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Brain Localization of Cholinergic Influence on Male Sex Behavior in Rats: Agonists

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HULL, E. M., D. BITRAN, E. A. PEHEK, G. M. HOLMES, R. K. WARNER, L. C. BAND AND L. G. CLEMENS. *Brain localization of cholinergic influence on male sex behavior in rats: Agonists.* PHARMACOL BIOCHEM BEHAV 31(1) 169–174, 1988.—Cholinergic agonists were microinjected into either the lateral ventricle or the preoptic area of sexually experienced male rats. In Experiment 1 carbachol, injected into the lateral ventricles, delayed the initiation of sexual behavior. When injected into the preoptic area, carbachol again delayed the onset of copulation, but these delays were shorter than after ventricular injections. In addition, preoptic injections reduced the number of intromissions preceding ejaculation. In Experiment 2 ventricular injections of the muscarinic agonist oxotremorine again delayed initiation of sexual behavior and also slowed its rate. However, oxotremorine injections into the preoptic area, through cannulae angled to miss all ventricles, only decreased the number of intromissions preceding ejaculation. These data suggest that cholinergic synapses in proximity to the ventricles may decrease sexual arousal, while cholinergic mechanisms in or near the preoptic area may reduce ejaculatory threshold.

Acetylcholine Carbachol Oxotremorine Sexual behavior Preoptic area Rat

MASCULINE sexual behavior of rats can be influenced by numerous pharmacological treatments [reviewed in (4,19)]. Whereas dopaminergic and serotonergic influences have been fairly extensively studied, the roles of cholinergic neurons in male copulatory behavior are more poorly defined. Suppression of copulation was observed with systemically administered eserine and physostigmine (acetylcholinesterase inhibitors that prolong the synaptic action of acetylcholine), with high doses of the agonists arecholine and nicotine, and also with the muscarinic receptor antagonists atropine and scopolamine (1, 3, 15, 22, 23). The fact that both increases and decreases in cholinergic activity reduced the number of animals copulating led to the proposal that any alteration of cholinergic function can impair sexual behavior. However, the drug doses administered in those experiments also impaired motor and autonomic functions, which may have interfered with copulation. A more recent study employing the selective muscarinic agonist oxotremorine reported reductions in ejaculation threshold (decreased ejaculation latency and decreased intromissions preceding ejaculation) at doses that did not produce motor impairment (1). The specificity for muscarinic receptors in the central nervous system was confirmed by the blockade of these effects by the muscarinic antagonist scopolamine, but not by methscopolamine, which does not cross the blood-brain barrier.

A problem with systemically administered drugs is that they may affect peripheral as well as numerous central synapses. A better resolution of cholinergic mechanisms that regulate masculine sexual behavior may be obtained by microinjecting drugs directly into structures of interest. The preoptic area is particularly important for the control of masculine sexual behavior. Lesions of the area permanently abolished or dramatically impaired sexual behavior (11,13), while electrical (17, 18, 24) or hormonal (5,7) stimulation of the preoptic area facilitated copulation. Furthermore, this area has been shown to possess both cholinergic (muscarinic) receptors (9) and the rate-limiting enzyme for the synthesis of acetylcholine, choline acetyltransferase (16). The present experiments were designed to determine whether microinjections of cholinergic agonists into the preoptic area (POA) would affect masculine sexual behavior.

Control injections were administered into the lateral ventricles (LV) to determine the specificity of any effects of the preoptic injections.

GENERAL METHOD

Animals

Adult male Long-Evans rats (300–350 g), purchased from Blue Spruce Farms (Altamont, NY), were caged singly in large plastic cages (40×20×20 cm) in a room where lights were off between 11.00 and 21.00 hr. Food and water were available ad lib. Animals were handled daily so that intracerebral injections could be accomplished without stress or anesthesia. Males received four preoperative and two postoperative tests before drug microinjection tests began. All tests were given weekly, between 14.00 and 16.00 hr. Stimulus females of the same strain were ovariectomized under Ketaset (5.6 mg/kg) plus Rompun (0.28 mg/kg) anesthesia and housed in a separate room. Forty-eight hours before a behavioral test females were injected subcutaneously with 20 μg estradiol benzoate in oil; four hours before the test they received a subcutaneous injection of 500 μg progesterone in oil.

Procedure

Implantation of outer guide cannulae was done under sodium pentobarbital anesthesia (55 mg/kg), using a Kopf stereotaxic frame. After shaving, cleaning, and exposing the skull, small holes were drilled over the appropriate structures. Cannulae were lowered to the predetermined depth, using the stereotaxic drive. Four small stainless steel screws, drilled barely through the skull, provided anchorage for the assembly. Dental cement surrounded the screws and the guide cannulae.

Guide cannulae were constructed from 23-gauge thin wall hypodermic tubing, cut to the length appropriate for each placement and sanded on a rotary sanding disk. Each guide cannula was fitted with an obturator constructed from 27-gauge hypodermic tubing. A collar of 23-gauge tubing was crimped around the top to prevent the obturator from extending more than 1 mm below the end of the guide cannula. A small length of polyethylene tubing was fitted over both the collar and the top of the guide tubing to make an airtight seal. The cannulae used for injections were made from 28-gauge hypodermic tubing. One end of a 1-m length of polyethylene tubing was fitted over the end of the injection cannula, thereby preventing it from extending more than 1 mm below the end of the guide cannula. At the time of drug administration the other end of the polyethylene tubing was connected to a 1-ml syringe held in a Kopf microinjection pump.

Drug injection was accomplished by removing the entire obturator assembly from the guide cannula and replacing it with the injection cannula. Injections were administered over a 30 sec interval, followed by an additional 30 sec with the injection cannula left in place. The injection cannula was then replaced with the obturator, and the male was returned to his cage. He was then taken to a testing room, and a stimulus female was introduced into his cage.

Each test lasted for 30 min after the male's first intromission, or for 30 min after introduction of the female if no intromission occurred. The following measures were recorded: latency to the first mount, latency to the first intromission, latency from the first intromission to the first ejaculation, postejaculatory interval between ejaculation and the next intromission, intromission ratio (number of intromissions/sum of mounts plus intromissions), intromission frequency (intromissions preceding each ejaculation: for ejaculations after the first, only those intromissions following the preceding ejaculation were counted), ejaculation frequency (the number of ejaculations during the test), and mean intermission interval (ejaculation latency/intromission frequency). Data from animals that failed to copulate were excluded from all analyses except ejaculation frequency. Intromissions were distinguished behaviorally from mounts by the presence of a rapid, springing dismount. Ejaculation patterns were characterized by longer deeper thrusts, slow dismounts, and a prolonged period of rest following ejaculation (postejaculatory interval). One-way analyses of variance with repeated measures were computed separately for preoptic and ventricle cannula placements. Newman-Keuls tests were used for post-hoc comparisons of each dose vs. vehicle.

Histology

After all behavioral tests were completed, males were anesthetized and sacrificed by decapitation. Brain slabs were removed and mounted in an American Optical cryostat. Coronal sections were cut at 40 μ, mounted on glass slides, stained with cresyl violet, and examined with a projection magnifier. Data from animals whose LV cannula missed the ventricle were omitted from analyses of ventricular injection effects. Data from animals whose POA cannulae ended more than 0.5 mm from their intended site were omitted from analyses of preoptic injection effects.

EXPERIMENT 1: EFFECTS ON COPULATION OF THE CHOLINERGIC AGONIST CARBACHOL MICROINJECTED INTO POA OR LV

Method

Ten males had histologically verified bilateral cannulae ending in the POA (coordinates: AP=+2.4, ML=+0.7, DV=−6.9, incisor bar=+5.0). Twelve males had verified bilateral cannulae ending in the LV (coordinates: AP=0.0, ML=+1.5, DV=−2.0, incisor bar=+5.0). Either 0.1 or 0.25 μg carbachol or the artificial cerebrospinal fluid vehicle was injected into each of the animals' two cannulae immediately before each test. Volume of the infusate was 0.5 μl. Order of treatments was counterbalanced.

Results

Carbachol microinjections into both the lateral ventricle and preoptic area increased intromission latency [LV: $F(2,22)=19.57$, $p<0.0001$; POA: $F(2,20)=4.35$, $p<0.05$] (see Fig. 1). Injections of the higher dose of carbachol into the ventricle delayed the onset of copulation much longer than did similar injections into the MPOA, $t(21)=4.66$, $p<0.001$. However, all animals eventually initiated copulation. A similar elevation of mount latencies resulted from carbachol injections [LV: Veh: 179.9±78.2, 0.1 μg: 512.8±75.1, 0.25 μg: 979.3±132.3, $F(2,22)=20.07$, $p<0.0001$; POA: Veh: 33.5±6.4, 0.1 μg: 280.2±88.3, 0.25 μg: 140.8±63.1, $F(2,20)=4.16$, $p<0.05$], indicating that the delay in intromitting did not result from failed attempts to achieve vaginal penetration.

Carbachol microinjections into the POA significantly decreased the number of intromissions preceding the first ejaculation, $F(2,20)=9.52$, $p<0.005$ (see Fig. 2). This effect was specific to the POA; injections into the LV failed to alter

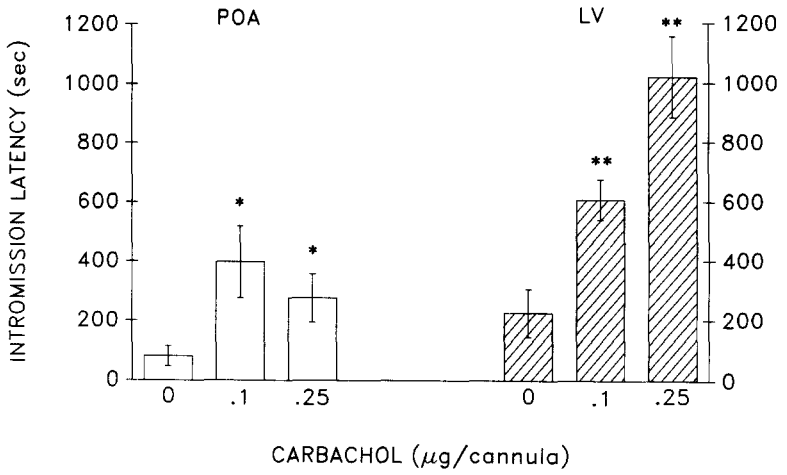

FIG. 1. The effect of carbachol microinjected bilaterally into the preoptic area or lateral ventricle on intromission latency. Values are means±S.E.M. $^{**}p<0.01$, $^{*}p<0.05$, relative to vehicle.

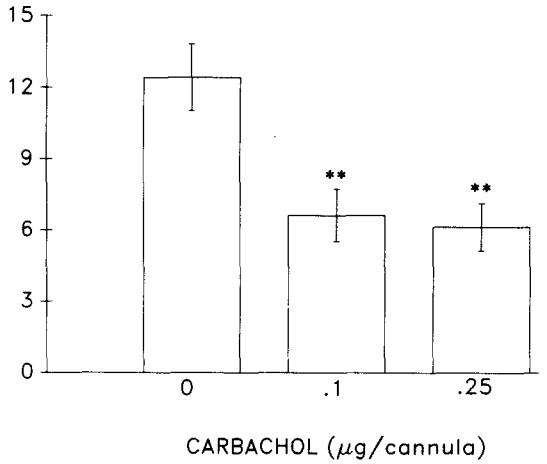

FIG. 2. The effect of carbachol microinjected bilaterally into the preoptic area on intromission frequency. Values are means±S.E.M. $^{**}p<0.01$, relative to vehicle.

this measure, F(2,22)=2.21, NS. No other parameters were significantly affected by injections into either site.

Discussion

The only effect of LV injections was a dose-dependent delay in the initiation of copulation. On the other hand, POA injections significantly decreased ejaculation threshold (i.e., decreased the number of intromissions preceding ejaculation). POA injections of carbachol also increased intromission latencies, compared to vehicle injections; however POA injections of the higher dose of carbachol resulted in much shorter delays than did similar LV injections. Histological examination disclosed that most POA cannulae had descended through the LV. Therefore, the drug may have diffused up the cannula track and into the ventricular circulation, thereby producing an effect qualitatively similar to that of LV injections. Clemens, Dohanich and Barr (6) reported that facilitation of feminine sexual behavior, associated with carbachol injections through similar vertical POA cannulae, was not observed in animals whose cannulae were angled to miss all ventricles. Therefore, in Experiment 2 the POA cannulae were inserted at an angle in order to miss the ventricles.

EXPERIMENT 2: EFFECTS ON COPULATION OF OXOTREMORINE MICROINJECTIONS INTO POA OR LV

Carbachol is a nonspecific cholinergic agonist, stimulating both muscarinic and nicotinic receptors. In order to assess the importance of muscarinic receptors in the regulation of masculine sexual behavior, the more specific muscarinic agonist oxotremorine was used in this experiment.

Method

Each of 23 animals received one angled guide cannula ending approximately 1 mm dorsolateral to the right POA (coordinates: AP=−0.6, ML=5.0, DV=−7.4, angle=30, incisor bar=−5.0) and one vertical cannula ending in the left LV (AP=−0.8, ML=1.5, DV=−3.2, incisor bar=−5.0). All animals received all doses, injected unilaterally into the POA or LV, on eight separate tests. Doses were 0.0, 0.5, 1.0, and 2.0 μg oxotremorine. Orders of doses and of POA vs. LV cannulae were completely counterbalanced.

Results

As in Experiment 1, drug injections into the LV increased intromission latency, F(3,60)=3.07, $p<0.05$ (see Fig. 3). A parallel increase in mount latency did not achieve statistical significance. However, unlike Experiment 1, drug injections into the POA did not delay the onset of copulation, F(3,48)=0.57, NS. In addition, LV injections of oxotremorine slowed the rate of intromitting [increased interintromission interval: F(3,60)=3.74, $p<0.05$, whereas POA injections of oxotremorine did not affect this measure, F(3,48)=1.95, NS] (see Fig. 4). As with carbachol in Experiment 1, oxotremorine injections into the POA decreased intromission frequency, F(3,64)=11.06, $p<0.001$, while oxotremorine injections into the LV failed to alter this parameter, F(3,45)=1.01, NS (see Fig. 5).

Discussion

Muscarinic effects on masculine sexual behavior were

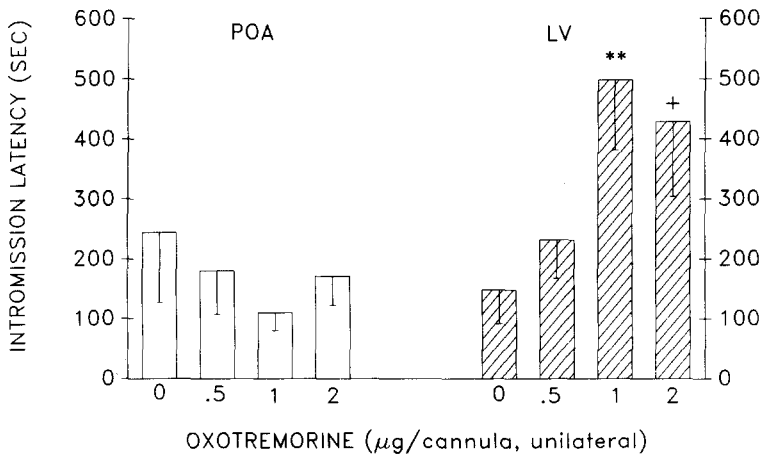

FIG. 3. The effect of oxotremorine microinjected into the preoptic area or lateral ventricle on intromission latency. Values are means±S.E.M. $^{**}p<0.01$, $+0.05<p<0.1$, relative to vehicle.

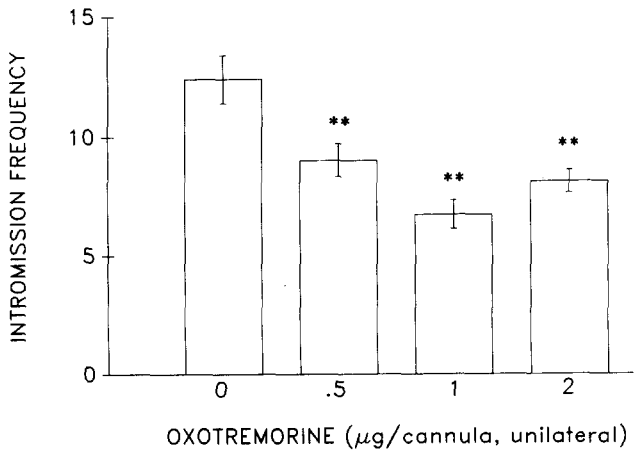

FIG. 4. The effect of oxotremorine microinjected into the preoptic area on intromission frequency. Values are means±S.E.M. $^{**}p<0.01$, relative to vehicle.

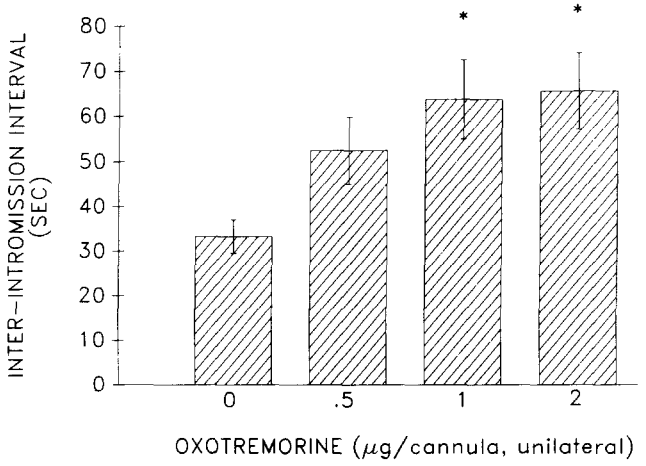

FIG. 5. The effect of oxotremorine microinjected into the lateral ventricle on interintromission interval. Values are means±S.E.M. $^{*}p<0.05$, relative to vehicle.

site specific. Microinjections of oxotremorine into the LV slowed both the onset of copulation (increased intromission latency) and its rate (increased interintromission interval); oxotremorine injections restricted to the POA only reduced the ejaculatory threshold (decreased intromission frequency). Therefore, it appears likely that the shorter delay in onset of copulation, observed after POA injections of carbachol in Experiment 1, resulted from diffusion of the drug up the cannula tracks to the LV, rather than from stimulation of cholinergic receptors in the POA. However, in both experiments injections of cholinergic agonists into the POA reduced ejaculatory threshold (intromission frequency), suggesting that this effect is specific to cholinergic mechanisms in or near the POA. Furthermore, while a contribution by nicotinic receptors cannot be ruled out on the basis of these experiments, it appears that stimulation of muscarinic receptors is sufficient both to decrease sexual arousal (increase intromission latency) and to reduce ejaculatory threshold (decrease intromission frequency).

GENERAL DISCUSSION

The main findings of these experiments are that sexual arousal is influenced by cholinergic synapses that appear to be in proximity to the ventricular circulation, while ejaculation threshold (intromission frequency) is affected by cholinergic mechanisms in or near the POA. Beach (2) proposed that masculine sexual behavior was divisible into at least two factors. A sexual arousal mechanism was hypothesized to lead to the onset of copulation and to its resumption after an ejaculation. A copulatory mechanism was proposed to maintain a series of intromissions and summate their excitatory effects until ejaculation was triggered. Sachs (21) extended this analysis by factor analyzing several sets of normative data. He suggested that a copulatory rate factor, a hit rate (intromission ratio) factor, and an intromission count factor (number of intromissions preceding ejaculation) should be included as subcomponents of the copulatory mechanism proposed by Beach. Sachs' initiation factor is

comparable to Beach's arousal mechanism, except that it includes only the initiation of copulation and not its resumption after ejaculation.

The present results suggest that cholinergic synapses within or near the POA contribute to the regulation of the intromission count factor. In both experiments cholinergic agonists injected into the POA decreased the number of intromissions preceding ejaculation (intromission frequency). Since neither interintromission interval nor ejaculation latency was altered significantly, the reduction in intromission frequency was not secondary to changes in those measures. In contrast, injections into the LV failed to affect intromission frequency, suggesting that the cholinergic regulation of this measure is achieved by mechanisms that interact with cholinoceptive cells in the POA. Several additional structures have been implicated in the control of intromission frequency. Lesions of the bed nucleus of stria terminalis (10), the corticomedial complex of the amygdala (12), and the stria terminalis connecting these two structures (20) have been reported to increase the number of intromissions preceding ejaculation. Furthermore, all of these areas exhibit moderate levels of cholinergic activity (14). It is thus tempting to speculate that input from the amygdala-stria terminalis system to the POA may modulate ejaculatory threshold via cholinergic synapses.

Cholinergic injections into the LV in the present experiments significantly lengthened intromission latency. When oxotremorine was introduced into the POA through angled cannulae, no delay in initiation was observed. Thus, it appears that one or more structures in close proximity to the ventricles (but not the POA) contribute to cholinergic influences on sexual arousal. Since the mean latency to intromit was approximately 4 min after vehicle injections into the LV, and since this latency was increased to 10 and 17 min following the two doses of carbachol, the drug must have affected the relevant structures within the first 4 min in order to prevent the normal onset of copulation. The relative rapidity of the drug effect suggest that it did not have to diffuse over a great distance to reach the effective structure(s).

The reduction in sexual arousal did not appear to result from any impairment in motor behavior. Before initiating copulation, drug-injected males walked about, sniffed the female, reared, groomed, or sat quietly. Nor did it result from inability to achieve vaginal penetration, since mount latencies were similarly increased. Once they began to copulate, their sexual behavior did not differ from that observed in control conditions. Thus sexual arousal was impaired, rather than a copulatory performance mechanism. However, the lack of effect on other copulatory parameters may have been due to diminished effectiveness of the drug by the time the males initiated sexual behavior. Mean intromission latencies after LV carbachol injections were 10 and 17 min, and tests continued for at least 30 min after the first intromission. Clemens *et al.* (6) and Dohanich, Barr, Witcher and Clemens (8) reported that the facilitative effects of carbachol and oxotremorine on feminine sexual behavior were greatest between 5 and 15 min after the intracerebral injections, and were no longer statistically significant 45 min, or in some cases 20 min, after injection.

In summary, cholinergic agonists microinjected into the LV of males rats retarded the initiation of copulation without markedly affecting motor behavior. On the other hand, the same agonists microinjected into the POA decreased the number of intromissions preceding ejaculation. These data suggest a cholinergic inhibition of sexual arousal in male rats, and a more localized cholinergic reduction of ejaculation threshold, by synapses in or near the POA.

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