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Behavioral Effects of Neonatal Treatment With Clomipramine, Scopolamine, and Idazoxan in Male Rats

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VELAZQUEZ-MOCTEZUMA, J., A. AGUILAR-GARCIA AND O. DIAZ-RUIZ. *Behavioral effects of neonatal treatment with clomipramine, scopolamine, and idazoxan in male rats.* PHARMACOL BIOCHEM BEHAV 46(1) 215–217, 1993.—It has been suggested that REM sleep deprivation due to the administration of clomipramine, during early developmental stages, results in dramatic behavioral changes during adulthood. One of the main alterations is the impairment of masculine sexual behavior (MSB). Clomipramine increase monoaminergic availability at the synaptic level and also suppresses REM sleep. This study was performed to compare the effect on masculine sexual behavior of three different neonatal treatments: clomipramine, which increases monoaminergic availability at the synaptic level and suppresses REM sleep; scopolamine, a cholinergic antagonist that suppresses REM sleep; and idaxozan, a selective adrenergic agonist. Subjects (Ss) were treated neonatally and tested for masculine sexual behavior during adulthood with standard techniques. Results obtained with clomipramine administration showed a marked impairment of MSB, mainly reflected by the decrease in the percentage of active Ss and the decrease in the percentage of Ss reaching ejaculation. In contrast, idaxozan and scopolamine produce a facilitation of MSB. The effect of idaxozan was not significantly different when compared to saline control rats. The effect of scopolamine was greater, and the percentage of Ss reaching ejaculation was significantly larger than saline control. These results suggest that the alterations of sexual behavior induced with neonatally administered clomipramine are not due to early REM sleep deprivation. As idaxozan did not replicate clomipramine results, it is also possible that noradrenergic transmission is not involved in the generation of these effects. It remains possible that the serotonin system could be responsible for the impairment of sexual behavior due to neonatal clomipramine treatment.

Forced swim test Clomipramine Scopolamine Idazoxan Sexual behavior REM sleep
Animal model of depression

IT has been proposed that the behavioral changes observed in rats neonatally treated with clomipramine reflect a state of depression (15,19). The main features on which this notion is based are the reduction of exploratory behavior in open field (5), the decrease of masculine sexual activity (5,8), the alterations in the sleep pattern, particularly the decrease in latency, as well as the increase in duration of REM sleep (5,18), the increased immobility time in the Porsolt test (15), decreased pleasure-seeking activities (17), and the positive effects of drugs that improve endogenous depression (16).

In the original reports, the authors were testing the hypothesis that the REM sleep phase plays an important role in normal brain development (5). They suggested that the behavioral changes observed during adulthood of clomipramine neonatally treated rats are due to early inhibition of the REM sleep state (6).

Clomipramine inhibits monoaminergic reuptake, increasing monoamine availability, mainly serotonin, at postsynaptic level (1). It is possible, however, that increased monoaminergic transmission induced by clomipramine elicits both the suppression of REM sleep and long-term behavioral effects during adulthood as two independent effects.

In this study we analyzed the effect of early REM sleep deprivation by cholinergic blockade with scopolamine, as well as the effect of increasing adrenergic availability with the alpha$_2$ receptor blocker idazoxan.

METHOD

Wistar rats were used in this study. Three days after birth, male pups were cross-fostered and female pups were eliminated from the study. Each lactating female received four pups. On postnatal days 8 through 21, each pup was injected SC with clomipramine hydrochloride (15 mg/kg, n = 14), scopolamine (3.39 mg/kg, n = 10), idazoxan (2.40 mg/kg, n = 10), or the equivalent volume of saline solution (75 μl, n = 21). At 35 days of age, animals were separated from their mothers and kept in a cage in groups of five, all with the same

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treatment. Rats were maintained in a room with an inverted light cycle (off: 9:00 a.m.; on: 7:00 p.m.), with food and water available ad lib.

Sexual Behavior

At 3 months of age, males were tested for masculine sexual behavior on three occasions, with at least a 1-week period between observations. During the dark phase and under dim red lights, males were placed in an arena. After a 5-min habituation period, a stimulus female brought into estrus by estradiol treatment was introduced. The observation period lasted for 30 min and the parameters recorded were: latencies and frequencies of mounts, intromissions, and ejaculations. The hit rate (number of intromissions/number of mounts + number of intromissions), the average interintromission interval (ejaculation latency/number of intromissions), and the average intercopulatory interval (ejaculation latency/number of mounts + number of intromissions) were calculated.

Forced Swim Test

After the sexual behavior tests were done, all animals were submitted to the forced swim test (FST), which consists in dropping a subject into a glass cylinder with a column of 15 cm of water, from which it cannot escape. The time during which the animal remained immobile was assessed during 15 min the first day and during 5 min the second day (24 h later). The animal was judged to be immobile when it was not making any movements or when it was making only slight postural adjustments that allowed it to keep its head or nose above the water level (9).

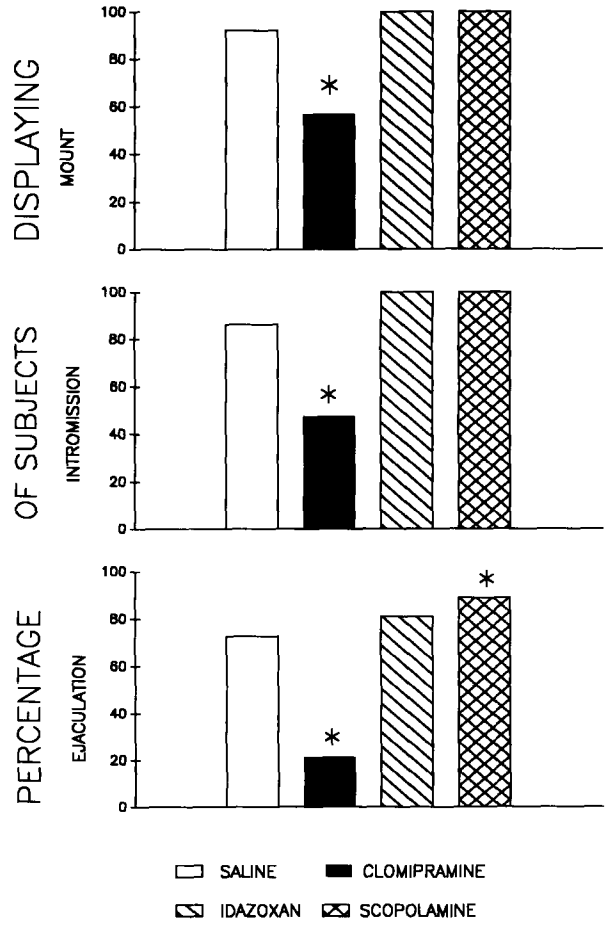

FIG. 1. Effect on sexual activity of neonatal treatment with clomipramine, idazoxan, or scopolamine. Chi-square, $*p < 0.001$ compared to saline group.

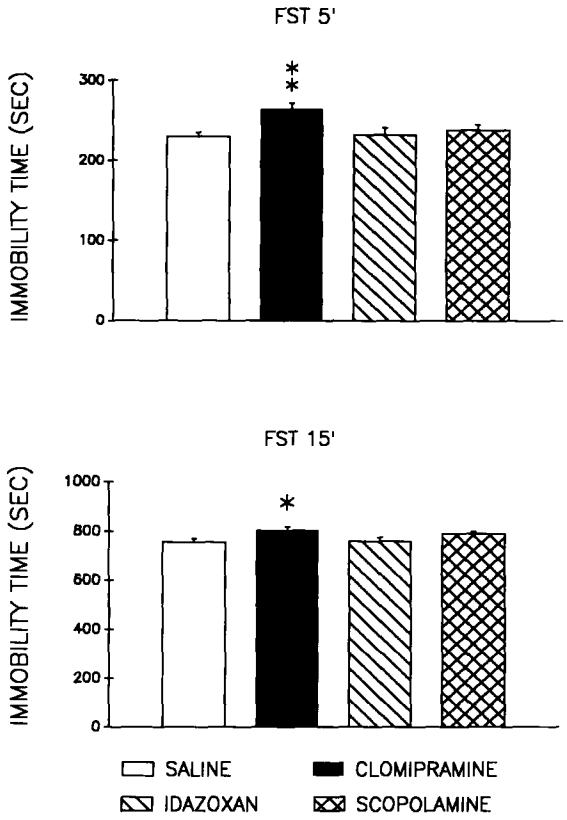

FIG. 2. Immobility time displayed during the forced swim test by adult rats neonatally treated with clomipramine, idazoxan, or scopolamine. Mean ± SEM. Student *t*-test, $*p < 0.02$ compared to saline group; $**p < 0.001$ compared to saline group.

Statistical Analysis

Sexual behavior data were analyzed by using the Kruskal–Wallis ANOVA, followed by the Mann–Whitney *U*-test. For proportions, chi-square test was used. Immobility during the FST was analyzed using the Student's *t*-test. For the sexual behavior test and immobility time assessment, the observers were unaware of the treatments.

RESULTS

Figure 1 shows the percentage of subjects displaying mounts, intromissions, and ejaculations. As can be seen, clomipramine treatment significantly reduced the percentage of subjects displaying mounts, intromissions, and ejaculations, while treatment with scopolamine and idazoxan seemed to induce a trend towards the augmentation of the percentage of active subjects. The scopolamine group displayed a significantly higher percentage of subjects reaching ejaculation.

When analyzing the behavioral features of active subjects,

there were no significant differences in any of the parameters recorded. Concerning immobility time during the FST, Fig. 2 shows the results obtained during the 5-min and the 15-min tests. Clomipramine-treated animals displayed a significant increase of immobility. On the other hand, the scopolamine and idazoxan treatments were unable to increase immobility time.

DISCUSSION

The present results strongly suggest that the behavioral features displayed during adulthood by rats neonatally treated with clomipramine are not due to early REM sleep deprivation and also are not due to increased availability of catecholamines at the synaptic level.

It is well known that different neurotransmitter systems are regulating the onset and maintenance of REM sleep [for review see (3)]. The cholinergic system plays a relevant role in this regulation. Cholinergic blockade at muscarinic receptors with atropine or with scopolamine completely suppresses the REM sleep stage [for review see (13)]. Moreover, elucidation of the involvement of the different muscarinic receptor subtypes in the control of REM sleep has been expanding constantly (2,4,10,12,14). In the present study, a dose of scopolamine higher than that used to eliminate REM sleep was administered. The effects observed, however, did not reproduce those observed with clomipramine, and in the case of sexual behavior there was stimulation instead of the inhibition observed with clomipramine. According to Mirmiran et al. (5,7), suppression of REM sleep by the selective alpha$_2$ agonist, clonidine, during early stages induces the same behavioral effects observed with the administration of clomipramine. Nevertheless, clonidine and clomipramine have the opposite action on the adrenergic system. As mentioned above, clomipramine increases synaptic availability of monoamines by blocking their reuptake; on the other hand, clonidine decreases synaptic availability of catecholamines by stimulation of the alpha$_2$ presynaptic receptors. In addition, REM sleep deprivation by the pendulum technique did not induce behavioral changes during adulthood (6).

In the present study, idazoxan, an alpha$_2$ receptor blocker, did not reproduce the effects of clomipramine. It has been reported recently that idazoxan is a more potent alpha$_2$ blocker than yohimbine (11). Alpha$_2$ blockade by idazoxan should increase the adrenergic activity. Nevertheless, no effects on adult behavior were observed due to the early administration of idazoxan.

In conclusion, the present results abolish the possibility that the effects of early administration of clomipramine on adult behavior are due to REM sleep deprivation or to the increased availability of catecholamines at the synaptic level. Further research is needed to evaluate the participation of the serotoninergic system.

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