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Cholinergic Brain Mechanisms and the Hormonal Regulation of Female Sexual Behavior in the Rat

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CLEMENS, L. G., R. R. HUMPHRYS AND G. P. DOHANICH. *Cholinergic brain mechanisms and the hormonal regulation of female sexual behavior in the rat.* PHARMAC. BIOCHEM. BEHAV. 13(1) 81–88, 1980.—Cholinergic muscarinic stimulation of the medial preoptic area or the mesencephalic reticular formation with carbachol or bethanechol facilitated lordosis in ovariectomized female rats treated with estrogen. Adrenalectomy did not abolish the facilitative influence of cholinergic stimulation in the preoptic area. Implants of carbachol in the neocortex failed to increase lordosis.

Sexual behavior Acetylcholine Muscarinic receptors

FEMALE sexual behavior in laboratory rats is regulated by the ovarian hormones, estrogen and progesterone. Sequential treatment of females with these hormones will induce high levels of sexual responding: lordosis [2,3].

A number of brain regions are believed to be important for sexual responses to ovarian hormones. Implantation of estrogen into the medial preoptic-anterior hypothalamus (POM-AH) will induce sexual receptivity in the ovariectomized female rat [22,37]. Several recent reports indicate that the ventromedial hypothalamus (VMH) may be more sensitive to estradiol in this regard then the preoptic region [1,8]. Both the POM-AH and the VMH have been shown to selectively concentrate estrogen [29].

One site of progesterone action for sexual receptivity appears to be the mesencephalic reticular formation (MRF). Implants of progesterone in this region induced high levels of sexual receptivity in female rats treated with estrogen [24, 33, 37, 38]. Analysis of tritiated progesterone uptake also indicated that the ventral mesencephalon accumulates more progesterone than do hypothalamic regions including the POM-AH area [36]. Several studies, however, have reported that implants of progesterone in hypothalamic sites also facilitate lordosis in estrogen-primed female rats [30], as well as male rats [35]. The importance of progesterone in the POM-AH area is further indicated by evidence that estrogen induces synthesis of progesterone receptors in hypothalamic neurons [25,32]. Apparently, the central sites of estrogen and progesterone action which control receptivity in the female are both widespread and overlapping.

Pharmacological manipulations suggest that lordotic behavior can also be enhanced by altering neurotransmitter processes. Meyerson [26,27] reported that compounds which reduce serotonergic activity facilitated lordosis in females treated with estrogen. Subsequent investigations have shown that POM-AH implants of agents which interfere with the synthesis or binding of serotonin increase lordotic behavior [34,39] whereas treatments which promote serotonergic function inhibit lordosis (for review [5, 6]). Catecholamines have also been reported to influence lordosis in female rats primed with estrogen [6, 7, 9, 10, 11, 12, 13, 15, 34]. Direct application of either norepinephrine or dopamine to the POM-AH [6, 11, 12] or to the arcuate-ventromedial hypothalamus [11,12] increased lordosis frequency. In the case of norepinephrine, stimulation of beta adrenergic receptors appears to activate lordosis while alpha adrenergic transmission may inhibit the occurrence of this behavior [11].

Cholinergic mechanisms also appear to have a facilitative influence on lordosis. Systemic treatment with nicotine facilitated lordosis in estrogen-treated females [13]. This effect appears to result from the selective stimulation of nicotinic receptors since it was blocked by pretreatment with the nicotinic antagonist mecamylamine.

In the present experiments, we have extended the study of cholinergic mechanisms and sexual behavior. The influence of direct muscarinic stimulation of the POM and the MRF on the lordotic behavior of estrogen-primed female rats was examined.

METHOD

Subjects were Sherman female rats (Camm Research Co., Wayne, NJ, 75–80 days of age) weighing 250–300 g. Animals were ovariectomized or ovariectomized/adrenalectomized at approximately 110 days of age. All animals were housed singly, in a room maintained on a 14:10 reversed light-dark

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cycle with lights on at 2100 hours. Food and water (0.9% saline for adrenalectomized animals) were available ad lib.

Experimental females were selected on the basis of their ability to show sexual responses to estrogen and progesterone. To make this selection, ovariectomized females were tested for lordosis prior to any intracerebral implantation. One week after ovariectomy, females were treated with 1 μg/day estradiol benzoate (EB) for three days followed by 0.5 mg progesterone on the fourth day. Approximately 4–6 hr after progesterone treatment each animal was given a test for sexual receptivity by placing her with a sexually vigorous male. The male was allowed to mount the female 10 times and the responses of the female to these mounts were recorded. A sexually receptive female will normally show a concave arching of the back (lordosis) when mounted by the male. The frequency of lordosis during a "10 mount test" was used to calculate a Lordosis Quotient: LQ=(lordosis frequency/10 mounts)×100. Proceptive or soliciting responses by the female were also noted when they occurred, e.g. hopping and darting, or ear wiggling. Females that achieved an LQ of 50 or more were retained for intracerebral implantation.

Double-barrel stainless steel cannulae were unilaterally or bilaterally implanted in the brain via a stereotaxic instrument. The outer cannulae were constructed from 21 gauge stainless steel tubing, while the removable inner cannulae were constructed from 27 gauge tubing. These inner cannulae were fitted with a 21 gauge hub 2 mm long, which allowed the inner cannula to penetrate the brain 1 mm beyond the end of the outer guide cannula. The inner cannula was loaded by tapping it five times into a thin layer of crystalline chemical spread on a glass plate. This method allowed for 9–15 μg of material to be tapped into the lumen of the inner cannula, as determined from weight estimates on a Cahn electrobalance.

Beginning 7 days after implantation, experimental females were treated with EB for 3 days (1 μg/day) and tested on the fourth day. Four hours prior to testing on the fourth day each female received 125 μg of dexamethasone (Sigma, St. Louis) to reduce the chance of adrenal activation [28]. While the dosage of EB used in these experiments does not normally induce high levels of sexual activity, all females were given a 10 mount test immediately prior to intracerebral treatment (pretest). Females which exhibited an LQ of 30 or greater on this pretest were considered receptive and eliminated from further testing. In all of the tests reported this occurred only 3 times. Remaining animals were given the intracerebral treatment and tested 30, 60, and 120 min later. At the completion of testing, the inner cannula was removed and inspected under a dissecting microscope. If any chemical remained in the lumen, the female was eliminated from the experiment since it was not certain crystals had effectively reached brain tissue. Only 2 animals were eliminated as such. Animals were tested once a week until completion of a particular sequence of treatments. After behavioral testing all animals were perfused intracardially with saline and 10% formalin solution. Brains were excised, embedded in paraffin, and sectioned at 30 μ. All sections were then stained with luxol fast blue for fibers and counterstained with cresyl violet for cell bodies.

Statistical Analyses

In each of the experiments treatment order was randomized within subjects. Friedman's two-way analysis of variance was used for determining significance within groups over repeated testing. Scores were analyzed in two ways. First, all animals with an implant in a specific brain region were included to determine whether the treatment had an effect. If a treatment yielded a significant effect ($p<0.05$), animals in that group were divided into "positive" and "negative" responders for histological representation. To be regarded as a positive responder an animal had to achieve an LQ of 30 or better in one of the tests following intracerebral treatment. With the exception of the cortical implants, which were included as control implant sites, there were very few negative sites, and separating animals on the basis of arbitrary criterion of positive or negative responders did not have any significant effect upon the outcome of the statistical tests. Significant levels for the positive animals only are reported. Given significance with Friedman's test, a sign test was used to determine significance levels between pre and post intracerebral treatment tests. Statistical comparisons between treatments were made with the Wilcoxon T. All p values are 2-tailed.

EXPERIMENT 1

To determine whether cholinergic stimulation of the progesterone-sensitive MRF would influence lordosis, 11 females were implanted unilaterally with cannulae in the MRF via coordinates: Ant. 1.4 mm, Lat. 1.0 mm, Vert. 2.75 mm [17]. Each animal was tested on two consecutive weeks. Before each test all females were given the standard EB pretreatment (1 μg/day for 3 days) and then treated intracerebrally on the fourth day with the cholinomimetic agent, carbachol (carbamylcholine chloride; Sigma, St. Louis). Sixty min before intracerebral treatment (ICT) half of the animals were given an intraperitoneal injection of the cholinergic antagonist [14], atropine sulfate (2.5 mg/animal; Sigma, St. Louis), and the remaining half were given an equal volume of saline. Systemic treatment was reversed on the subsequent week.

To determine whether cholinergic stimulation would be effective at any brain locus, another group of 6 animals was unilaterally implanted in the neocortex. Animals in this group were tested weekly over three consecutive weeks until each animal had been tested with carbachol, progesterone, and cholesterol. The order of experimental testing for these three conditions was randomized for each animal.

Results

Direct cholinergic stimulation of the MRF resulted in a facilitation of lordosis in 10 of 11 estrogen-primed animals (Table 1). None of the animals achieved lordosis during the 10 mount test preceding ICT. A significant increase in lordosis was observed on all tests after ICT with carbachol ($p<0.001$; PT vs 30, 60, and 120 min tests). When the animals were pretreated with the cholinergic antagonist, atropine sulfate, they failed to achieve significant increases in lordosis on any of the post ICT tests.

Figure 1 shows the histological placement of the lordosis positive and negative implant sites. The ten positive sites for cholinergic implants were caudal and immediately dorsal to the interpeduncular nucleus; one nonresponsive site was found more ventral and caudal to the interpeduncular nucleus.

In contrast to the MRF implants, animals with cortical implants failed to show statistically significant changes in LQ

TABLE 1

EFFECTS OF UNILATERAL IMPLANTATION OF CARBACHOL IN THE MESENCEPHALIC RETICULAR FORMATION ON THE LORDOTIC BEHAVIOR OF OVARIECTOMIZED, ESTROGEN-PRIMED RATS

Treatment	N	PT	Post ICT tests 30 min	60 min	120 min
Carbachol + saline	10	0	71.0 ± 7.8*	71.1 ± 7.8*	64.0 ± 8.4*
Carbachol + atropine	10	0	6.0 ± 3.3†	9.0 ± 2.7†	16.0 ± 9.5†

All females were treated with 1 μg EB for three days and 125 μg dexamethasone 4 hr prior to behavioral testing. Atropine sulfate (2.5 mg) or saline was injected 1 hr before testing. Atropine/saline treatments were reversed for each female over the two weeks of testing. Pretests (PT) were given immediately before intracerebral treatment (ICT) with crystalline carbachol (10–15 μg).

Values are mean lordosis quotients ± SEM. *$p<0.001$, PT vs 30, 60, and 120 min tests, sign test following Friedman's analysis of variance; †$p<0.01$, carbachol + saline vs carbachol + atropine at 30, 60, and 120 min, Wilcoxon T.

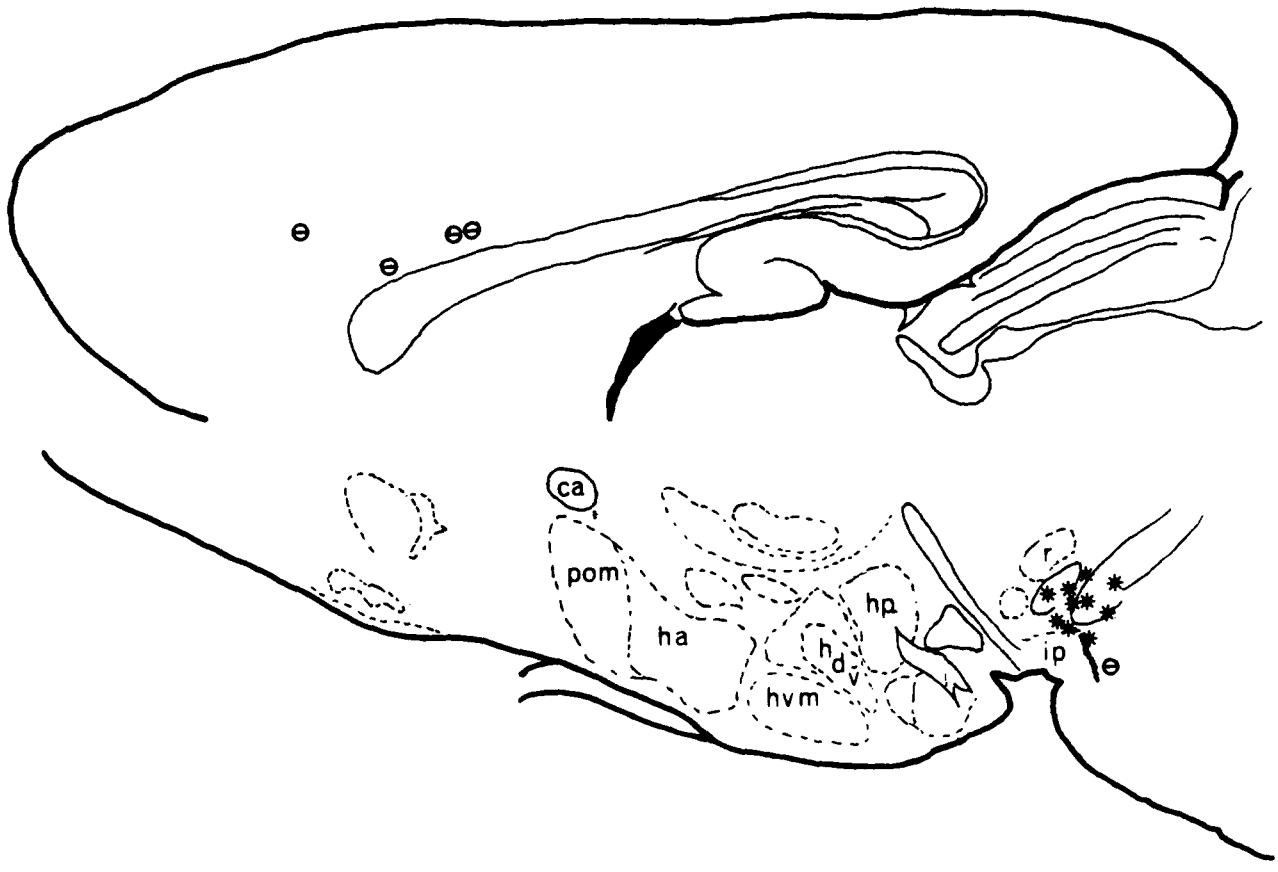

FIG. 1. Histological placement of unilateral implants (Lat. 1.0 mm) in the mesencephalic reticular formation (MRF) or neocortex of ovariectomized female rats from Experiment 1. Ten of 11 animals achieved the criterion of positive responder following implantation of carbachol in the MRF. None of the cortical treatments significantly facilitated lordosis. Only placements for 4 cortical animals receiving carbachol are shown. *positive responder; ⊖ negative responder. Abbreviations: ca, anterior commissure; ha, anterior hypothalmus; hdv, dorsomedial hypothalamus; hp, posterior hypothalamus; hvm, ventromedial hypothalmus; ip, interpeduncular nucleus; pom, medial preoptic area; r, red nucleus.

TABLE 2

EFFECTS OF NEOCORTICAL IMPLANTATION OF CARBACHOL, PROGESTERONE, AND CHOLESTEROL ON THE LORDOTIC BEHAVIOR OF OVARIECTOMIZED, ESTROGEN-PRIMED RATS

			Post ICT tests		
ICT	N	PT	30 min	60 min	120 min
Carbachol	4	0	5.0 ± 5.0	2.0 ± 2.5	7.0 ± 7.5
Progesterone	4	10.0 ± 4.0	5.0 ± 2.8	40.0 ± 12.9	30.0 ± 21.2
Cholesterol	5	4.0 ± 4.8	8.0 ± 4.8	10.0 ± 5.4	10.0 ± 6.3

All females were treated with 1 μg EB for three days and 125 μg dexamethasone 4 hr prior to behavioral testing. Animals were tested over three consecutive weeks until each female had been tested with carbachol, progesterone, and cholesterol. The order of experimental treatment was randomized for each female. Pretests (PT) were given immediately before intracerebral treatment (ICT).

Values are mean lordosis quotients ± SEM. Scores presented are only for females which displayed a lordosis quotient less than 30 on the PT.

following carbachol, progesterone, or cholesterol treatment (Table 2, Fig. 1). Under the carbachol and progesterone conditions 4 of the 6 animals had pretest scores below 30. Only scores and histology from these low pretest animals are reported in Table 2 and Fig. 1. The 2 animals which achieved higher than 30 on the pretest both responded at their pretest levels after experimental treatment. In the cholesterol tests 5 of 6 scored below 30 on the pretest.

EXPERIMENT 2

The next step in our analysis of cholinergic influences upon lordosis was to extend the investigation to include the POM which has been shown to be a brain area involved in mediation of female sexual behavior. In addition, the experiment was designed to determine whether muscarinic and/or nicotinic receptors were implicated in the facilitation of lordosis.

Eleven ovariectomized female rats, selected on the basis of their response to estrogen and progesterone, were bilaterally implanted. Six were implanted in the MRF and 5 were implanted in the POM via coordinates: Ant. 7.2 mm, Lat. 1.0 mm, Vert. 2.0 mm. Beginning 1 week after implantation each female was tested weekly for 2 consecutive weeks. Before each test all females were given the standard EB pretreatment (1 μg/day for 3 days) and then treated intracerebrally on the fourth day with the muscarinic agonist bethanechol (carbamyl β-methyl-choline Cl; Sigma, St. Louis). On the first test, half the animals in each group received a systemic injection of atropine sulfate (2.5 mg/animal) and the remaining half received saline 1 hr before ICT. The systemic treatments were reversed on the second weekly test. As in the previous experiment, each animal was given a 10 mount pretest before the ICT and then tested 30, 60, and 120 min later.

Results

Direct stimulation of the MRF or the POM with bethanechol resulted in a significant increase in lordotic behavior (Fig. 2). For the MRF implants, significant elevations were observed on all post ICT tests ($p<0.001$; PT vs 30, 60, and 120 min tests). For POM implants, only the 60 and 120 min test means were significantly higher than the pretest mean ($p<0.001$; PT vs 60 and 120 min tests). Pretreatment with the muscarinic antagonist, atropine sulfate, inhibited the facilitative effects of bethanechol in both MRF and POM animals (Fig. 2).

In comparing the responses of the POM and MRF animals, the increase in lordosis frequency occurred more rapidly from MRF stimulation than from POM since the 30 min test with the POM failed to reach statistical significance.

Four of the 5 POM implants achieved the criterion of 3 or more lordosis responses during the bethanechol+saline tests. All five implants were located in the anterior portion of the POM (Fig. 3). All 6 animals with MRF implants reached the criterion of positive responder. These implants were found to lie along the dorsal-caudal border of the interpeduncular nucleus (Fig. 3).

EXPERIMENT 3

While the preceding experiments all contained dexamethasone treatment to minimize the possibility of adrenal activation, independent measurement of adrenal activity was not possible. Therefore, a second control experiment was carried out with adrenalectomized animals to verify that POM stimulation with bethanechol did not induce lordosis via adrenal steroids. Animals were bilaterally adrenalectomized at the time of ovariectomy. Seven days later all animals were bilaterally implanted in the POM and subsequently treated with intracerebral bethanechol following treatment with EB and atropine sulfate as outlined in previous experiments. These animals were not administered dexamethasone.

Results

Cholinergic stimulation of the POM with the muscarinic agonist, bethanechol, resulted in significant increases in lordotic behavior in adrenalectomized females on all post ICT tests (Table 3; $p<0.001$; PT vs 30, 60, and 120 min tests). Pretreatment with atropine sulfate antagonized this facilitative effect (Table 3). Although mean LQ values for this experiment were lower on all tests than corresponding scores for intact groups, these differences were not statistically significant.

Examination of histological preparations revealed that implants were localized within the anterior portion of the POM (Fig. 4).

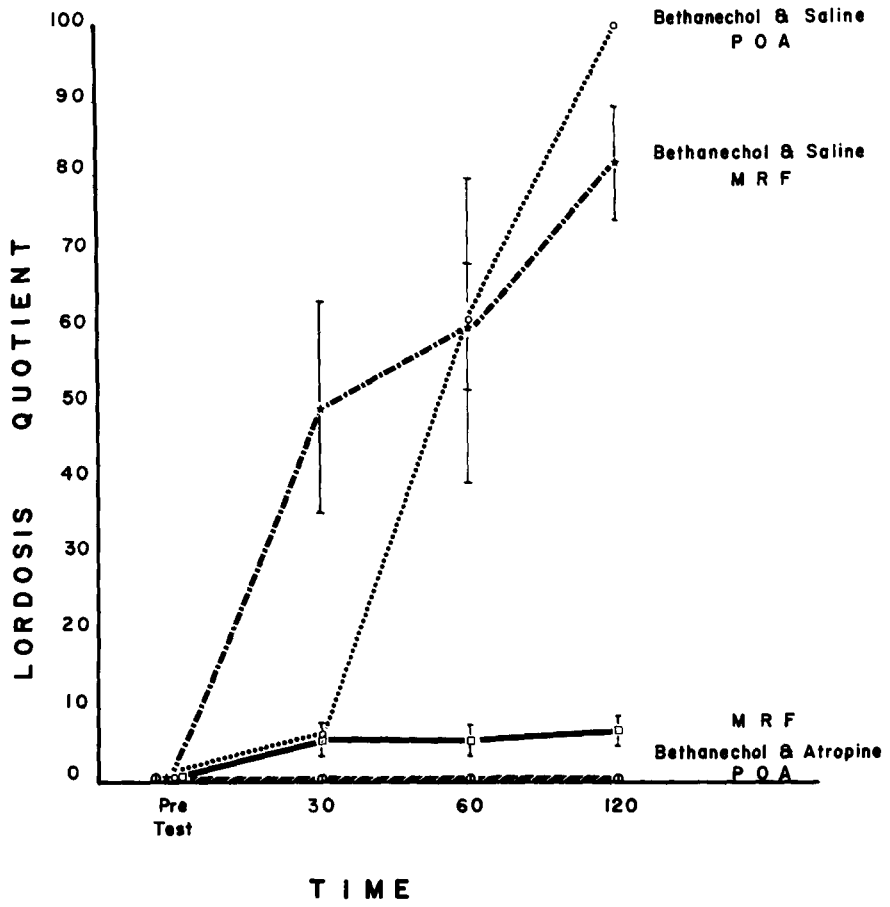

FIG. 2. Mean lordosis quotients ± SEM in ovariectomized female rats treated intracerebrally with crystalline bethanechol (10–15 μg/cannula). Bilateral cannula implants were placed in the medial preoptic area (POA, n=5) or the mesencephalic reticular formation (MRF, n=6). All females were treated with 1 μg EB for three days and 125 μg dexamethasone 4 hr before behavioral testing. Atropine sulfate (2.5 mg) or saline was injected 1 hr before testing. Atropine/saline treatments were reversed for each female over the two weeks of testing. Pretests (PT) were given immediately before intracerebral treatment. POA, bethanechol+saline: $p<0.001$, PT vs 60 and 120 min tests, sign test following Friedman's analysis of variance; MRF; bethanechol+saline: $p<0.001$, PT vs 30, 60, and 120 min tests; POA: $p<0.01$, bethanechol+saline vs bethanechol+atropine at 60 and 120 min, Wilcoxon T; MRF: $p<0.01$, bethanechol+saline vs bethanechol+atropine at 30, 60, and 120 min.

TABLE 3

EFFECTS OF BILATERAL IMPLANTATION OF BETHANECHOL IN THE MEDIAL PREOPTIC AREA ON THE LORDOTIC BEHAVIOR OF OVARIECTOMIZED/ADRENALECTOMIZED ESTROGEN-PRIMED RATS

			Post ICT tests		
Treatment	N	PT	30 min	60 min	120 min
Bethanechol + saline	7	0	32.0 ± 8.6*	54.2 ± 16.6*	48.5 ± 13.9*
Bethanechol + atropine	7	0	2.5 ± 2.5†	7.5 ± 4.9†	13.7 ± 9.4†

All females were treated with 1 μg EB for three days. Dexamethasone was not administered as previously. Atropine sulfate (2.5 mg) or saline was injected 1 hr before testing. Atropine/saline treatments were reversed for each female over the two weeks of testing. Pretests (PT) were given immediately before intracerebral treatment (ICT) with crystalline bethanechol (10–15 μg/cannula).

Values are mean lordosis quotients ± SEM. *$p<0.001$, PT vs 30, 60 and 120 min tests, sign test following Friedman's analysis of variance; †$p<0.02$, bethanechol + saline vs bethanechol + atropine at 30, 60, and 120 min, Wilcoxon T.

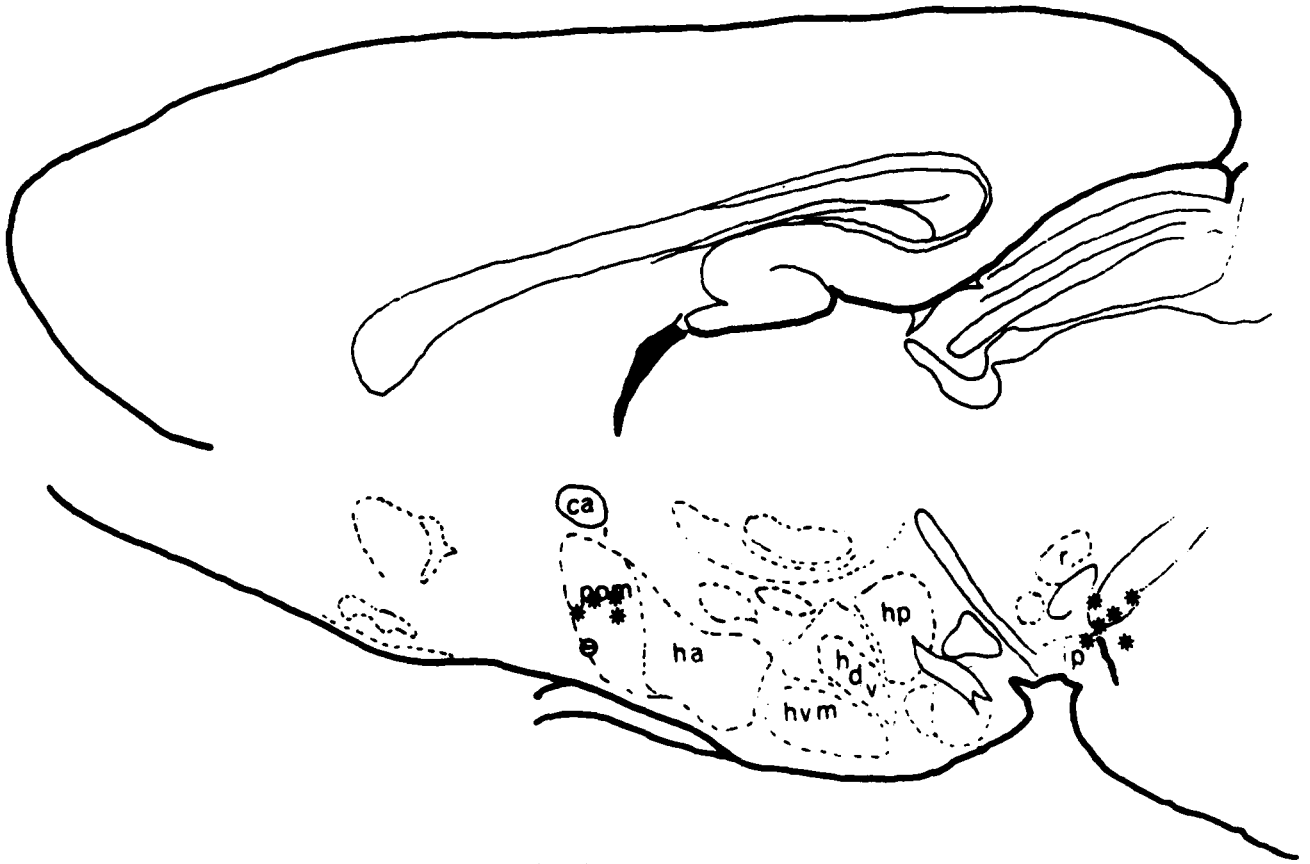

FIG. 3. Histological placement of bilateral bethanechol implants (Lat. 1.0 mm) in ovariectomized female rats from Experiment 2. Four positive sites and 1 negative site were found in the pom. Six positive sites were found dorsal to the ip. See Fig. 1 for abbreviations. *positive responder; ⊖negative responder.

DISCUSSION

Implantation of cholinergic agents into the mesencephalic reticular formation or medial preoptic area increased the probability of lordosis in estrogen-primed female rats. Crystalline implants of muscarinic receptor agonists, bethanechol and carbachol, in the MRF facilitated lordosis while implants of carbachol were effective in the POM. These facilitative effects in both brain areas were blocked by pretreatment with the muscarinic antagonist atropine sulfate.

We have previously demonstrated [4,33] that intracerebral treatment with progesterone in the MRF, near the interpeduncular nucleus, facilitated lordosis in the estrogen-primed female rat. In addition to selective concentration of progesterone by neurons in this region [36], this area has also been shown to have a high concentration of choline acetyltransferase (ChAc), an enzyme responsible for the synthesis of acetylcholine [16,18]. The findings of the present study indicate that activation of cholinergic receptors in this region with cholinergic agonists has a facilitative effect upon sexual receptivity. This raises the possibility that progesterone may achieve its facilitative effects upon lordosis by activation of a cholinergic system.

The role of estrogen in the MRF is, at present, uncertain. The mesencephalic area stimulated by our implants does not concentrate estrogen heavily [29]. In addition, estrogen implants in the POM which activate lordosis were ineffective when placed in the MRF [37]. Yet, some estrogenic priming appears to be necessary before MRF implants of progesterone will induce lordotic behavior [37].

Several lines of evidence are available to suggest that cholinergic facilitation of lordosis in the POM may have its basis in estrogen activation of sexual receptivity. Neurons in this region selectively concentrate estrogen [29] and intracerebral implants of estrogen in this area facilitate lordosis [22,37]. More recently, it has been demonstrated that estrogen treatment increased POM levels of ChAc [23]. Taken together such findings offer support for the idea that the estrogenic induction of lordosis may be mediated, in part, by cholinergic mechanisms located in the POM.

Establishing a relationship between estrogen action and neurotransmitter function is complicated by the fact that one aspect of estrogen action is to increase the number of progesterone receptors in the hypothalamus [25]. Furthermore, implantation of progesterone in the POM activates lordosis in estrogen-primed female rats [30,34]. Therefore, alteration of responsiveness to cholinergic stimulation of the POM or MRF following estrogen treatment may reflect activation of both estrogenic and progestagenic mechanisms.

The control conditions used in the present set of experiments indicate that the cholinergic stimulation effects were not due to nonspecific chemical effects. The facilitative effects of carbachol and bethanechol were blocked by the muscarinic blocking agent atropine sulfate. An additional experiment using adrenalectomized animals indicated that the facilitative effects could not be attributed to secretion of adrenal steroids subsequent to intracerebral treatment.

The results of the present study conflict with those of Lindstrom and his co-workers [19, 20, 21]. Lindstrom [20]

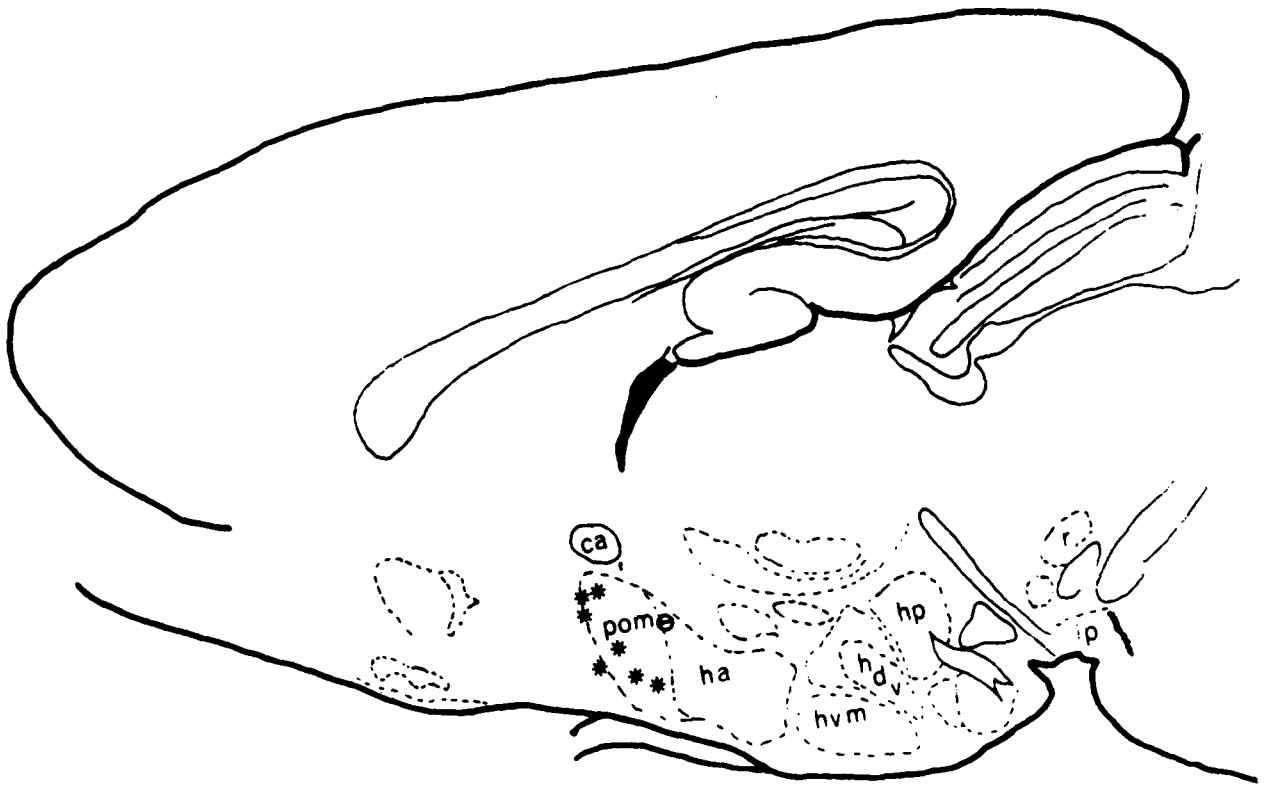

FIG. 4. Histological placement of bilateral bethanechol implants (Lat. 1.0 mm) in ovariectomized/adrenalectomized female rats from Experiment 3. Seven positive sites and 1 negative site were found in the pom. See Fig. 1 for abbreviations. *positive responder; ⊖negative responder.

reported that while the cholinergic agonists pilocarpine and oxotremorine facilitated lordosis in estrogen-primed female rats, this facilitation was blocked by adrenalectomy. In the present study adrenalectomy did not eliminate the facilitative effects of bethanechol. A reconciliation of these conflicting results is not possible since major methodological differences exist between the present study and those of Lindstrom.

Rogers and Law [31] found that crystalline implants of cholinergic compounds in various brain areas induced a low level of lordosis in female rats. More recently it has been reported that nicotine will facilitate lordosis in estrogen-primed rats [13]. This effect of nicotine was blocked by the nicotinic antagonist mecamylamine. Results of the present study support these earlier findings and indicate a facilitative role of cholinergic activity in the control of lordosis in rats.

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