PHYSIOLOGY

Activation of Maternal Behavior of Albino Rats after Combined Treatment with Dopamine and Opioid Receptor Antagonists in Low Doses

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We studied the effect of D1/D2 antagonist haloperidol on maternal motivation in nursing albino rats. Haloperidol in a dose of 0.2 mg/kg significantly attenuated parental reactions and motor and exploratory activities. In a lower dose (0.1 mg/kg) the drug produced the same effect on maternal behavior (number of approaches to newborns) without reducing motor activity. The effect of low-dose haloperidol was different after naloxone treatment (0.2 mg/kg intranasally): the number of pup transfers increased significantly. The detected phenomenon indicates good prospects of combined treatment with agents modifying the cerebral dopaminergic and opioid systems as the method for correction of disorders in maternal behavior.

Key Words: *dopaminergic system; haloperidol; opioid system; naloxone; maternal behavior*

Maternal reactions are important components in the complex of congenital behavioral programs of humans and all higher animals. Many CNS structures, endocrine (progesterone, estrogens, prolactin) and neurochemical systems are involved in their realization [7]. Dopamin- and opioidergic neurons are particularly significant among the latter systems. The capacity of opioid antagonists to stimulate and of agonists to attenuate the maternal behavior was analyzed in our previous studies [2,3]. Activation of the dopaminergic system in the dorsolateral striatum associated with triggering of the parental reactions was detected [8]. Systemic treatment with apomorphine stimulated many components of maternal behavior [13]. Injection of selective D1 and D2 receptor antagonists SCH 23390 and clebopride caused a significant reduction in maternal activity of females [5]. This latter experimental situation can be regarded as a model of postpartum depression, signs of which are observed in 13% women [6].

We carried out a pharmacological analysis of the effects of systemic treatment with low doses of D1/D2 antagonist haloperidol on the intensity of parental reactions of nursing albino rat females. Correction of the detected shifts in maternal behavior by pretreatment with a threshold dose of naloxone was attempted.

MATERIALS AND METHODS

Maternal behavior was evaluated in primiparous outbred albino rat females (n=32; 270-320 g). Each

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female with its litter was kept in a separate cage with free access to water and food. The behavior was evaluated over 6 days (days 4-9 postpartum). In two first experimental series haloperidol in doses of 0.2 or 0.1 mg/kg was injected intraperitoneally 20 min before testing on days 2 and 4 of observation. On days 4, 6, 8, and 9 postpartum the animals were injected with equivalent volumes of the solvent. In the two next series of experiments we analyzed the capacity of opioid receptor antagonist naloxone in threshold doses (0.2 mg/kg intranasally) to modify the effects of high and low doses of haloperidol. Naloxone was given 10 min before injection of D1/D2 antagonist.

The level of maternal motivation was evaluated under conditions of open field (round arena, 80 cm in diameter). In each test the female was placed 3 times at the periphery of the arena for 2 min at 1-min intervals. During the first 2 min, standard values of motor and exploratory activity (ambulation, rearing, ventures from the arena wall, grooming) were evaluated at red illumination. For the next 2 min (at the same illumination) a Petri dish with three pups was placed into the center of the arena and the latency (LP) of the first approach to the dish, total number of these approaches, number of transfers of pups, LP of transfer of the first and last pups from the dish were recorded. During the third 2-min period the pups were again placed into the Petri dish, but the parental reactions were observed at bright illumination.

The parameters of maternal reactions of albino rats were stable during repeated testing, and the behavioral effects of single low doses of haloperidol and naloxone lasted no longer than several hours [2,4], therefore the same females were used as "control" and "experimental" alternatively (on different days of observation) and injected with the solvent or the test drugs respectively. The significance of shifts was evaluated using paired statistical tests (Student, Wilcoxon, ANOVA after Friedman).

RESULTS

The choice of haloperidol dose for injection was based on published data and our findings [4,10,11]. Four series of experiments were carried out for the analysis of haloperidol effects on maternal behavior. In two series D1/D2 antagonist was injected, in two other series haloperidol injection was preceded by a threshold intranasal naloxone dose (0.2 mg/kg). Our previous studies [2] showed that this dose of naloxone had a negligible effect on the intensity of maternal reactions (in contrast to, *e.g.*, 1 mg/kg).

The mean control level (postpartum days 4, 6, 8, and 9) of the number of approaches to the pups during each 2-min period varied from 4.0 to 5.9 in different series; the number of pup transfers varied from 1.2 to 2.5. The mean LP of the first approach varied from 5 to 27 sec; LP before transfer of the last pup from the dish was 90-118 sec. The manifestations of maternal motivation somewhat increased at bright illumination [3].

Injection of haloperidol in a dose of 0.2 mg/kg significantly reduced motor activity of females during the first 2 min in the open field (without pups): ambulation decreased by 34.3% ($p<0.01$). The manifestations of maternal motivation also significantly decreased (Fig. 1). The LP of approach to pups increased at red illumination from 15.4±6.0 (AM± SEM) to 35.4±8.1 sec ($p<0.05$). The number of approaches to pups decreased at red and bright illumination (from 5.3±0.8 to 3.4±0.5 and from 5.1±1.0 to 1.4±0.3, respectively; $p<0.02$). The number of transfers at bright illumination decreased from 1.2±0.5 to 0.3±0.2 ($p<0.05$). These data indicate inhibition of parental behavior under the effect of dopamine receptor antagonists [5,10].

Injection of haloperidol in a dose of 0.1 mg/kg virtually did not change motor and exploratory activities of females during the first 2 min of testing: ambulation was 103.2% of control level. Evaluation of maternal reactions at red illumination showed that the number of approaches to the pups decreased from 5.9±0.3 to 4.8±0.5 ($p<0.05$; Fig. 2) and

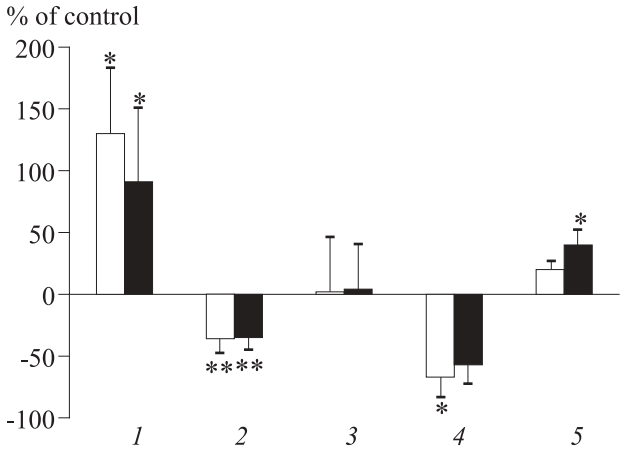

Fig. 1. Dynamics of parameters characterizing maternal motivation of nursing albino female rats after intraperitoneal injection of haloperidol (0.2 mg/kg; light bars) and haloperidol after intranasal naloxone (0.2 mg/kg; dark bars). Here and in Fig. 2: *1*) LP of the first approach to pups at red illumination; *2*) number of approaches at red illumination; *3*) number of transfers at red illumination; *4*) number of transfers at bright illumination; *5*) LP of the last pup transfer from Petri dish at bright illumination. Control: injection of the solvent. Each group consisted of 8 animals. *$p<0.05$, **$p<0.02$ compared to the control.

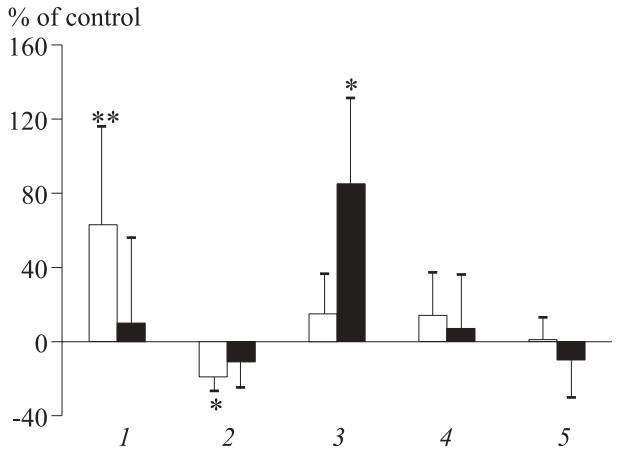

Fig. 2. Time course of parameters characterizing maternal motivation of nursing albino female rats after intraperitoneal injection of haloperidol (0.1 mg/kg; light bars) and haloperidol in the same dose after intranasal naloxone (dark bars).

LP of the approach increased from 7.5±2.2 to 12.2± 4.0 sec (p<0.02). On the other hand, a definite trend to an increase in the number of transfers and reduction of LP of the first transfer (by 20-30%) was observed.

Injection of haloperidol (0.2 mg/kg) after intranasal administration of naloxone in the threshold dose continued inhibiting motor activity of females in the absence of pups. Evaluation of maternal behavior showed a significant reduction in the number of approaches to the pups at red and bright illumination (from 4.0±0.5 to 2.6±0.4 and from 4.3±0.6 to 2.0±0.5, respectively; p<0.02; Fig. 1). Prolongation of LP before the first approach to the pups was observed in both experiments (from 26.8±8.3 to 51.1±16.1 and from 14.3±6.9 to 45.8±14.2 sec; p<0.05). Reduction of the number of transfers at bright illumination was negligible (p=0.12); on the other hand, the LP before transfer of the last newborn from Petri dish increased significantly (from 84.4±14.4 to 118.5±10.2 sec; p<0.05). The effects detected in this series of experiments are very close to the effects of haloperidol alone (0.2 mg/kg). It seems that in this case pretreatment with nonspecific opioid receptor antagonist naloxone virtually did not modify the effect of haloperidol on the behavior of nursing females.

Haloperidol (0.1 mg/kg) injected after the threshold dose of naloxone did not change motor and exploratory activity of rats. However, evaluation of maternal behavior at red illumination showed an increase in the number of the pup transfers (from 1.3±0.4 to 2.4 ±0.6; p<0.05; Fig. 2) and shortening of LP of first pup transfer (from 73.3±10.9 to 55.2± 12.0 sec; p<0.05). The decrease in the number of approaches to pups and lengthening of LP of the first approach observed after haloperidol alone (0.1 mg/kg) did not reach the level of significant changes. The results indicate reversion of the effect of haloperidol in a low dose after pretreatment with naloxone in the threshold dose (under less stressogenic conditions at red light).

Dopamine plays an important role in the expression of maternal behavior. The dopaminergic system is linked with the "motivation awakening" of postpartum female and the mesolimbic neurons in this system are activated under the effect of signals from the pups [9]. Blocking of dopamine receptors leads to reduction of parental motivation and disturbs maternal behavior, which was demonstrated in our study. Our results are in line with published data indicating, among other things, that haloperidol impairs some aspects of parental care, e.g. construction of a nest [10].

Reversion of the effects of low dose of haloperidol after pretreatment with naloxone in a threshold dose is particularly interesting. It is shown that two agents, none of which activates maternal reaction if used alone, stimulate parental behavior if used together (Fig. 2).

The mechanism of this phenomenon is most likely of a presynaptic nature. High level of opioid ligand binding was demonstrated for the substantia nigra and ventral tegmental area neuron terminals; activation of the presynaptic opioid receptors promoted inhibition of dopamine release [11]. Consequently, treatment with morphine and endorphin antagonists (naloxone) can abolish the inhibition of dopamine release. Moreover, autoreceptors are present on the dopaminergic presynaptic terminals, whose activation by the negative feedback mechanism reduces the mediator release [1]. The use of appropriate antagonists (in low doses) causes an increase in dopamine release.

In our case the summation and mutual amplification of the effects of the two drugs is highly probable: naloxone reduces the opioidergic inhibition of dopamine release, while haloperidol attenuates the inhibitory effect of autoreceptors. The effect of haloperidol in a higher dose seems to develop mainly through the postsynaptic D1 and D2 receptors. They are blocked and the level of maternal motivation decreased. Pretreatment with naloxone in this case does not modify the effects of D1/D2 antagonist (Fig. 1). Our results, along with our previous data [2,3] create the basis for the development of methods for correction (stimulation or attenuation) of the level of maternal motivation in clinical practice.

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