Effect of Single and Repeated Injections of Selective D_2-Antagonist Clebopride on Maternal Behavior of Albino Rats

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This study examined the effect of clebopride at low concentration that did not modify the motor activity on the parental care in female albino rats. Single injection of the drug attenuated the parental care reactions on postinjection minute 20, but not one day thereafter. The daily injection of the drug during the *post partum* period (1-6 days) resulted in significantly more pronounced and stable effects. The data obtained substantiated the views on the major contribution of D_2-receptors in the development of behavioral manifestations of puerperal depression.

Key Words: *parental care; D_2-antagonist*; *clebopride*; *puerperal depression*

The parental (maternal) care reactions are extremely important biological features directed to maintain the behavioral components of mammals. The combined effects of pregnancy, labor, and lactogenic hormones [3,6,12] as well as the sensory stimuli provided by pups [12] are needed to efficiently stimulate these reactions. Anatomically, the parental care originates from such structures as the medial preoptic area, *n. accumbens*, and the ventral tegmental area (VTA) [3,8,9]. The neurotransmitter basis of maternal behavior is mostly composed of dopaminergic and the brain opioid receptor systems [1,4,11]. The agonists and antagonists of the corresponding receptors are considered as promising agents to cope with disturbances in parental care (post partum depression) [14] and to model the related symptoms in experimental animals [1,5]. This problem is also examined in the studies that analyze the effects of antipsychotic preparations and antidepressants administered to women during postpartum (PP) period [7,10].

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We previously studied these problems with haloperidol, a non-specific antagonist of dopamine receptors with the greatest affinity to D_2-sites. In high doses it suppressed both parental care of albino rats and their motor activity. In contrast, the low doses of haloperidol specifically affected only the paternal care [1]. In this study, the same method was used to examine clebopride, a selective D_2-antagonist, after its single and repetitive administration for 6 days.

MATERIALS AND METHODS

The study was carried out on primipara random-bred albino rats (n=36) with the mean body weight of 280 g. Each dam and her brood were kept in individual case on unrestricted food and water. The behavioral tests were performed in an open field arena (OpenScience). During a test, a rat was placed three times at the edge of the arena, thereafter the motor activity was measured without pups under the red light (2 min). Then the pups were placed into the center of arena in a Petri dish, and the parental care was examined successively under red (2 min) and bright (2 min) illumination. We measured the latency of the first ap-

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proach to the pups, the number of visits, the latency of transferring the pups, the number of transfers, *etc*. The methodical details are described somewhere [1,2,5].

Examination of parental behavior was performed on PP days 4 to 9. Clebopride, a selective D_2-antagonist (Sigma) was injected intraperitoneally in doses of 0.2 and 0.5 mg/kg (1 ml/kg). In experimental series I and II clebopride was injected 20 min before testing on PP days 5 and 7 and in other days the dams received physiological saline. The averaged data recorded on the injection days were compared with the control averaged data sampled during other examination days. Significance was assessed with Student's and Wilcoxon paired tests. The rats of experimental group I and II were treated with, respectively, 0.5 mg/kg (n=9) and 0.2 mg/kg (n=10) clebopride. In series III with repeated administration of the drug, clebopride (0.2 mg/kg) was injected daily on PP days 1 to 6. Control dams (n=8) received the solvent in an equivalent volume. On PP days 4-6, when the parental care parameters were measured, the injections were performed immediately after testing. There were no injections on days 7-9.

The data were processed statistically by the Student and Mann–Whitney tests for independent samples using Statistica 6.1 software.

RESULTS

In experimental series I, a selective D_2-antagonist clebopride was injected to lactating rat dams in a relatively high dose of 0.5 mg/kg. In this series, the drug significantly moderated not only the parental care, but also total behavioral activity (Fig. 1, *a*). Clebopride decreased the examined parameters as follows: the running length (horizontal motor activity for 2 min) from 34.2±3.3 to 21.3±3.0 crossed arena segments, the number of departures from arena wall from 2.8±0.5 to 1.9±0.5, and the number of visits to arena center from 1.6±0.3 to 0.8±0.2. The parameters were measured after injection of physiological saline and the drug, respectively. Clebopride significantly decreased the number of approaches to pups under dim red light (from 5.1±0.5 to 3.6±0.4) or bright white illumination (from 5.1±0.5 to 2.5±0.3). In the latter case, (but not under the red light) clebopride decreased the number of pup transfers from 2.6±0.6 to 1.0±0.4 and increased the latency of the first approach to pups from 5.6±1.2 sec to 25.3±7.7 sec (p<0.05).

In experimental series II, two injections of the smaller dose of clebopride (0.2 mg/kg on PP days 5 and 7) produced virtually no effect on motor activity of the rat dams tested without pups. There was a slightly decreasing trend only in the value of the running length (p=0.11, Fig. 1, *b*). In the presence of pups under red light, clebopride also produced no effect (p>0.2 for all parameters). The only significant effect was a decrease in the number of approaches to the pups under bright white illumination from 4.8±0.2 to 2.7±0.4. Thus, only threshold effects of a small dose of clebopride (0.2 mg/kg) could be revealed as trends under any illumination, while one of them (decrease in the number of approaches to the pups) became significant under bright white light known to be a stressor for the rats. We previously showed that

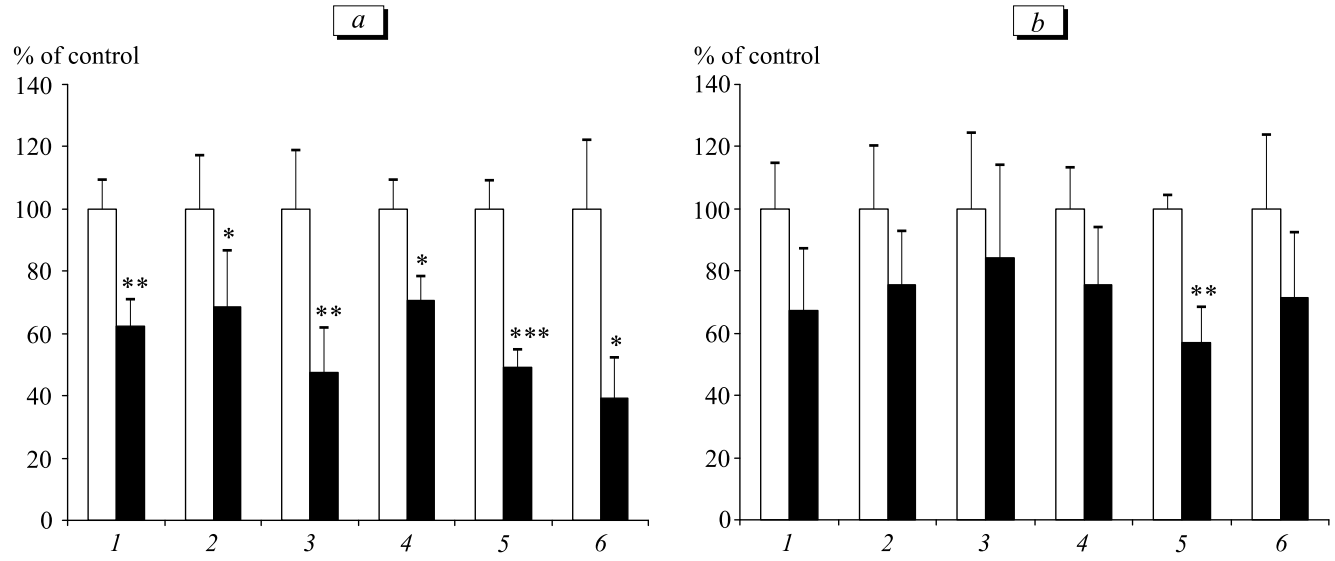

Fig. 1. Averaged parameters of female behavior in the days with clebopride injection (dark bars: *a*, 0.5 mg/kg, n=9; *b*, 0.2 mg/kg, n=10) and with injection of physiological saline (open bars). *1*) running length (the number of crossed segments); *2*) the number of departures from arena wall; *3*) the number of visits to arena center; *4*) the number of approaches to pups under red light; *5*) the number of approaches to pups under bright light; *6*) the number of pup transfers under bright light. *p<0.05, **p<0.01, ***p<0.001 compared to the control.

under stress conditions, the inhibitory effect of some drugs (*e.g.* agonists of opioid receptors) increases and outweighs the parental care despite additional activation of this care by a stressor [1]. In series III with repetitive (cumulative) administration of clebopride, we used a dose of 0.2 mg/kg, which according to the above experiments (series II) produced practically no behavioral effects after two injections made with a 2-day interval.

Moreover, two injections of clebopride in a high dose of 0.5 mg/kg (series I) produced no long-term behavioral effects, and the parameters of motor activity and parental care returned virtually to the initial values in a day after injection. Really, the second injection of 0.5 mg/kg clebopride made on PP day 7 immediately decreased the number of approaches to the pups under bright illumination to the value of 2.2±0.5. However, on the previous day 6 and on the next day 8, the values of this parameter were 5.3±0.6 and 5.4±0.6, respectively, which insignificantly differ from the control value of 5.1±0.5, but significantly differ from the value obtained on day 7 (p<0.01).

In series III with 6 daily injections of clebopride (0.2 mg/kg during PP days 1-6) we averaged two successive values of every behavioral parameter obtained on PP days 4-5, 6-7, and 8-9 (in the last case, on postinjection days 2-3). During PP days 1-6 (*i.e.*, during injection period), none of the parameters of motor activity of lactating dams demonstrated a decreasing trend. Moreover, the running length in the experimental group surpassed the control value (Fig, 2, *a*; p=0.16-0.26 on various days). Assessment of parental reactions under the red light revealed the significant intergroup differences only on PP days 4-5 and only for the number of pup transfers (in experimental group this number was only 12% in comparison with the control, Fig. 2, *b*). Under the bright illumination, a significant intergroup difference was revealed in one more parameter: the experimental dams not only four times less often transferred the pups (Fig. 2, *c*), but they also two times less often visited them (p<0.02). However, in this case the differences were significant only on PP days 4-5. On PP days 6-7, the parental behavioral parameters in the experimental group approximated the control values (p=0.25-0.31). On PP days 8-9, the intergroup differences virtually disappeared.

Some authors reported that either blockade of dopamine receptors or destruction of dopaminergic

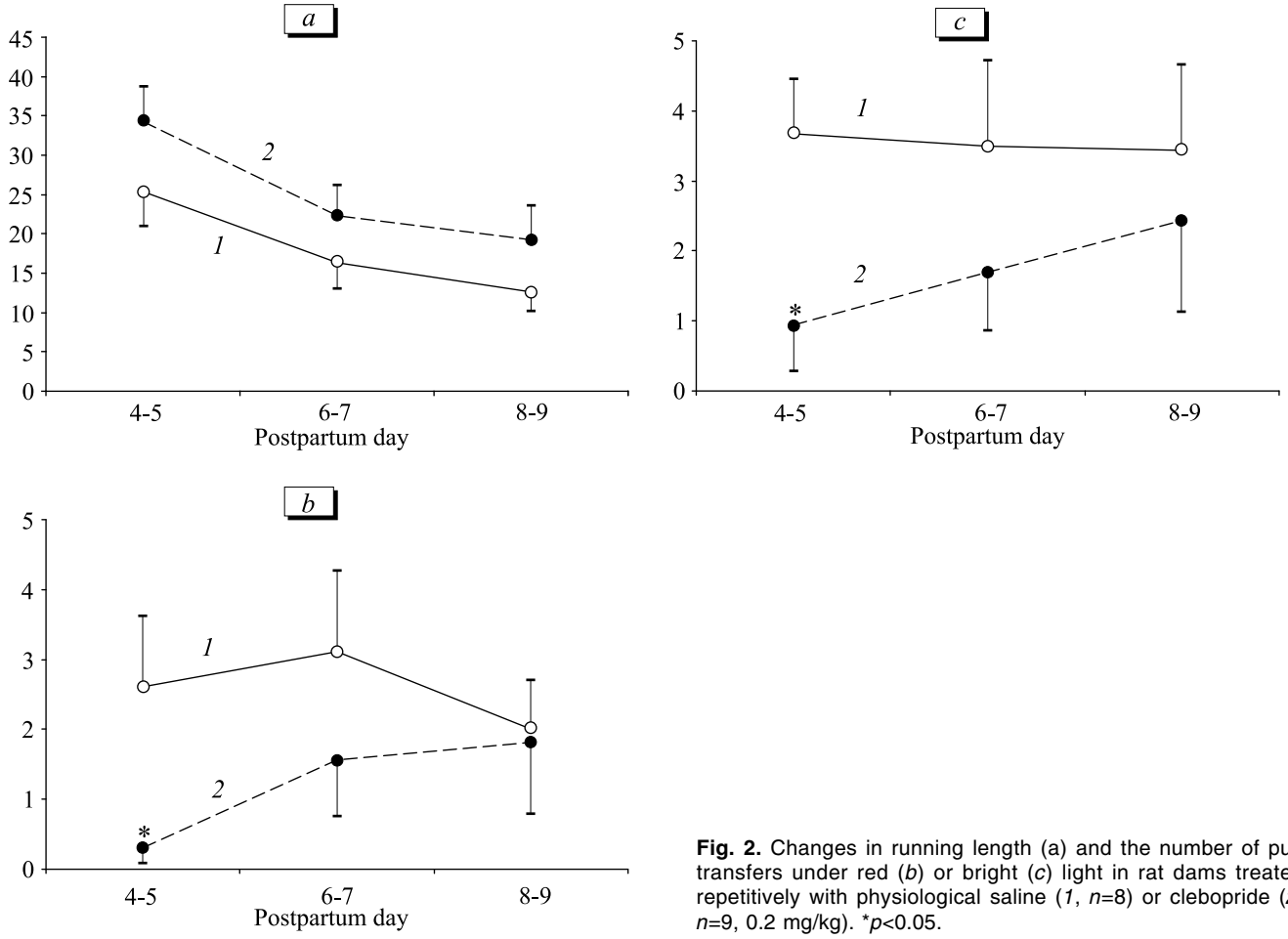

Fig. 2. Changes in running length (a) and the number of pup transfers under red (*b*) or bright (*c*) light in rat dams treated repetitively with physiological saline (*1*, n=8) or clebopride (*2*, n=9, 0.2 mg/kg). *p<0.05.

neurons (predominantly in VTA) diminished parental motivation in females and inhibited the corresponding behavioral manifestations [4,9]. Our study corroborates these data and established the dose of a selective D_2-antagonist, which after a single systemic injection reduced the intensity of parental care while producing no significant effect on motor activity of rat dams.

Since in clinical practice the puerperal depressive syndrome and the corresponding use of the antipsychotic medication by recently confined women relate to persistent changes in activity of the dopaminergic system, we performed the experiments with repeated administration of clebopride to lactating dams. As a result, we observed more pronounced (in comparison with the experiments with a single injection of the tested drug) inhibition of the parental care, which was documented with a greater number of parameters and was observed not only under the bright illumination, but also under the red light in the open field. Since the effects of daily injections of the drug were tested one day postinjection, these observations attest to more long-term and persistent behavioral alterations than those induced by a single injection. At this, the signs of puerperal depression were not accompanied with moderation of exploratory activity or with the changes in anxiety level. Moreover, in these experiments clebopride did not inhibit utilization of sucrose (data are not presented).

The significant negative effect of repeated injections of clebopride on parental care was revealed only during the first two days of recording (postpartum days 4-5). Further weakening of the intergroup differences may result from acquired tolerance to the drug followed by its withdrawal (postpartum days 7-9). The learning processes should be taken into consideration as well, because they can compensate for initially low level of parental motivation of the experimental dams. Similar phenomenon we observed in previous study with WAG/Rij rat females whose behavior can be considered as a genetic model of the puerperal depression: after repeated placing of the dams into the open field environment, their parental care reactions became less significantly different from the control (Wistar) rats [2,5].

Overall, our data corroborate the view on prevailing contribution of D_2-receptors to the development of behavioral manifestations of parental care. Moreover, they substantiate a novel model of puerperal depression provoked with a pharmacological agent, which can be further employed to test the medical drugs with potential ability to correct this sever psychotic state.

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