Pharmacology Biochemistry & Behavior, Vol. 31, pp. 175–178. © Pergamon Press plc, 1988. Printed in the U.S.A. 0091-3057/88 $3.00 + .00

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

ELAINE M. HULL,*[1] ELIZABETH A. PEHEK,* DANIEL BITRAN,* GREGORY M. HOLMES,* ROBERT K. WARNER,* LINDA C. BAND,* TERENCE BAZZETT* AND LYNWOOD G. CLEMENS†

**Department of Psychology, State University of New York at Buffalo, Amherst, NY 14260 and †Department of Zoology, Michigan State University, East Lansing, MI 48824*

Received 8 September 1987

HULL, E. M., E. A. PEHEK, D. BITRAN, G. M. HOLMES, R. K. WARNER, L. C. BAND, T. BAZZETT AND L. G. CLEMENS. *Brain localization of cholinergic influence on male sex behavior in rats: Antagonists.* PHARMACOL BIOCHEM BEHAV 31(1) 175–178, 1988.—The muscarinic receptor antagonist scopolamine was microinjected into either the preoptic area or the lateral ventricle, preceding sexual behavior tests. In Experiment 1 unilateral ventricular injections of scopolamine delayed the initiation of copulation, while unilateral preoptic injections had no effect. In Experiment 2 bilateral injections into the preoptic area produced dose-related decreases in the percentages of animals intromitting and ejaculating. In Experiment 3 scopolamine, injected alone into the preoptic area, again decreased the percentages of animals mounting, intromitting, and ejaculating. The muscarinic agonist oxotremorine, injected alone into the preoptic area, decreased ejaculatory threshold (i.e., decreased the number of intromissions preceding ejaculation) as previously reported. Concurrent oxotremorine and scopolamine injections into the preoptic area were not different from vehicle; thus, scopolamine blocked oxotremorine's effect. These data suggest that some cholinergic activation of the preoptic area is critical for normal copulation, since bilateral blockade of muscarinic receptors there dramatically decreased the number of animals copulating. However, increased cholinergic activity there only reduced ejaculation threshold.

Acetylcholine Scopolamine Oxotremorine Sexual behavior Preoptic area Rat

SUPPRESSION of masculine sexual behavior has been reported after systemic injections of both cholinergic agonists and antagonists (1, 3, 6, 9, 10). The observation that both increases and decreases in cholinergic activity reduced copulation suggested that any alteration in cholinergic function could impair sexual behavior. However, an alternative explanation is that cholinergic mechanisms in various sites may affect behavior in different ways. Thus, systemically administered agonists and antagonists might have similar behavioral effects as the result of modulating different neural mechanisms. Indeed, we have shown that the cholinergic agonists carbachol and oxotremorine, injected into the lateral ventricles of sexually experienced male rats, delayed their initiation of sexual behavior (5). However, one of these agonists, oxotremorine, injected into the preoptic area through cannulae that did not traverse the ventricles, only reduced the number of intromissions preceding ejaculation.

It is of interest to determine whether a cholinergic antagonist, microinjected into the lateral ventricle or the preoptic area, might also produce site specific effects and whether these effects would be opposite or similar to those of the agonists.

The present series of experiments was designed to: 1) determine the behavioral effects of injections of the cholinergic (muscarinic) antagonist scopolamine into the lateral ventricle or the preoptic area; and 2) determine whether the effects of the muscarinic agonist oxotremorine could be blocked by the antagonist scopolamine.

GENERAL METHOD

Methods of constructing and implanting cannulae, microinjecting drugs, behavioral testing and histological examination were described in the previous article (5).

EXPERIMENT 1: EFFECTS ON COPULATION OF THE CHOLINERGIC ANTAGONIST SCOPOLAMINE MICROINJECTED INTO POA OR LV

Method

Fifteen sexually experienced male Long-Evans rats received two stainless steel guide cannulae, one aimed at the left lateral ventricle (AP=−0.8, ML=1.5, DV=−3.2, incisor bar=−5.0), the other at the right preoptic area (AP=−0.6, ML=5.0, DV=−7.4, angle=30, incisor bar=−5.0). The preoptic guide cannula was angled so as not to traverse the ventricle. One of three doses of scopolamine (1, 2, or 5 μg) or the artificial cerebrospinal fluid vehicle was injected into one of the animals' two cannulae 15 min before each weekly test. Each animal was tested 8 times; orders of drug doses and of

[1]Requests for reprints should be addressed to Dr. Elaine M. Hull, Department of Psychology, Park Hall, State University of New York at Buffalo, Amherst, NY 14260.

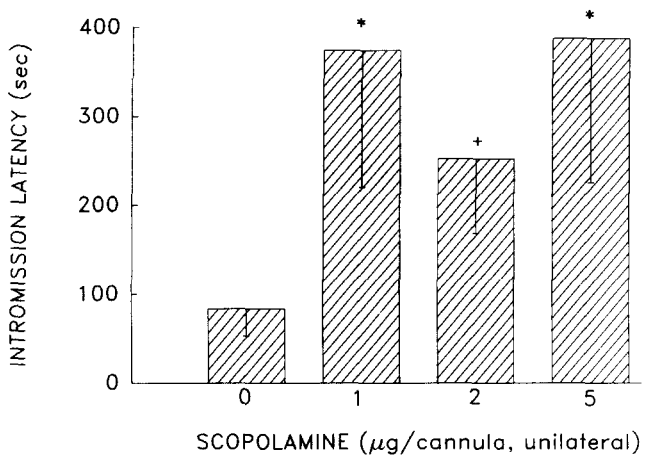

FIG. 1. The effect of scopolamine microinjected into the lateral ventricle on intromission latency. Values are means±S.E.M *$p<0.05$, $+0.05<p<0.1$, relative to vehicle.

injection sites were completely counterbalanced. Volume of the infusate was 0.5 μl; it was administered over a period of 30 sec, with the injection needle left in place for an additional 30 sec. One-way analyses of variance with repeated measures were computed separately for results following ventricle and preoptic injections. Post hoc comparisons utilized the *t*-Statistic in Comparing Treatment Means with a Control (12). Because of the variability of the latency data, they were subjected to a log transformation prior to computing analyses of variance. Only data from animals with histologically confirmed cannula placements were analyzed (10 for lateral ventricle comparisons and 12 for preoptic comparisons).

Results

Scopolamine injected into the lateral ventricle increased the latency to intromit, $F(3,18)=3.47$, $p<0.05$ (see Fig. 1). However, once copulation began, no other measures were affected by ventricular injections of scopolamine. No dose of scopolamine injected into the preoptic area affected any copulatory measure.

Discussion

Injections of the cholinergic antagonist scopolamine into the lateral ventricle produced the same effect as had the cholinergic agonists carbachol and oxotremorine: delayed initiation of copulation (5). The similarity of effects of agonists and an antagonist is reminiscent of the impairment of copulation by both agonists and antagonists administered systemically in high doses (1, 3, 6, 9, 10).

Since unilateral preoptic injections of scopolamine failed to alter any parameter of copulatory behavior, we wished to determine whether bilateral blockade of preoptic cholinergic receptors might be more effective than unilateral blockade. It is possible that there is sufficient redundancy in the system so that cholinergic activity on one side of the preoptic area is sufficient for normal regulation of sexual behavior. Since most cannula tips in Experiment 1 ended between the medial and lateral preoptic areas, it is unlikely that drug injected into the right preoptic area diffused across the midline to the opposite side. Preliminary data using both Evans blue dye and radioactively labeled scopolamine injections have indicated that the drug diffuses about 0.5 mm from the tip of the cannula into surrounding tissue, less than 2 mm along the cannula track, and does not enter the ventricular circulation when injected through cannulae angled to miss the ventricles (Clemens, Myers, Brigham, Holmes and Hull, unpublished observations). The second experiment was designed to assess the effectiveness of bilateral blockade of cholinergic receptors in the preoptic area.

TABLE 1

EFFECTS OF BILATERAL SCOPOLAMINE INJECTIONS INTO THE PREOPTIC AREA ON COPULATION, EXPERIMENT 2

	Scopolamine (μg/cannula)				
	0	1	2	5	10
% Mounting	100	100	89	78	67
% Intromitting	100	89*	78*	67*	56*
% Ejaculating ≥1	100	78*	78*	67*	56*
% Ejaculating ≥2	89	78	78	67	56
% Ejaculating ≥3	67	33*	56	44*	22*

*$p<0.05$, relative to vehicle.

EXPERIMENT 2: EFFECTS ON COPULATION OF BILATERAL PREOPTIC INJECTIONS OF SCOPOLAMINE

Method

Eleven animals had histologically confirmed bilateral cannulae ending in the preoptic area. Stereotaxic coordinates were the same as for preoptic cannulae in the preceding experiment, except that they were bilateral. One of four doses of scopolamine (1, 2, 5, or 10 μg) or 0.5 μl of the saline vehicle was injected through each of the animals' two cannulae 5 min before each of 5 weekly tests. Cochran's Q-tests were used for statistical analysis of nominal data, followed by McNemar post hoc comparisons when Q-tests were significant.

Results

Bilateral injections of scopolamine into the preoptic area produced significant dose-dependent decreases in the numbers of animals intromitting, Cochran's $Q(4)=10$, $p<0.05$, ejaculating at least once, $Q(4)=9.78$, $p<0.05$, and ejaculating at least three times, $Q(4)=10$, $p<0.05$ (see Table 1). The number of animals mounting was not significantly affected, nor were any other copulatory measures.

Discussion

Scopolamine impaired several measures of a copulatory performance mechanism [as opposed to measures of sexual arousal, described in (2,8)], when injected bilaterally into the preoptic area. Numbers of animals intromitting, ejaculating at least once, and ejaculating at least three times, were reduced in dose dependent fashion. Since 1, 2, and 5 μg unilateral injections in the previous experiment failed to affect any copulatory measure, it appears that cholinergic receptors on both sides of the preoptic area must be blocked in order to observe an effect on sexual behavior.

It is of interest that the specific measures affected by cholinergic agonists injected into the preoptic area (5) differed from those affected by scopolamine in this experiment.

TABLE 2

EFFECTS OF OXOTREMORINE AND SCOPOLAMINE INJECTIONS INTO THE PREOPTIC AREA ON COPULATION, EXPERIMENT 3

	Vehicle	OXO	SCOP	OXO + SCOP
Intromission Frequency	9.0 ± 1.0	6.2 ± 0.7*	7.0 ± 1.1	8.7 ± 1.1
% Mounting	70	83	35*	61
% Intromitting	61	74	17†	52
% Ejaculating	61	70	17†	48

*$p<0.05$, †$p<0.01$, relative to vehicle. OXO is oxotremorine (1 μg). SCOP is scopolamine (10 μg).

Both of the agonists, carbachol and oxotremorine, decreased intromission frequency, whereas that measure was not affected by scopolamine. Ahlenius and Larsson (1) reported a pattern of results similar to these, using systemic injections of oxotremorine and scopolamine. Specifically, oxotremorine decreased intromission frequency, whereas scopolamine injected alone failed to influence behavior, except that relatively high doses reduced the number of animals copulating.

EXPERIMENT 3: SCOPOLAMINE BLOCKADE OF OXOTREMORINE'S EFFECTS IN THE PREOPTIC AREA

This experiment was designed to verify that the effect of preoptic injections of oxotremorine, described in the previous article (5), was due to stimulation of muscarinic synapses.

Method

Twenty-three sexually experienced male rats had histologically confirmed bilateral angled cannulae ending in the preoptic area. Scopolamine (10 μg), oxotremorine (1 μg), the combination of the two, or the saline vehicle was injected in a volume of 0.5 μl into each of the animals' two cannulae immediately before each of four weekly tests. Order of treatments was counterbalanced.

Results

Oxotremorine injected alone into the preoptic area decreased the number of intromissions preceding ejaculation, but did not affect any other measure, $F(3,39)=3.18, p<0.05$; OXO vs. vehicle: $t(10)=2.79, p<0.02$ (see Table 2). This effect was blocked by concurrently administered scopolamine [OXO + SCOP vs. vehicle: $t(10)=0.3$, NS], which by itself did not affect this measure, $t(2)=3.02$, NS.

On the other hand, scopolamine injected alone into the preoptic area decreased the numbers of animals mounting, $Q(3)=11.36, p<0.01$, intromitting, $Q(3)=16.61, p<0.01$, and ejaculating, $Q(3)=14.82, p<0.01$ (see Table 2). Furthermore, these effects were blocked by concurrently administered oxotremorine, since results on oxotremorine plus scopolamine trials were not different from those on vehicle trials for any measure.

Discussion

As previously reported (5), microinjection of the muscarinic agonist oxotremorine into the preoptic area reduced ejaculatory threshold, as measured by a decrease in the number of intromissions preceding ejaculation. No other copulatory measure was affected. This effect was blocked by concurrent administration of the muscarinic antagonist scopolamine, confirming that the reduction in ejaculatory threshold was mediated by muscarinic cholinergic receptors.

On the other hand, scopolamine, injected alone into the preoptic area, significantly decreased the percentages of animals mounting, intromitting, and ejaculating. These effects were blocked by concurrently administered oxotremorine. The effects of scopolamine in this experiment are similar to those observed in Experiment 2, except that in addition to the decreases in animals intromitting and ejaculating, there was also a significant decrease in the percentage of animals mounting. Thus, whereas in previous experiments injections of both oxotremorine and scopolamine into the preoptic area affected only measures of a copulatory mechanism (oxotremorine: decreased intromission frequency; scopolamine: decreased percentages of animals intromitting and ejaculating), in this experiment a measure of sexual arousal (percentage of animals mounting) was also impaired. However, even here the percentages of animals intromitting and ejaculating were reduced more dramatically than the percentage of animals mounting; twice as many males mounted as those that intromitted and ejaculated. Thus, it would appear that measures of a copulatory mechanism are more readily altered by preoptic injections of a cholinergic antagonst than is sexual arousal. However, a profound reduction of cholinergic activity in the preoptic area can affect even the initiation of copulation. We have reported a similar pattern of results for preoptic injections of a dopaminergic agonist (apomorphine) and antagonist cis-flupenthixol) (7). In those experiments injection of the agonist and low doses of the antagonist affected only measures of a copulatory mechanism, whereas higher doses of the antagonist prevented copulation by most animals. Thus, we propose that small alterations of cholinergic and dopaminergic mechanisms in the preoptic area may serve to modify copulatory performance, whereas more severe reductions in either transmitter system may significantly impair sexual arousal.

GENERAL DISCUSSION

Our finding that relatively large doses of scopolamine injected into the preoptic area decreased the number of animals copulating, without affecting copulatory rate or ejaculatory threshold, is in agreement with reports that large doses of systemically administered scopolamine also merely decreased the percentages of animals copulating (1,6). However, systemically administered scopolamine in those experiments would have blocked postganglionic parasympathetic synapses, resulting in unpleasant peripheral effects, such as a dry mouth (4) or inability to attain an erection (11). Injections confined to the preoptic area should not have interfered with copulation in that way, although peripheral effects of preoptic injections were not directly assessed in these experiments.

We suggest that some cholinergic activation in the preoptic area is critical for normal copulation. Blocking muscarinic receptors there decreased the percentage of animals copulating; among those that did at least mount, fewer gained intromission or ejaculation. Furthermore, since the percentage of animals mounting was less frequently and less dramatically decreased than were the percentages of animals intromitting and ejaculating, a copulatory mechanism appears to be more vulnerable to disruption by preoptic injections of

a cholinergic antagonist than is an arousal mechanism. On the other hand, enhancing cholinergic activity in the preoptic area served only to decrease the number of intromissions preceding ejaculation (decreased ejaculatory threshold).

In contrast to the differential effects of agonists and an antagonist in the preoptic area, both types of drug injected into the lateral ventricle only delayed the onset of copulation. Since copulation usually begins within a few minutes after introduction of the female, a drug induced delay implies that the drug had reached the relevant site(s) within those few minutes. Therefore, those sites are probably within close proximity to the ventricular circulation. Most animals did eventually copulate, and when they did all parameters of their sexual behavior were normal. Furthermore, motor behavior appeared to be normal even during the prolonged delay. Thus, both enhancing and inhibiting cholinergic activity in structure(s) near the ventricular circulation can delay initiation of copulation.

ACKNOWLEDGEMENTS

We wish to thank Thomas Myers and Dave Brigham for assistance in measuring diffusion of tritiated scopolamine in brain tissue, and Dorothy Sideris for assistance with histology. This work was supported by NIMH grants MH4082601 and MH38526-01A1 and BRSG grants 2S07RR0706619 and 2S07RR0706620 to E.M.H. and by a PHS award HD06760 to L.G.C.

REFERENCES

1. Ahlenius, S.; Larsson, K. Central muscarinic receptors and male rat sexual behavior: Facilitation by oxotremorine but not arecoline or pilocarpine in methscopolamine pretreated animals. Psychopharmacology (Berlin) 87:127–129; 1985.
2. Beach, F. A. Characteristics of masculine "sex drive." In: Jones, M. R., ed. Nebraska symposium on motivation. vol. 4. Lincoln: University of Nebraska Press; 1956:1–32.
3. Bignami, G. Pharmacological influences on mating behavior in the male rat. Psychopharmacology (Berlin) 10:44–58; 1966.
4. Drips, R. D. Preanesthetic medication. In: Goodman, L. S.; Gilman, A., eds. The pharmacological basis of therapeutics. 3rd ed. New York: Macmillan Co.; 1965:57–60.
5. Hull, E. M.; Bitran, D.; Pehek, E. A.; Holmes, G. M.; Warner, R. K.; Band, L. C.; Clemens, L. G. Brain localization of cholinergic influence on male sex behavior in rats: Agonists. Pharmacol. Biochem. Behav 31:169–174; 1988.
6. Leavitt, F. I. Drug-induced modifications in sexual behavior and open field locomotion of male rats. Physiol. Behav. 4:677–683; 1969.
7. Pehek, E. A.; Warner, R. K.; Bazzett, T.; Bitran, D.; Band, L. C.; Eaton, R. C.; Hull, E. M. Microinjection of cis-flupenthixol, a dopamine antagonist, into the medial preoptic area impairs sexual behavior of male rats. Brain Res. 443:70–76; 1988.
8. Sachs, B. D. Conceptual and neural mechanisms of masculine copulatory behavior. In: McGill, T. E.; Dewsbury, D. A.; Sachs, B. D., eds. Sex and behavior: Status and prospectus. New York: Plenum Press; 1978:267–295.
9. Soulairac, M.-L. Etude experimentale des regulations hormono-nerveuses du comportement sexuel du rat male. Ann. Endocrinol. Paris 24(Suppl.)1–98; 1963.
10. Soulairac, M.-L., Soulairac, A. Monoaminergic and cholinergic control of sexual behavior in the male rat. In: Sandler, M.; Gessa, G. L., eds. Sexual behavior: Pharmacology and biochemistry. New York: Raven Press; 1975:99–116.
11. Vliet, L. W.; Meyer, J. K. Erectile dysfunction: Progress in evaluation and treatment. Johns Hopkins Med. J. 151:246–258; 1982.
12. Winer, B. J. Statistical principles in experimental design. 2nd ed. New York: McGraw Hill; 1971.