Effect of Opioid Antagonist Naloxone on Maternal Motivation in Albino Rats

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We studied the effect of nonselective antagonist of opioid receptor naloxone on the behavior of albino female rats on days 4-6 after delivery. Intraperitoneal injection of naloxone (5 mg/kg) significantly stimulated maternal reactions (increased the number of approaches to pups, decreased the latency of their transfer into new location). Intranasal naloxone (1 mg/kg) produced similar changes. Naloxone in intraperitoneal dose of 1 mg/kg and intranasal dose of 0.2 mg/kg virtually did not modify maternal behavior.

Key Words: *cerebral opioid system; naloxone; maternal behavior; intranasal administration*

Postpartum psychosis and depression are the most prevalent forms of disorders in maternal behavior. These conditions can be caused by high activity of endogenous opioid system or excess of endorphin-like peptides (fragments of alimentary proteins) in the body [9]. It was shown in animal experiments that opioid receptor agonists (morphine and its derivatives) in analgetically inert doses attenuate female reaction to progeny [13]. Hence, it can be expected that opioid antagonists can produce a positive (activating) effect on components of the maternal behavior.

Here we analyzed the effects of a nonselective antagonist of opioid receptors (naloxone) administered via invasive (intraperitoneal) and noninvasive (intranasal) routes on maternal motivation.

MATERIALS AND METHODS

Maternal behavior was evaluated in primiparous outbred albino rat females (n=56) weighing about 250 g. The females were kept together with the litter in cages with free access to water and food. Maternal behavior was observed on postpartum days 4-6. Ten min before testing the females were injected with distilled water (days 4 and 6) and aqueous solution of naloxone (Sigma) (day 5). The intraperitoneal dose was 1 ml/kg, intranasal 100 μl/animal.

Maternal reactions were evaluated in the open field test (round arena, 80 cm in diameter). The female was placed into the center of the field 3 times during the test (2 min each time with 1-min intervals). During the first session standard parameters of spontaneous exploratory activity (running, rearing, grooming, *etc.*) were recorded at red light. During the second session a Petri dish with three rat pups was placed into the center of the arena and parameters of maternal behavior (latency of the first approach to the dish, total number of these approaches, number of transfers of pups, latency of transfer of the third (last) pup from the dish) were recorded at red light. During the last session the same parameters were evaluated at bright illumination.

It was previously shown that maternal behavior in rats virtually did not change during repeated testing [3]. However, behavioral effects of single low doses of opioid agonists and antagonists are short-lasting (several hours). We therefore considered the mean level of behavioral parameters on days 4 and 6 postpartum (control) and on day 5 (after naloxone injection). The significance of the effects was evaluated

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using paired parametrical (Student's) and nonparametrical (Wilcoxon's and signs) tests.

RESULTS

A total of 5 experimental series were carried out. In two of them nonselective antagonist of opioid receptors naloxone was injected intraperitoneally in doses of 1 and 5 mg/kg, in three series intranasally in doses of 0.2, 1.0, and 5.0 mg/kg. The mean control level for each group (days 4 and 6 postpartum) was 4.1-5.7 approaches to pups over 2 min in different experimental series, 2.0-3.6 transfers of pups. The latency of the first approach varied from 5 to 15 sec; the latency of transfer of the last pup was 70-100 sec. Bright light activated the majority of maternal reactions: the number of approaches and transfers increased in comparison with red light sessions, and the latency of last pup transfer was shorter (p<0.05-0.01).

Naloxone dose of 1 mg/kg (intraperitoneally) is most often used in pharmacological experiments for blocking the effects of opioid agonists. In experimental series I we evaluated the effect of this dose on the exploratory and motor activities of rat females and on their maternal reactions. The changes we observed can be characterized as the minimum. Ambulation and other parameters of spontaneous behavior remained at the level of control (p>0.4); virtually no trend to more pronounced manifestation of maternal motivation was observed; the number of approaches to pups somewhat increased at bright light (p=0.13).

A higher dose of intraperitoneal naloxone (5 mg/kg) also did not modify female activity in the open field test without pups. In testing with pups under conditions of red light the drug caused significant changes in all studied parameters of maternal behavior (Figs. 1, 2): the latency of the first approach to the dish with pups decreased from 11.6±3.6 to 3.2±0.6 sec (p<0.05), while the latency of the transfer of the last pup decreased from 96.0±8.2 to 69.7±11.9 sec (p<0.02). The most pronounced changes were observed in the number of approaches to the pups, which increased from 4.1±0.4 to 6.4±0.6 (p<0.01). The number of transfers increased from 2.0±0.4 to 3.4±0.7 (p<0.05). Similar, but less pronounced shifts were observed at bright illumination; the changes were significant only for the number of approaches to the dish with the pups (from 4.7±0.3 to 6.4±0.5; p<0.02). These data indicate a positive effect of naloxone on maternal behavior.

In the next series of experiments we tested the effect of intranasal naloxone. Noninvasive intranasal route of opioid penetration into the body attracts special attention now and is actively studied with morphine derivatives [7,8], but not with naloxone. On the other hand, clinical studies showed that this route of antagonist administration can be used for detection of heroin dependence and for alleviation of opiate overdose aftereffects [5].

Intranasal administration of naloxone in a dose of 1 mg/kg (in contrast to intraperitoneal injection) led to apparent activation of maternal reactions. The number of pup transfer increased most demonstratively (from 2.2±0.5 to 3.4±0.6, p<0.05, at red light and (from 2.7±0.6 to 4.4±0.8, p<0.05, at bright light; Fig. 1). The latency of the last pup transfer at red light also decreased significantly (from 97.1±10.2 to 74.8±13.9 sec, p<0.02; Fig. 2). The effect disappeared at the lower intranasal dose (0.2 mg/kg): changes in the parameters characterizing maternal behavior did not reach the level of significance and was at least p=0.25 in all cases.

Increasing the dose of intranasal naloxone to 5 mg/kg restored the effect. Although no changes in motor and exploratory activities were observed during

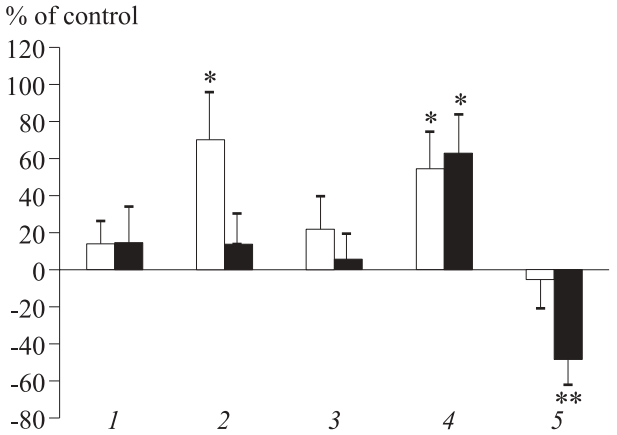

Fig. 1. Effect of naloxone on the number of pup transfers at red dim (light bars) and bright illumination (dark bars). Here and in Fig. 2: *1*) 1 mg/kg naloxone intraperitoneally; *2*) 5 mg/kg naloxone intraperitoneally; *3*) 0.2 mg/kg naloxone intranasally; *4*) 1 mg/kg naloxone intranasally; *5*) 5 mg/kg naloxone intranasally. *p<0.05, **p<0.02 compared to the control (water injection/instillation).

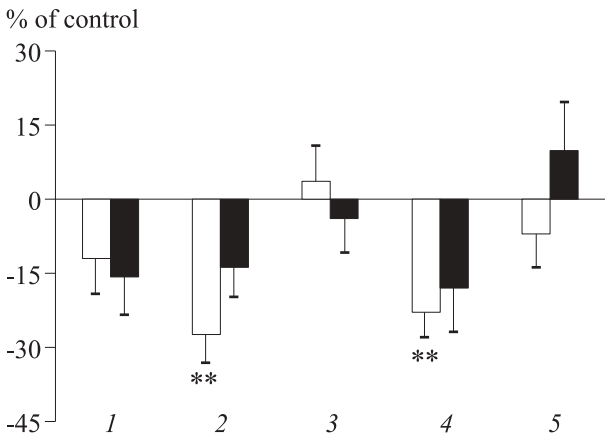

Fig. 2. Effect of naloxone on the latency of transfer of the last pup from Petri dish under conditions of red and bright illumination.

the first 2 min of testing, a significant attenuation of maternal reactions in the presence of pups was observed. The number of approaches to pups decreased from 4.8±0.5 to 3.7±0.6 ($p<0.02$) at red light and from 5.4±0.4 to 4.3±0.5 ($p<0.05$) at bright illumination, the number of pup transfers decreased from 3.3±0.6 to 1.7±0.4 ($p<0.02$; Fig. 1), while the latency of the first approach to pups increased from 7.2±1.7 to 20.5±6.9 sec ($p<0.05$). Decreased maternal motivation after administration of 5 mg/kg intranasal naloxone indicates that this drug can exhibit the effects of an opioid receptor agonist. These effects were reported previously [1]. The mechanism of this phenomenon is primarily due to the capacity of naloxone and similar compounds in high doses to stabilize the stimulated opioid receptors in the active state.

The increase of maternal motivation after moderate dose of naloxone is the main effect observed in our study. This effect manifested in doses of 1 mg/kg (instillation into the nasal cavity) and 5 mg/kg (intraperitoneal injection), which, in turn, proves higher efficiency of intranasal route. Similar difference was observed when different routes of administration of morphine and opioid and some other regulatory peptides were compared [4]. Importantly, hypothalamic structures are the first exposed to the drug penetrating through the nasal mucosa. Hence, the physiological and behavioral responses to the drug depend on the route of its administration, though the direction of changes is the same [2]. In our case antagonist injected intraperitoneally most markedly modified the number of approaches to the pups, while after intranasal administration it most markedly changed the number of pup transfers.

The preoptic and supraoptic zones of the hypothalamus, amygdala, ventral tegmental area, and nucleus accumbens are the key cerebral structures responsible for parental reactions [11,12]. The concentrations of opioid receptors are high in the majority of these structures. This determines the capacity of endogenous and exogenous opioids to regulate the level of maternal motivation. The effect of morphine, inhibiting all manifestations of parental behavior and maternal aggression, is studied best of all [10,13]. Our experiments demonstrated similar effects of systemic administration of β-casomorphines (opioid fragments of caseins, penetrating into female blood from the mammary gland) [3].

Effects of opioid antagonists on the level of maternal motivation are less studied; usually the drugs of this group are used for suppressing the aftereffects of morphine and endorphins [13]. Attempt at the analysis of antagonist activity was undertaken only for naloxone [6]: the drug was injected subcutaneously (0.5 and 5 mg/kg) and intracerebroventricularly (5 and 50 μg). Both intracerebroventricular doses and higher subcutaneous dose stimulated maternal motivation: the total time spent in the nest and duration of feeding increased.

Hence, naloxone increases such components of parental behavior as the reaction latency, transfers and approaches to pups; it is effective (in low doses) after intranasal administration. This noninvasive route of drug administration is clinically perspective, in our case, for the correction of maternal motivation in postpartum psychosis and depression.

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