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Effect of Oxotremorine on the Acquisition of a Conditioned Avoidance Response Is Modified by the Estrous Cycle, Ovariectomy, and Estradiol Replacement in Rats

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DÍAZ-VÉLIZ, G., N. DUSSAUBAT AND S. MORA. *Effect of oxotremorine on the acquisition of a conditioned avoidance response is modified by the estrous cycle, ovariectomy, and estradiol replacement in rats.* PHARMACOL BIOCHEM BEHAV 51(2/3) 279–283, 1995.—This study was designed to evaluate the influence of the hormonal status of the rat on the effects of a potent reversible muscarinic agonist, oxotremorine, on the acquisition of conditioning avoidance responses (CARs) and the performance of some spontaneous motor behaviors. Oxotremorine (OXO 50 and 100 μg/kg, intraperitoneally) given 5 min before testing improved active conditioned avoidance in intact female rats at estrus and in ovariectomized rats after estradiol replacement, and impaired performance in female rats at diestrus and after ovariectomy without estradiol replacement. No significant differences due to hormonal status of the rat in some spontaneous motor behaviors were observed. In fact, OXO in this dose range failed to induce significant changes in spontaneous motor activity, the number of rears diminished, and the time spent in grooming behavior increased in all groups studied. These results provided behavioral evidence for the hypothesis that central cholinergic activity is function of the hormonal status of the animal. Relationships between ovarian hormones and cholinergic system are discussed.

Cholinergic system Oxotremorine Estrous cycle Conditioned avoidance responses Estradiol Ovariectomy

IN FEMALE RATS, the acquisition of conditioned avoidance responses is influenced by the hormonal changes that occur during the estrous cycle. Previously, we reported that this behavioral response is facilitated at diestrus but is deteriorated at estrus and metestrus (8). Furthermore, ovariectomy enhances the performance of avoidance and the systemic administration of a single dose of estradiol benzoate (EB; 2 μg) reduces this behavior (9). Other authors have reported effects of sex, estrous cycle, and gonadal hormones upon acquisition of CARs without conclusive results (1,20,24).

The important role of central cholinergic neuronal systems in learning and memory has been recognized for a number of years (3,12,22). There is evidence suggesting that brain cholinergic muscarinic systems are important for acquisition (2), consolidation (25), and retrieval (5) of avoidance behavior in animals. It has been further postulated that cholinergic activity within the rat brain has a significant influence on female sexual behavior (7,10). Moreover, intracerebral administration of muscarinic agonists such as carbachol and oxotremorine facilitate lordosis in ovariectomized rats primed with low doses of estrogen (10).

Evidence is also available that the cholinergic system is organized in a sex-dependent way, and that is influenced by the female's estrous cycle (14,16). In addition, it has been reported that administration of estradiol increases choline acetyltransferase activity (13,15) and the number of cholinergic muscarinic binding sites in the rat brain (11,18). These various findings strongly suggest that estrogen may participate in the regulation of cholinergic neurons function.

The purpose of this study was to investigate further

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whether hormonal status of a rat might influence nonsexual behavioral effects of a cholinergic drug. We studied the influence of a potent reversible muscarinic agonist, oxotremorine (OXO), upon avoidance conditioning and spontaneous motor responses, in intact cycling female rats at two stages of the estrous cycle and also in ovariectomized rats primed or not with estrogen.

METHOD

Animals

A total of 120 female Sprague-Dawley rats, weighing 180–200 g, were housed in groups of six per cage under a 12 L : 12 D (lights were on from 0800 to 2000 h) in a temperature-controlled vivarium, with free access to food and tapwater.

Vaginal smears were taken daily from 60 intact female rats to determine the different stages of the estrous cycle. Only rats exhibiting three or more consistent 4-day estrous cycles were used. Because a previous report (8) showed great differences in the acquisition of CARs between diestrous and estrous females, only these phases were considered for the pharmacologic treatment.

Another group of 60 female rats was bilaterally ovariectomized under light ether anesthesia. Fourteen days after surgical removal of the ovaries, rats were randomly divided into two groups that received either 2 μg estradiol benzoate or corn oil vehicle (0.2 ml/rat), injected subcutaneously (SC) into the dorsal region of the neck 48 h before the administration of oxotremorine.

Drugs

Oxotremorine sesquifumarate (OXO; Research Biochemical Inc., Natick, MA) was dissolved in 0.9% saline. Rats were injected with OXO [50 or 100 μg/kg, intraperitoneally (IP)] 5 min before the behavioral testing in a volume of 1 ml/kg. Saline injections in equal volume served as controls. Each animal was injected with the muscarinic agonist or saline only once and tested between 1000 and 1400 h.

Active Avoidance Conditioning

The conditioning experiments were carried out with a two-way shuttle-box (Lafayette Instrument Co., Lafayette, IN) composed of two stainless-steel modular testing units, which were equipped with an 18-bar insulated shock grid floor, two 28-V DC lights, and a tone generator (2800 Hz; Mallory Sonalert, Lafayette, IN). Electric shocks were provided to the grid floor by a Master shock supply (Lafayette Instrument Co.). The rats were individually placed in the shuttle-box and were trained over 50 trials. Each trial consisted of the presentation of a tone which after 5 s was overlapped with a 0.20-Ma foot-shock until the animal escaped to the opposite chamber with a maximum shock duration of 10 s. A conditioned avoidance response (CAR) was defined as a crossing within the first 5 s (tone).

Spontaneous Motor Activity

The animals were individually placed in a Plexiglas cage (30 × 30 × 30 cm), inside a soundproof room. Spontaneous locomotor activity was monitored automatically through an Opto-Varimex Mini (Columbus Instruments, Columbus, OH) and the following responses were recorded simultaneously: number of times each animal reared and the time (in seconds) spent in grooming behavior. Each animal was observed continuously for 30 min, via a videocamera connected to a VHS tape-recorder. Scores were generated from live observations, and video sequences were used for subsequent reanalysis.

Statistical Analysis

Results are expressed as mean ± SEM. All data were analyzed by two-way analysis of variance (ANOVA) followed by posthoc Newman-Keuls's multiple comparison test. A probability of 0.05 was accepted as statistically significant.

RESULTS

Active Avoidance Conditioning

Figure 1 shows that the avoidance conditioning in saline control animals was similar in rats at diestrus and OVX female rats, but this behavior was seriously deteriorated ($p < 0.001$) in rats at estrus and after a single injection of EB to OVX rats. Analysis of variance revealed a significant effect of OXO treatment [$F(2, 108) = 6.511, p < 0.01$] and hormonal status of the rat [$F(3, 108) = 25.628, p < 0.01$] on the acquisition of CARs. The interaction between dose of OXO and hormonal status was also significant [$F(6, 108) = 13.038, p <$

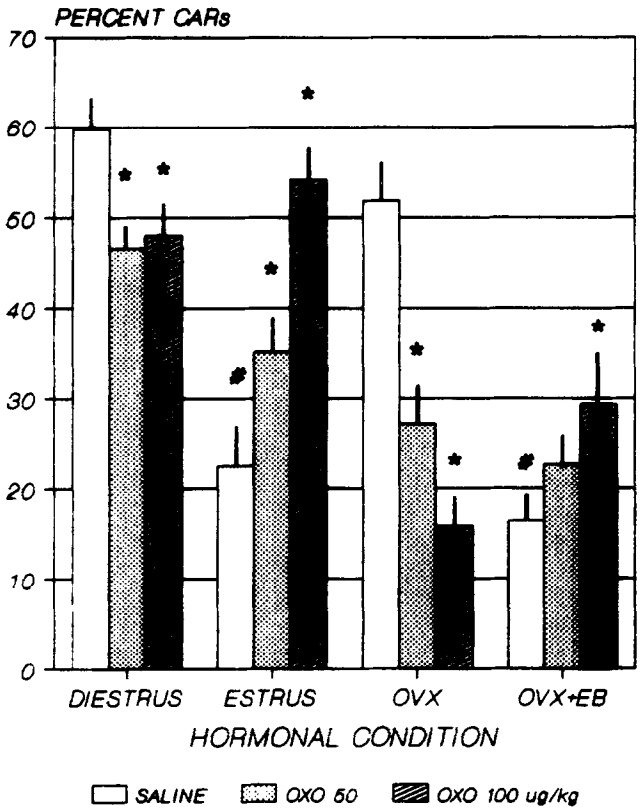

FIG. 1. Influence of hormonal status (intact female rats at diestrus and estrus, ovariectomized = OVX; and ovariectomized with estradiol replacement = OVX + EB) on the effects of oxotremorine (OXO) on the acquisition of conditioned avoidance responses (CARs). Each bar represents the mean ± SEM of the percentages of CARs of 50 trials. Comparisons were made using two-way ANOVA followed by posthoc Newman-Keuls test. *Significantly different compared with its saline control group; #$p < 0.001$ comparing diestrus vs. estrus or OVX vs. OVX + EB rats. $n = 10$/group.

0.01]. Posthoc comparisons of means using Newman-Keuls analysis indicated that administration of both doses of OXO significantly improved the acquisition of CARs in intact female rats at estrus ($p < 0.05$ and $p < 0.001$, respectively). Only at the highest dose (100 μg/kg) did OXO enhance this behavior in OVX rats with estradiol replacement ($p < 0.05$). However, both doses of OXO significantly impaired the conditioned response in female rats at diestrus ($p < 0.05$) and in OVX rats without estradiol treatment ($p < 0.001$).

Spontaneous Motor Bevavior

Locomotor activity. In the overall ANOVA, neither hormonal status [$F(3, 108) = 0.357$, NS] nor OXO treatment [$F(2, 108) = 1.8967$, NS] exerted a statistically significant effect on locomotor activity (data not shown).

Rearing behavior. Analysis of variance showed only a significant OXO treatment effect for rearing behavior [$F(2, 108) = 43.130$, $p < 0.01$]. Newman-Keuls multiple comparisons indicated that both doses of OXO significantly decreased this behavior in rats from all experimental groups studied compared with the control animals ($p < 0.001$) (Fig. 2).

Grooming behavior. Figure 3 shows the data from the time spent in grooming behavior. ANOVA revealed significant effects of OXO treatment [$F(2, 108) = 56.466$, $p < 0.01$]. Newman-Keuls multiple comparisons indicated that OXO was able to stimulate this behavior significantly in all the experimental groups ($p < 0.001$).

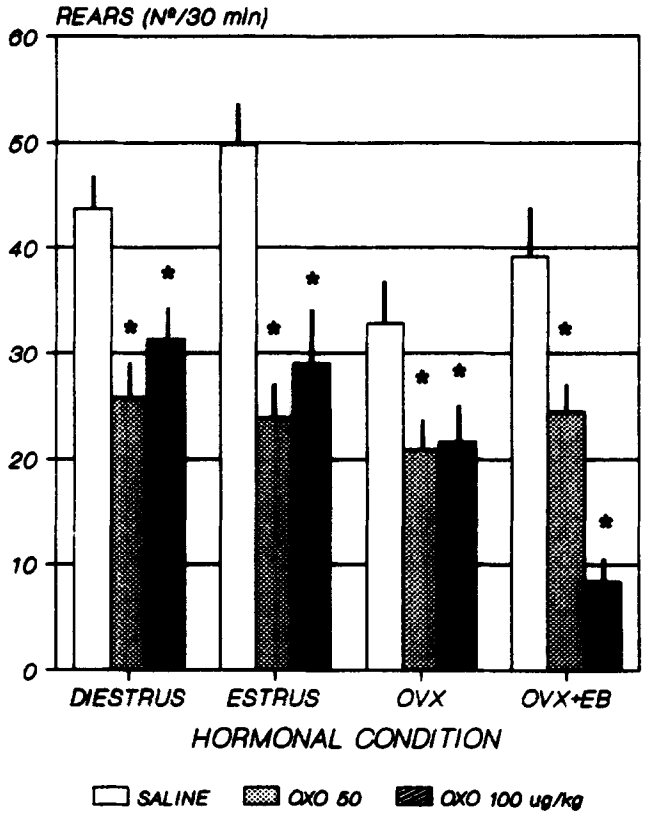

FIG. 2. Influence of hormonal status (intact female rats at diestrus and estrus, ovariectomized = OVX; and ovariectomized with estradiol replacement = OVX + EB) on the effects of oxotremorine (OXO) on rearing behavior. Each bar represents the mean ± SEM of the number of rears during 30 min of observation. Comparisons were made using two-way ANOVA followed by posthoc Newman-Keuls test. *$p < 0.001$ compared with its saline control group. $n = 10$/group.

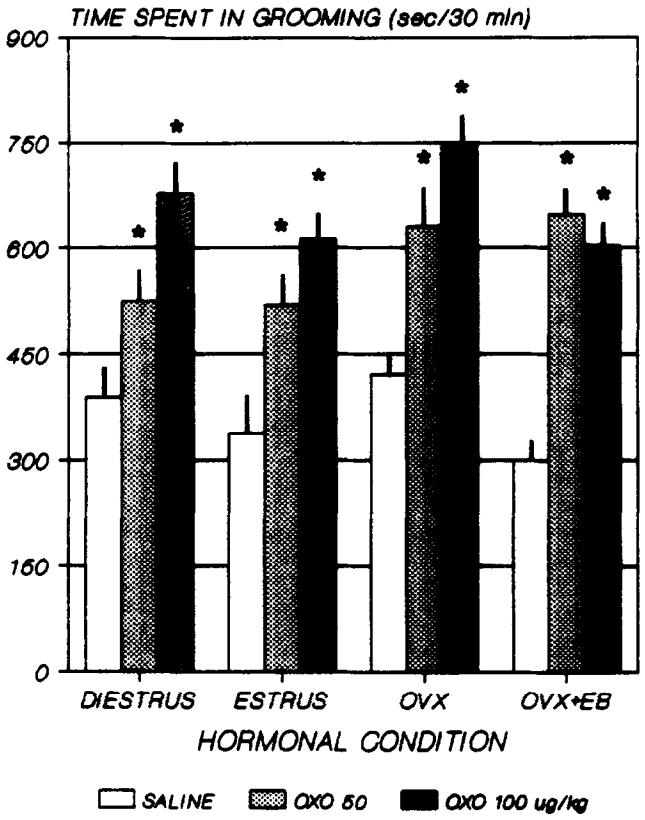

FIG. 3. Influence of hormonal status (intact female rats at diestrus and estrus, ovariectomized = OVX; and ovariectomized with estradiol replacement = OVX + EB) on the effects of oxotremorine (OXO) on grooming behavior. Each bar represents the mean ± SEM of the time (s) spent in grooming behavior during 30 min of observation. Comparisons were made using two-way ANOVA followed by posthoc Newman-Keuls test. *$p < 0.001$ compared with its saline control group. $n = 10$/group.

DISCUSSION

The main findings of the present study were that the acquisition of CARs is influenced by hormonal changes that occur during the estrous cycle, after ovariectomy, and after estradiol administration, and that the hormonal status of the animal can have direct consequences for the action of drugs influencing cholinergic transmission.

The results presented here support previous evidence revealing that avoidance conditioning is influenced by the female's estrous cycle and by the estradiol administration to ovariectomized rats (8,9). We have reported that whereas acquisition of CARs is facilitated during diestrus and almost suppressed at estrus, estradiol replacement induces a severe impairment of this avoidance response in OVX rats (8,9). In the current study large effects of OXO on conditioned behavior were found in females rats according to their hormonal status. Interestingly, administration of OXO improved the conditioned avoidance behavior in cycling female rats at estrus

and in OVX rats primed with estrogen, but the same dose of this cholinergic drug impaired the acquisition behavior in rats at diestrus and in OVX rats without EB replacement. These findings suggest that the action of OXO could be influenced by estradiol levels, as the stimulant effects of OXO were observed only late after the peak plasma concentration of estradiol—that is, during estrus (6) and 48 h after a single injection of EB to OVX rats (9). These results cannot be explained by changes in pain sensitivity or motor activity. Although conditioned avoidance can vary according to the foot-shock intensity (4), in our experimental conditions no significant differences were observed between the foot-shock thresholds applied to the different groups. On the other hand, the improvement in the acquisition of a conditioned response induced by OXO in intact rats at estrus and in OVX rats treated with EB cannot be explained by an increase in motor activity, considering that the doses of OXO used in this study significantly enhanced conditioning without modifying motor activity.

Pharmacologic studies have demonstrated that the central cholinergic neuronal system may be functionally involved in learning and memory processes (3,12,22). In general, drugs that increase central cholinergic activity enhance performance on memory tasks (2,5,12), presumably by mimicking the action of acetylcholine or increasing acetylcholine concentrations at the muscarinic receptor. It has been postulated that positive interactions with cognitive performance might well be expected from agonists at the M2 muscarinic receptors and that it is possible completely to antagonize the amnestic effects of a nonselective muscarinic antagonist with an M2 agonist (23). In the present study, OXO, a full agonist at the M2 receptor (23) was able to antagonize the alterations in avoidance conditioning during estrus and in OVX rats primed with EB. The effects of OXO on cholinergic function might be related to the reactivity of muscarinic receptors which, in turn, could be modulated by estradiol. Evidence is available that the cholinergic system is organized in a sex-dependent way and that it is influenced by the female's estrous cycle (14,16). Studies in the rat brain have shown that the activity of choline acetyltransferase is responsive to the levels of circulating female gonadal hormone estradiol (13,15). In addition, the administration of estradiol increases the number of cholinergic muscarinic binding sites in the rat brain (11,18). Furthermore, muscarinic receptor binding in certain brain regions is highest at proestrus, when estrogen levels are also highest (17). In our experimental conditions the impairment in acquisition correlated with a decay in estrogen levels during estrus or 48 h after estradiol in OVX rats. This impairment could be considered part of an estrogen withdrawal syndrome that is prevented by OXO. Although the gonadal hormonal status appears to be able to modulate behavioral responses to central cholinergic stimulation in animal models, the precise mechanism that underlies behavioral differences across the estrous cycle in female rats or after estradiol treatment remains unclear. It is possible that female sex hormones may act directly at the level of cholinergic receptors, or influence other neurotransmitter systems that may in turn modulate cholinergic activity. In addition, alteration of the metabolism of drugs by various hormonal conditions has not been ruled out as a possible mechanism.

The present data show that the acquisition of CARs was the most prominent gonadal hormone-dependent response to the cholinergic experimental manipulations. Although rearing behavior decreased and grooming behavior was enhanced after treatment with OXO, these effects were not influenced by the hormonal status of the rat. These results suggest that cognitive abilities are more sensitive than motor behaviors to manipulation of the cholinergic system. There are reports considering the possibility that dopamine receptors could be more involved in the expression of both rearing (19) and grooming behavior (21).

In conclusion, the present findings provide compelling evidence that OXO can significantly enhance or impair performance of a conditioned avoidance behavior in rats, according to the hormonal status of the rats at the time of behavioral testing. This study also contributes to a better understanding of the complex interaction between gonadal hormones and neurotransmitter systems.

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