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Muscarinic and Nicotinic Influences on Masculine Sexual Behavior in Rats: Effects of Oxotremorine, Scopolamine, and Nicotine

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RETANA-MARQUEZ, S., E. DOMINGUEZ SALAZAR, AND J. VELAZQUEZ-MOCTEZUMA. *Muscarinic and nicotinic influences on masculine sexual behavior in rats: Effects of oxotremorine, scopolamine, and nicotine.* PHARMACOL BIOCHEM BEHAV **44**(4) 913–917, 1993.—In this study, the role of cholinergic systems in the regulation of male sexual behavior was analyzed by different approaches. Both muscarinic agonists and antagonists, as well as a nicotinic agonist, were administered to sexually experienced male rats. In Experiment 1, oxotremorine (OXO), a muscarinic agonist, decreased the intromission frequency and ejaculatory latency in a dose-dependent way. Moreover, an increase in ejaculatory frequency was observed. In Experiment 2, the muscarinic antagonist scopolamine (SCO) produced a dose-related impairment of sexual behavior, decreasing the percentage of sexually active males. The smaller doses of SCO delayed the initiation of sexual behavior and decreased ejaculatory frequency. In an attempt to analyze the effect of muscarinic supersensitivity on sexual behavior, in Experiment 3 a long-term blockade of muscarinic receptors (SCO for 17 days) was followed by OXO administration. Animals displayed a significant increase of mount frequency, which results in the decrease of both the hit rate and ejaculatory frequency. In Experiment 4, six doses of nicotine were acutely administered. Only the higher doses (0.4, 0.8, and 1.6 mg/kg) induced a decrease in intromission frequency, although no significant differences were found in any other parameter. These results strongly suggest that cholinergic participation in masculine sexual behavior regulation is mediated mainly through muscarinic system.

Cholinergic regulation Muscarinic receptors Scopolamine Oxotremorine Nicotine Sexual behavior

THE participation of the cholinergic systems in the regulation of male sexual behavior remains poorly understood. Behavioral studies have shown that both the muscarinic and nicotinic systems are involved [for review, see (3)]. Oxotremorine, a selective muscarinic agonist, produces a reduction in the ejaculation threshold when administered systemically (1), as well as when microinjected directly into the medial preoptic area (mPOA) (3,7). Oxotremorine and carbachol (another cholinergic agonist) delay the initiation of masculine sexual behavior when injected into the lateral ventricle (3,7). These effects are blocked by the muscarinic antagonist scopolamine but not by methscopolamine, which does not cross the blood–brain barrier, suggesting that these effects are mediated by muscarinic receptors located in the CNS (3,7).

Regarding the behavioral effects of muscarinic antagonists, the existing literature is controversial. It has been reported that scopolamine blocks the facilitatory effect of oxotremorine on masculine sexual behavior (1,8). However, when this drug was administered alone at doses that did not produce motor impairment it did not modify any of the sexual behavior parameters (1). When infused intraventricularly, scopolamine increased the intromission latency. Further, when directly injected into the mPOA scopolamine produced a dose-related decrease of the percentage of active subjects. In addition, the number of intromissions preceding ejaculation increased but not significantly (3,8).

Concerning nicotinic participation in the regulation of masculine sexual behavior, the lack of information is even greater. It has been reported that a relatively low dose of nicotine seems to have a facilitatory effect, increasing the ejaculatory frequency and decreasing intromission frequency, ejaculation latency, and postejaculatory interval (18). However, the effect of a wider range of doses remains to be tested.

In this study, four different approaches were conducted in an attempt to further elucidate the participation of the cholinergic system in the regulation of masculine sexual behavior.

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GENERAL METHOD

Male Wistar adult rats (300–350 g) were housed, five per cage (50 × 30 × 20 cm), under constant temperature and humidity conditions. The colony room was maintained on a 12 D : 12 L reverse cycle (light off 9:00 a.m.). Food and water were available ad lib.

Testing was performed under dim red illumination 3 h after the onset of the dark phase of the cycle. Masculine sexual behavior was assessed by placing the male in a Plexiglas arena (45 cm in diameter) during 5 min before a stimulus receptive female was presented. The female rat was brought into sexual receptivity by sequential treatment with estradiol benzoate (5 μg/0.1 ml oil, SC, once daily). To avoid stimulus habituation, the female rat was changed every 5 min (20). After introduction of the female, the tests lasted 30 min. All subjects (Ss) were previously tested and only those that displayed at least one ejaculation were included in the study. Animals were randomly assigned to one of the four experiments. In experiments in which Ss received several doses of a single drug, only one trial was given at a particular dose. The following parameters were recorded: mount (ML), intromission (IL), and ejaculation (EL) latencies; mount (MF), intromission (IF), and ejaculatory (EF) frequencies; postejaculatory interval (PEI). Also, the following indexes were obtained: hit rate (HR) (# of intromissions/# of intromissions plus # of mounts); average interintromission interval (AIII) (ejaculation latency/# of intromissions); average intercopulatory interval (AICI) (ejaculation latency/# of intromissions plus # of mounts). The full description of masculine sexual behavior parameters has been detailed elsewhere (16).

Scopolamine HCl (SCO HCl), scopolamine methyl bromide (SCO MBr), oxotremorine sesquifumarate (OXO), and nicotine ditartrate (NIC) were obtained from Sigma Chemical Co. (St.Louis, MO). All drugs were dissolved in 0.9% saline solution and administered IP in a volume of 0.66 ml/kg or SC in a volume of 0.1 ml.

Statistical analysis was performed using the Kruskall-Wallis one-way analysis of variance (ANOVA) followed by the Mann-Whitney *U*-test. χ^2 was used to compare proportions.

EXPERIMENT 1

In this study, we analyzed the effect of the stimulation of muscarinic receptors located in the CNS on masculine sexual behavior. Several doses of the muscarinic agonist OXO were tested in animals previously treated with the peripheral muscarinic blocker SCO MBr.

Method

Animals (n = 10) were IP injected with SCO MBr (3 mg/kg) 15 min before administration of OXO. Each animal received three different doses of OXO (0.2, 0.4, and 0.8 mg/kg) or saline, with a period of 1 week between injections. Behavioral tests started 30 min after OXO.

Results

OXO effects were not prevented by previous administration of the peripheral muscarinic antagonist SCO MBr. OXO administration induced a dose-related decrease of IF [Kruskal-Wallis, $H(3) = 19.21$, $p < 0.001$], as well as a decrease in EL [Kruskal-Wallis, $H(3) = 12.31$, $p < 0.01$] (see Fig. 1). However, these effects were limited to the first copulatory series. At all doses tested, OXO elicited a significant increase in EF [Kruskal-Wallis, $H(3) = 11.6$, $p < 0.01$] (Fig. 2).

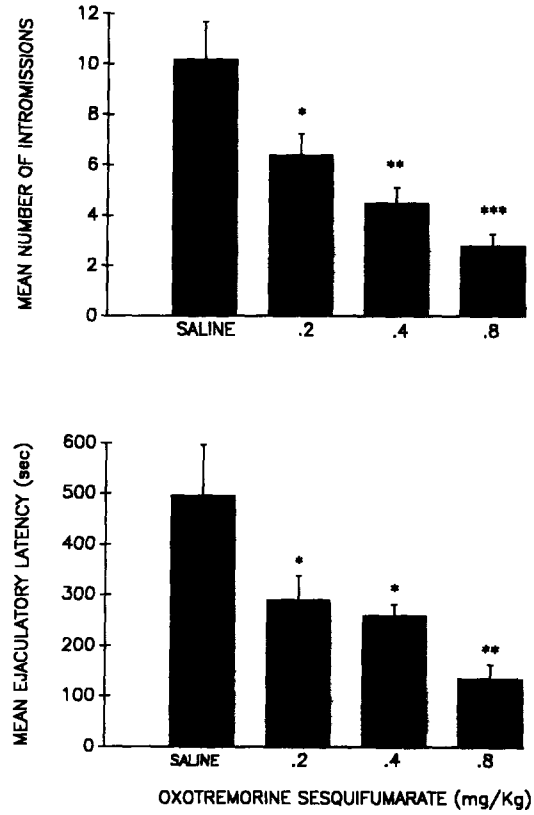

FIG. 1. Effect of IP administration of oxotremorine (OXO) on the number of intromissions preceding ejaculation as well as on ejaculation latency. Data are expressed as mean ± SEM. n = 10. *$p < 0.05$, **$p < 0.005$, ***$p < 0.001$, Mann-Whitney *U*-test, compared to control.

EXPERIMENT 2

The effect of muscarinic blockade on masculine sexual behavior remains controversial. Atropine and scopolamine seems to have no effect or to completely impair masculine sexual behavior (1,2,9,17). It is possible that the source of these conflicting reports may be the differences in doses and routes used.

In this study, we analyzed the effect on masculine sexual behavior of a wider range of scopolamine doses administered IP.

Method

Six different doses of SCO HCl were administered IP. Adult, male rats were randomly assigned to two different groups. One group (n = 10) received the higher 3 doses (0.4, 0.8, and 5 mg/kg) while the other group (n = 15) received the lower doses (0.2, 0.1, and 0.05 mg/kg). Both groups received a saline control injection. SCO HCl administration were made 45 min before the behavioral test. The sequence of

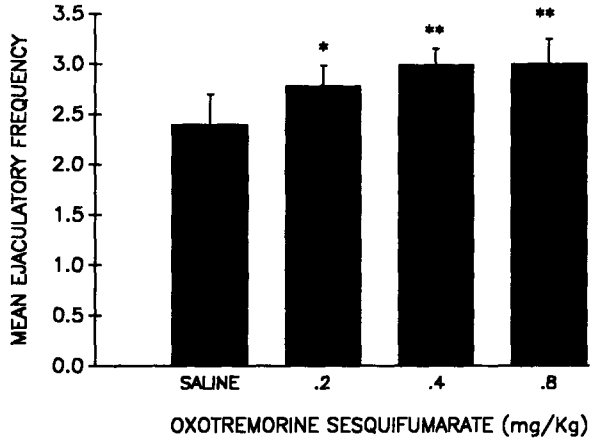

FIG. 2. Dose-dependent increase of ejaculatory frequency in rats previously treated IP with oxotremorine (OXO). Data are expressed as mean ± SEM. $n = 10$. $^*p < 0.05$, $^{**}p < 0.025$, Mann-Whitney U-test, compared to control.

injections was randomized and a 1-week period elapsed between observations.

Results

SCO HCl produced a dose-related impairment of masculine sexual behavior. As doses increased, a decrease in the percentage of Ss presenting either intromission and/or ejaculation was observed, $\chi^2(3) = 20.12, p < 0.001$. This decrease was significant even at the 0.1-mg/kg dose. At the dose of 0.8 mg/kg, all Ss failed to ejaculate and at a dose of 5 mg/kg none of the Ss displayed activity (Fig. 3). Moreover, when analyzing the behavior of active Ss a clear increase of mount [Kruskal-Wallis, $H(3) = 9.58$, $p < 0.05$] and intromission latencies [Kruskal-Wallis, $H(3) = 12.5$, $p < 0.01$] was observed, although only during the first copulatory series (Fig. 4).

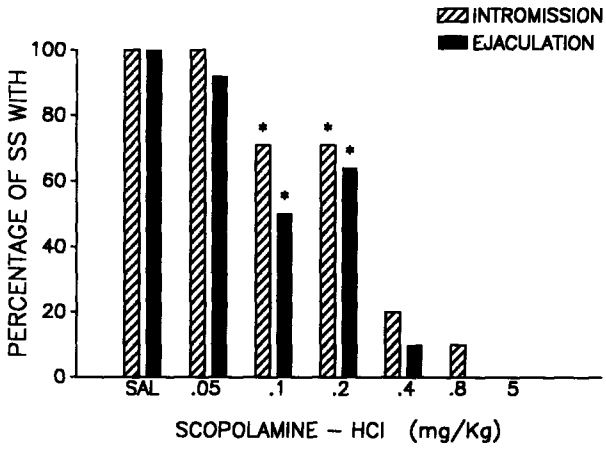

FIG. 3. Inhibitory effect of IP treatment with scopolamine (SCO) HCl on the percentage of animals displaying intromission as well as the percentage of animals reaching ejaculation. Lower doses (0.05, 0.1, and 0.2), $n = 10$. Higher doses (0.4, 0.8, and 5) $n = 15$. Saline, $n = 25$. $^*p < 0.001$, χ^2, compared to control.

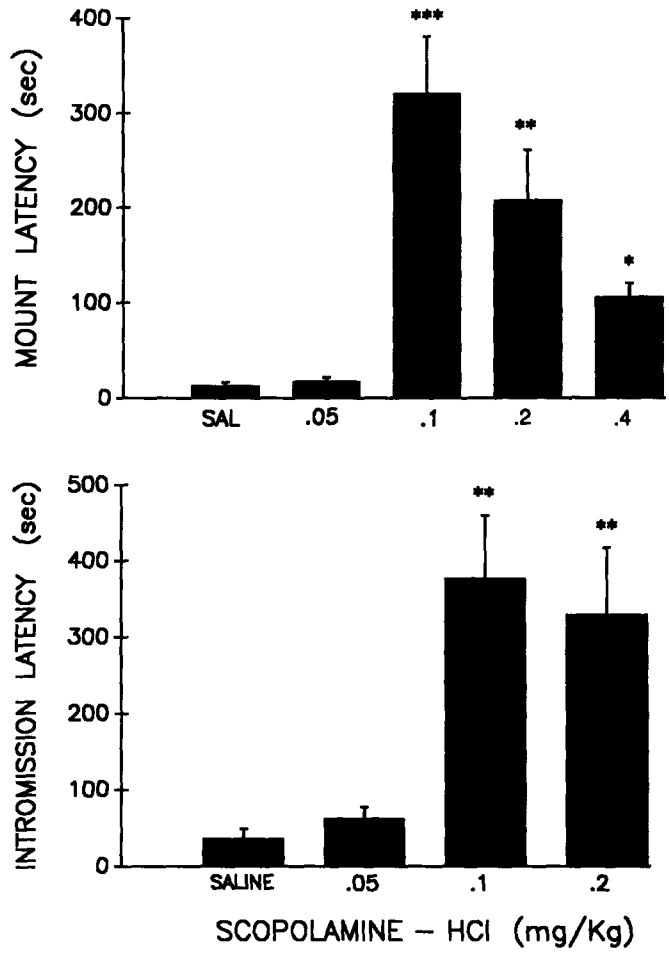

FIG. 4. Effect of scopolamine (SCO) HCl administrated IP on mount and intromission latencies of the first copulatory series. Data are expressed as mean ± SEM. Lower doses (0.05, 0.1, and 0.2), $n = 10$. Higher doses (0.4, 0.8, and 5) $n = 15$. Saline, $n = 25$. $^*p < 0.025$, $^{**}p < 0.005$, $^{***}p < 0.001$, Mann-Whitney U-test, compared to control.

Figure 5 shows the results of the lower doses of SCO HCl on ejaculatory frequency. As can be observed, a trend to decrease is present already at the lower dose of 0.05 mg/kg. This decrease was significant at the doses of 0.1 and 0.2 mg/kg [Kruskal-Wallis, $H(3) = 9.93, p < 0.02$].

EXPERIMENT 3

It has been reported that rats selectively bred for cholinergic supersensitivity showed a significant decrease in the number of intromissions preceding ejaculation (19). The main feature of these rats is the increased number of muscarinic receptors (14,15). It would be possible that the alterations in sexual behavior may be due to the altered muscarinic sensitivity. It is known that chronic blockade of receptors induces supersensitivity (11). This experiment was performed as an attempt to clarify if pharmacologically induced muscarinic supersensitivity could reproduce the alterations of masculine sexual behavior observed in genetically supersensitive rats.

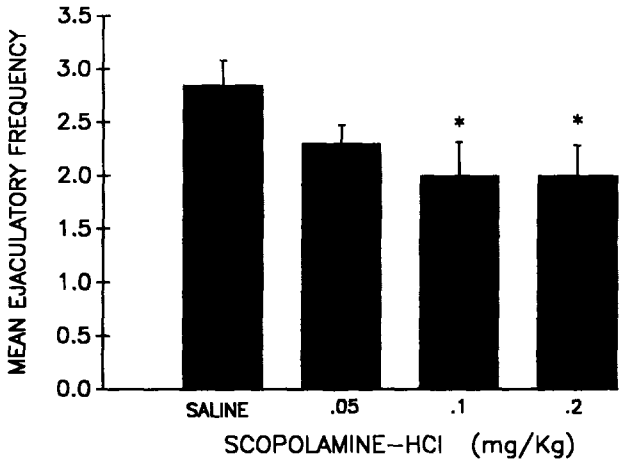

FIG. 5. Inhibitory effect of scopolamine (SCO) HCl administration on ejaculatory frequency. Saline, $n = 25$. SCO HCl, $n = 10$. Data are expressed as mean ± SEM. *$p < 0.025$, Mann-Whitney U-test, compared to control.

Method

Thirty rats were randomly assigned to three different treatments. SCO HCl (2 mg/kg/day), SCO MBr (3 mg/kg/day), and saline were SC injected daily for 17 days. Forty-eight hours after the last injection, all Ss were treated with SCO MBr (3 mg/kg, IP) and 15 min later with a low dose of OXO (0.2 mg/kg, IP). Recording of masculine sexual behavior began 30 min after OXO administration.

Results

Both SCO HCl and SCO MBr chronic treatment induced a statistically significant increase in ejaculation latency [Kruskal-Wallis, $H(2) = 5.99$, $p < 0.05$], in mount frequency [Kruskal-Wallis, $H(2) = 8.84$, $p < 0.02$, and in the AIII [Kruskal-Wallis, $H(2) = 10.85$, $p < 0.01$], as well as a significant decrease in HR [Kruskal-Wallis, $H(2) = 15.07$, $p < 0.001$] and ejaculatory frequency [Kruskal-Wallis, $H(2) = 6.2$, $p < 0.05$]. Animals treated with SCO HCl showed also a trend to decrease the number of the intromissions preceding ejaculation as well as the refractory period (see Table 1).

EXPERIMENT 4

It has been reported that acute administration of nicotine (0.6 mg/kg) seems to have facilitatory effects on masculine sexual behavior (18). This experiment was performed to study a wider range of doses of nicotine and evaluate its effects on male sexual behavior.

Method

Six doses of NIC were administered to two groups of sexually experienced males. One group ($n = 13$) received low doses (0.05, 0.1, and 0.2 mg/kg) and the other group ($n = 10$) received high doses (0.4, 0.8, and 1.6 mg/kg). Both groups received also a saline control injection. All injections were made IP 30 min before sexual behavior recording. The sequence of the doses was randomized. A period of 1 week elapsed between injections.

Results

High doses of NIC elicited a statistically significant decrease in the number of intromissions preceding ejaculation [Kruskal-Wallis, $H(6) = 22.11$, $p < 0.01$] (see Fig. 6), although no significant differences were found in AIII and EL. At lower doses, NIC was unable to produce any noticeable changes in any of the masculine sexual behavior parameters recorded.

GENERAL DISCUSSION

The present results support the notion that the cholinergic systems participate in the regulation of masculine sexual behavior, mainly through the muscarinic system. Despite methodological differences with previous reports, the notion that OXO has a facilitatory effect on masculine sexual behavior is confirmed and extended. OXO seems to decrease the ejaculatory threshold because it decreases IF and EL (1,16). The present results indicate that the total copulatory potential, that is, number of ejaculations in a time-limited test (16), increases in a dose-dependent manner.

In contrast with OXO effects, SCO HCl decreases the copulatory potential (decreases EF). The main effect of muscarinic blockade, however, seems to be the dose-related decrease of active rats, which can be obtained both with systemic administration as well as with microinjections in the mPOA (1,2,9,17). As active Ss displayed an increase in mount latency, it could be possible that SCO HCl alters the motiva-

TABLE 1

EFFECT OF CHRONIC SCO HCl, SCO HBr, OR SALINE ADMINISTRATION ON MASCULINE SEXUAL BEHAVIOR IN RATS

	Saline	SCO HCl	SCO MBr
ML (s)	72.88 ± 56.67	4.22 ± 0.59	50.66 ± 40.85
IL (s)	177.77 ± 133.26	90.11 ± 67.98	168.22 ± 61.73
EL (s)	222.5 ± 43.38	418.44 ± 65.23	615.87 ± 143.41
MF	1.66 ± 0.98	5.77 ± 0.81	15.12 ± 2.67
IF	6 ± 0.71	4.88 ± 0.56	6 ± 1.36
HR	0.83 ± 0.08	0.46 ± 0.03	0.29 ± 0.04
AIII (s)	35.5 ± 50.06	85.45 ± 6.86	111.45 ± 23.96
AICI (s)	28.11 ± 2.97	39.57 ± 4.49	27.55 ± 3.49
PEI (s)	491.6 ± 71.65	464.55 ± 45.66	528.66 ± 92.76
EF	2.5 ± 0.42	1.77 ± 0.14	1.62 ± 0.32

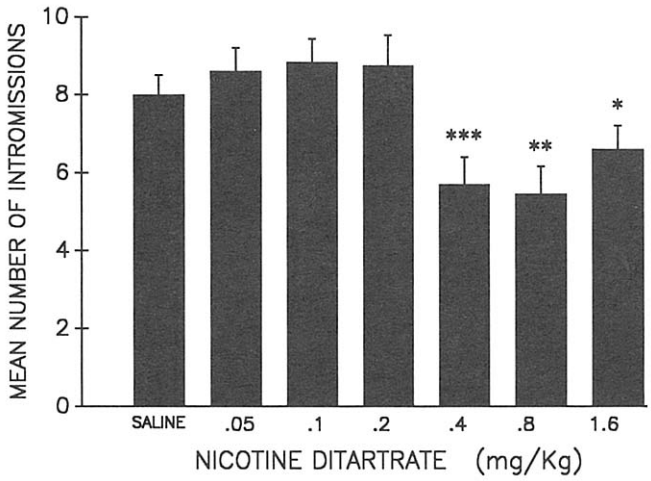

FIG. 6. Effect of several IP doses of nicotine (NIC) on the number of intromissions preceding ejaculation. Lower doses (0.05, 0.1, and 0.2), $n = 13$. Saline, $n = 23$. Higher doses (0.4, 0.8, and 1.6), $n = 10$. Data are expressed as mean ± SEM. $*p < 0.05$, $**p < 0.02$, $***p < 0.01$, Mann-Whitney U-test, compared to control.

tional component of sexual behavior, as it does when infused intraventricularly (8).

Further, it is known that both muscarinic receptors (5,13) as well as the rate-limiting enzyme for the synthesis of acetylcholine, choline acetyltransferase (10), are present in the mPOA, a brain structure of central importance for the expression of male sexual behavior (3,4,6,12,16).

Chronic blockade of muscarinic receptors failed to reproduce sexual behavior features of cholinergic supersensitive rats and did not facilitate OXO effects. The behavioral features altered were the same with central-peripheral blockade (SCO HCl) as well as with only peripheral blockade (SCO MBr). Moreover, the main alteration after chronic blockade was an important increase in MF, which resulted in alterations of the HR, EL, and AIII. These results suggest that the effects of OXO in these rats was located peripherally. It could be possible that due to the induced supersensitivity administration of SCO MBr before OXO administration was unable to completely block muscarinic peripheral receptors.

Regarding nicotine, only a minor facilitatory effect was detected. It seems that the nicotinic system does not have an important influence on the regulation of masculine sexual behavior.

Summarizing, our results support the notion that cholinergic regulation of masculine sexual behavior is mediated by muscarinic receptors. Nevertheless, further research is needed to fully elucidate the mechanisms through which this influence is exerted.

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