other, nonopiate, mechanisms, determining the animals' nociceptive reactivity. Enhancement of the analgesic effects of morphine also has been found in animals exposed to various kinds of stress [2, 5], and whose state was characterized by readjustment of their endogenous opioidergic neurotransmission. The long-term potentiation of the analgesic effects of morphine discovered in the present investigation after periodic systemic injections of naloxone indicates that, besides the ways of modification of morphine analgesia discussed above, it may also arise through the increased effectiveness of interaction of morphine with opiate receptors which have undergone compensatory readjustment under these conditions.

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MODULATION OF THE HYPOGLYCEMIC ACTION OF INSULIN BY LATERALIZED TRANSCEREBRAL ELECTRICAL STIMULATION

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The neurophysiological basis of some complex neuropsychopathological syndromes consists of assemblies of neurons which organize determinant dispatch stations [1]. On empirical grounds disorganization of these determinant foci is considered to lie at the basis of the therapeutic action of insulin coma therapy [2]. Meanwhile, destabilization of these systems by the oriented modulation of interhemispheric interaction by means of lateralized subsensory electrical stimulation has been used as a method of treatment of mental disorders, through the creation of antisystems in the opposite hemisphere [4, 6]. With a combination of insulin coma therapy and lateralized electrical stimulation in the same patients, potentiation of the action of the former by right-hemispheric electrical stimulation was found for the first time [5], although the mechanism of this phenomenon was not clear.

This phenomenon was reproduced experimentally in the present investigation in order to study some components of the complex mechanisms of the hypoglycemic action of insulin. It will be recalled that previous attempts to potentiate the hypoglycemic action of insulin experimentally by bilateral transcerebral electrical stimulation proved unsuccessful [3].

EXPERIMENTAL METHOD

Experiments were carried out on 30 mature male chinchilla rabbits weighing 2.8-3 kg, kept on the standard diet. The sensitivity of the rabbits to the hypoglycemic action of exogenous insulin and the rate of clearance of insulin from the blood stream [8] were studied. Blood levels of sugar (by the Hagedorn–Jensen method) and insulin (double-antibody radioimmunoassay) were determined [7]. The investigations were carried out 7 days before and again 1 day after electrical stimulation. A continuous series of negative square pulses with a strength of 1.2-1.8 times below the threshold of motor responses, with a frequency of 1 to 30 Hz was applied for 10 min. Needle electrodes were inserted intradermally, unilaterally: the cathode in the frontal region, the anode in the region of the mastoid process. There were two series of experiments, in each of which three groups (with five rabbits in each group) took part. In series I the blood sugar level was measured before and after

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