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Inhibition of Lordotic Behavior in Female Rats Following Intracerebral Infusion of Anticholinergic Agents

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CLEMENS, L. G. AND G. P. DOHANICH. *Inhibition of lordotic behavior in female rats following intracerebral infusion of anticholinergic agents.* PHARMAC. BIOCHEM. BEHAV. 13(1) 89–95, 1980.—Cholinergic antagonists were bilaterally infused into forebrain areas of ovariectomized female rats brought into sexual receptivity by administration of estrogen and progesterone. Infusion of hemicholinium-3 (HC-3, 5 or 7.5 μg/cannula), an acetylcholine synthesis inhibitor believed to interfere with choline uptake, decreased the incidence of lordotic behavior displayed by females. This inhibition was prevented by infusion of choline chloride (120 μg/cannula) along with HC-3. Atropine sulfate, an acetylcholine receptor blocker, also reduced lordotic behavior in females following intracerebral infusion (20 μg/cannula). These results lend support to the suggestion that lordotic behavior is facilitated by central cholinergic activity.

Sexual behavior Acetylcholine Hemicholinium-3 Atropine sulfate

THE sexually receptive female rat displays an arching of her back with elevation of the head and perineum when mounted by the male. This response by the female, lordosis, facilitates vaginal penetration by the male and is known to be activated by the ovarian hormones estrogen and progesterone [5].

Recent reports have implicated several neurotransmitters in the mediation of hormonally-induced lordosis. An inhibitory role for serotonin has been suggested [8, 10, 24, 25, 32, 34] while norepinephrine may function to facilitate lordosis [7,11]. Dopamine, in contrast, appears to exert either facilitative or inhibitory actions on lordotic behavior depending on the experimental contingency [8, 9, 12, 18].

Centrally-active acetylcholine (ACh) may represent another neural influence on female receptivity. Lindstrom and Meyerson [22] reported that systemic administration of cholinergic agonists such as pilocarpine, oxotremorine, and arecoline reduced the incidence of lordosis in female rats primed with estrogen and progesterone. This inhibition may have been mediated by a serotonergic action, however, since the inhibitory effect of pilocarpine was prevented by prior treatment with a serotonin synthesis inhibitor [20]. Alternatively, there is evidence indicating a facilitative effect of ACh on female receptivity. Stimulation of cholinergic receptors by treatment with nicotinic or muscarinic agonists [6,15] has been shown to facilitate lordosis in female rats primed with estrogen. High levels of lordotic behavior were elicited in estrogen-primed females following muscarinic stimulation of the mesencephalic reticular formation or the medial preoptic area [6]. Activation of lordosis by centrally-administered cholinergic agents occurred with short latency and persisted in the absence of adrenal progestins [6].

The present study was designed to extend our analysis of cholinergic facilitation of receptivity. In the experiments reported here, behavioral effects of cholinergic antagonists, hemicholinium-3 and atropine sulfate, were examined in ovariectomized female rats made receptive by administration of estrogen and progesterone. In order to limit drug actions to the brain, compounds were infused directly into brain regions known to selectively concentrate estrogen. Based on our previous work with cholinergic agonists [6] it was predicted that disruption of normal cholinergic activity in estrogen-progesterone treated females would result in a reduction in the incidence of lordosis.

GENERAL METHOD

Animals

Sherman female rats were obtained from Camm Research Co., Wayne, NJ, at 60–70 days of age. The animals were housed singly with free access to food and water. A reversed light-dark cycle was maintained in the vivarium (14 hr light:10 hr dark, lights off at 1100 hr). All surgical manipulations were conducted under ether anesthesia and were followed by a minimum recovery period of one week. Only ovariectomized females displaying high levels of sexual receptivity (LQ≥70, see below) on a preimplant test following estrogen and progesterone priming were included in the study.

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Stereotaxis and Infusion

Cholinergic drugs were infused intracerebrally via a double-barrel cannula assembly. Each assembly consisted of a 23 gauge outer barrel (guide) and a removable 27 gauge inner barrel (insert). Guides were bilaterally implanted 1 mm dorsal to the target sites and anchored to the skull with machine screws and dental acrylic. Each insert extended 1 mm beyond the end of the guide into the site. Prior to intracerebral infusion, the insert was removed and replaced by a 28 gauge infusion insert which also extended into the target site. The infusion insert was connected by 20 PE polyethylene tubing to a syringe mounted on a reciprocal microinfusion pump (Harvard Apparatus). A 0.5 μl volume of solution was delivered over 30 sec through each cannula (1 μl/animal). Following infusion, the original insert was returned to the guide.

Solutions

For intracerebral infusions, hemicholinium-3 (Aldrich Chemical, Milwaukee), choline chloride (Sigma Chemical, St. Louis), and atropine sulfate (Sigma Chemical, St. Louis) were dissolved in an artificial cerebrospinal fluid (CSF). The CSF (pH=6.8) consisted of redistilled H_2O with the following ionic concentrations: 164.8 mM Na^+, 3.0 mM K^+, 0.8 mM Mg^{++}, 1.3 mM Ca^{++}, and 137.1 mM Cl^-. Estradiol benzoate (EB) and progesterone (P) were injected intramuscularly in 0.1 ml volumes of sesame seed oil.

Behavioral Testing

Behavioral tests were conducted in a Plexiglas arena (45×50×58 cm) occupied by a Long-Evans male rat. Each female rat was mounted a total of 10 times during a single test. Any female failing to receive 10 mounts within 10 min was transferred to another arena with a different male where the test was completed. The number of lordosis responses observed per 10 mounts was recorded and a lordosis quotient was computed for each female: LQ=(number of lordoses/10 mounts)×100.

Histology

After the final behavioral test, all females were anesthetized with ether and perfused intracardially with 0.9% saline followed by 10% buffered formalin. Frozen coronal sections (50 μ) were taken and implant locations were verified following neutral red staining.

EXPERIMENT 1

In the first experiment we examined the incidence of lordosis in receptive female rats following intracerebral infusion of hemicholinium-3 (HC-3). This anticholinergic has been shown to retard the synthesis of ACh by competitively inhibiting transport of the precursor, choline, across neuronal membranes [16, 17, 23]. HC-3 was bilaterally infused near the dorsal-medial border of the bed nucleus of the stria terminalis, an area shown to heavily concentrate tritiated estradiol [26].

Method

Ovariectomized female rats were bilaterally implanted with cannulae according to stereotaxic coordinates provided by Albe-Fessard *et al.* [1]. The tip of each insert was located at anterior 7.9 mm, lateral 1.0 mm, horizontal 5.5 mm (Fig. 1). One week after surgery the lordotic behavior of all females was evaluated in order to identify any deleterious effects of the implant procedure. Each female was primed with 2 μg EB 72, 48, and 24 hr before the test. A 500 μg injection of P was administered 4–6 hr before testing. Since all females displayed high levels of receptivity this postimplant test was omitted from the remaining experiments. During the following two weeks animals were again primed with 2 μg EB 72, 48, and 24 hr before behavioral testing. Approximately 4–5 hr after receiving 500 μg P and before intracerebral treatment, each female was "pretested" for lordosis. After the pretest, each female was bilaterally infused with one of three solutions: 7.5 μg HC-3/cannula, 5 μg HC-3/cannula, CSF vehicle. The effects of intracerebral treatment on lordotic behavior were tested 15 and 30 min after infusion. Each feamle received the same hormonal and intracerebral treatment on two consecutive weeks.

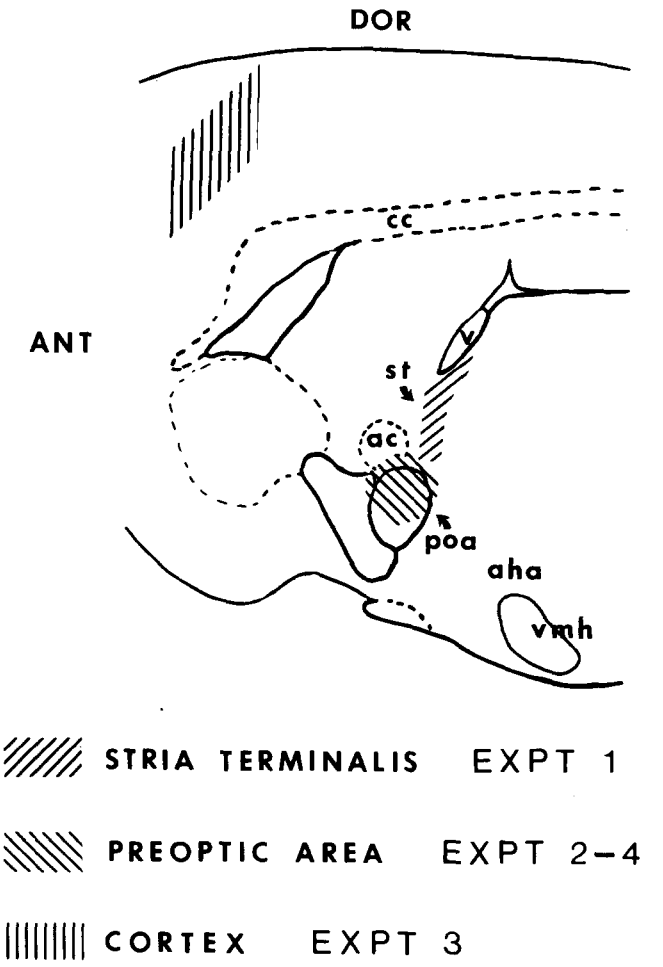

FIG. 1. Distribution of insert cannula tips as histologically verified in female rats from 4 experiments. Only rats with implants in the areas indicated were included in data analysis. The plate represents a sagittal section taken at 1.0 mm lateral to the midline. Adapted from Albe-Fessard *et al.* [1]. Abbreviations: ANT, anterior; DOR, dorsal: ac, anterior commissure; aha, anterior hypothalamic area; cc, corpus callosum; poa, preoptic area; st, stria terminalis; v, ventricle; vmh, ventromedial hypothalamus.

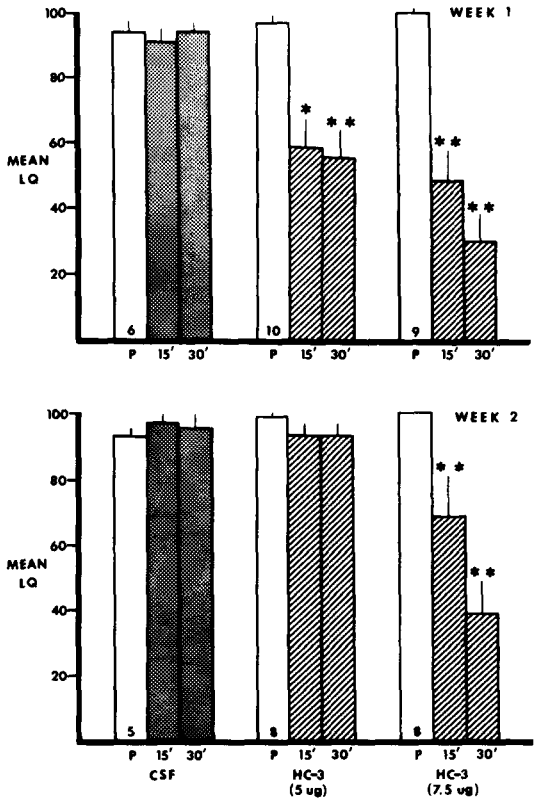

FIG. 2. Mean lordosis quotients (LQ) with standard errors recorded from female rats treated intracerebrally with either HC-3 or CSF vehicle on two weekly tests. All females were primed with 2 μg EB for three days and 500 μg P 4–5 hr before testing. Unshaded bars represent preinfusion scores. The effects of intracerebral treatments were tested 15 and 30 min after infusion. The change in LQ following HC-3 was compared to the change observed following CSF at corresponding test times (Mann-Whitney U; $*p<0.05$, $**p<0.01$).

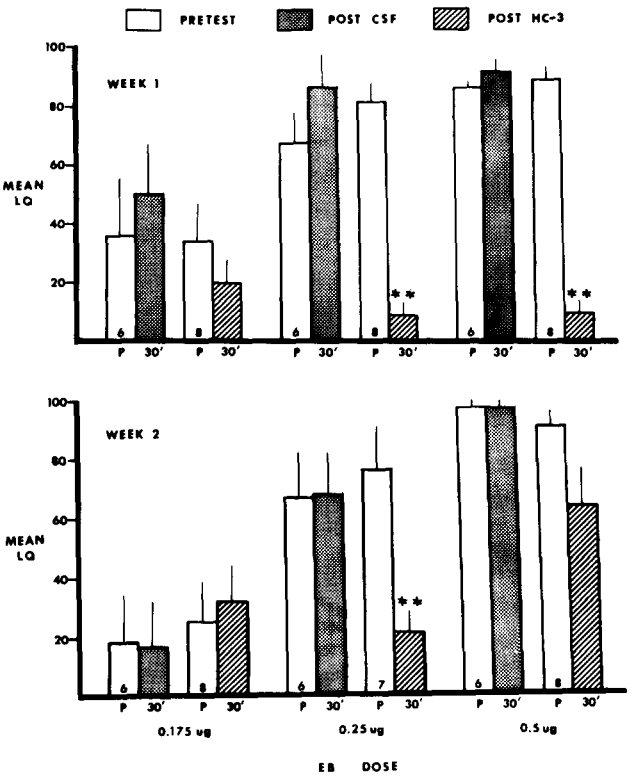

FIG. 3. Mean lordosis quotients (LQ) with standard errors recorded from female rats treated intracerebrally with either HC-3 (5 μg/cannula) or CSF vehicle on two weekly tests. Females were primed with either 0.175 μg, 0.25 μg, or 0.5 μg EB for three days and 500 μg P 4–5 hr before testing. Unshaded bars represent preinfusion scores. The effects of intracerebral treatments were tested 30 min after infusion. The change in LQ following HC-3 was compared to the change observed following CSF at corresponding EB doses (Mann-Whitney U; $*p<0.05$, $**p<0.01$).

Results

Intracerebral infusion of HC-3 significantly reduced the incidence of lordosis in females treated with EB (2 μg) and P (500 μg) (Fig. 2). The change in LQ following HC-3 infusion (pretest minus test) was compared to the change in LQ observed following CSF infusion at corresponding times after intracerebral treatment (Mann-Whitney U test). During the first week of treatment, significant reductions in LQ were recorded at 15 and 30 min following infusion of 5 μg HC-3/cannula (at 15 min, $p<0.05$; at 30 min, $p<0.01$) or 7.5 μg HC-3/cannula (at 15 min, $p<0.01$; at 30 min, $p<0.01$). During the second week of treatment, similar reductions in LQ were observed only following infusion of the higher dose of HC-3 (7.5 μg/cannula: at 15 min, $p<0.01$; at 30 min, $p<0.01$). Infusion of 5 μg HC-3/cannula failed to alter lordotic behavior during the second week of treatment.

EXPERIMENT 2

Significant reductions in lordosis were observed in Experiment 1 following a single infusion of HC-3 into the stria terminalis of receptive females. However, behavioral inhibition was evident only at the higher dose of HC-3 (7.5 μg/cannula) following a second treatment on the subsequent week. We suspected that estrogen and progesterone in the quantities administered may have induced a behavioral tolerance to HC-3 by the second week of testing. Protection conferred by steroids against neurotropic agents is well documented [28,29]. To determine whether the dosage of EB used in Experiment 1 was interfering with HC-3 treatment, lower dosages of EB were utilized in Experiment 2. In addition, the possibility of a facilitative action of HC-3 was examined by testing females primed with a combination of EB plus P which normally induces only a low level of receptivity.

Method

Ovariectomized female rats were bilaterally implanted so that the tip of the insert cannula rested on the dorsal border of medial preoptic area (coordinates: anterior 7.9 mm, lateral 1.0 mm, horizontal 4.0 mm). We have found this estrogen-concentrating region [24] to also be sensitive to anticholinergic intervention (Fig. 1).

During the two weeks following implantation, animals were primed with either 0.175 μg, 0.25 μg, or 0.5 μg EB 72, 48, and 24 hr before behavioral testing. Approximately 4–5 hr after receiving 500 μg P, each female was pretested and afterward bilaterally infused with either HC-3 (5 μg/cannula) or CSF vehicle. The effects of these intracerebral treatments

were tested 30 min after infusion. Each animal received the same priming dose of EB and intracerebral treatment on both weeks.

Results

Preoptic infusion of HC-3 (5 μg/cannula) caused substantial reductions in lordotic behavior of females primed with EB and P (Fig. 3). The change in LQ following HC-3 infusion (pretest minus test) was compared to the change in LQ observed following CSF infusion at corresponding doses of EB (Mann-Whitney U). During the first week of treatment, significant reductions in LQ were seen following HC-3 infusion in females receiving 0.5 μg EB plus P ($p<0.01$) or 0.25 μg EB plus P ($p<0.01$). During the second week of treatment, a significant reduction in LQ following HC-3 infusion was observed only in those females receiving 0.25 μg EB plus P ($p<0.01$). A second exposure to HC-3 failed to significantly reduce lordotic behavior in females receiving 0.5 μg EB plus P. The tolerance to HC-3 displayed by females primed with this highest EB dose was similar, though not as pronounced, as that seen in Experiment 1 at a 2 μg priming dose of EB. Finally, no significant elevations in lordotic behavior occurred in HC-3-treated females receiving 0.175 μg EB plus P.

EXPERIMENT 3

The third experiment evaluated the specificity of the HC-3 effects reported in Experiments 1 and 2. First, we attempted to counteract the inhibitory action of HC-3 by simultaneous administration of choline chloride. Choline, possibly the rate limiting factor in ACh synthesis, is not produced centrally but rather is transported to the neuron in a lipid-bound form [3]. The quaternary ammonium compound, HC-3, is postulated to competitively inhibit the transport of choline across neuronal membranes to the site of ACh synthesis [16, 17, 23]. Accordingly, the depletion in brain ACh content reported following intraventricular infusion of HC-3 [13] can be prevented if choline chloride is infused along with HC-3 [31]. A dose of 120 μg/cannula of choline chloride was administered in order to reverse the effects of 5 μg/cannula of HC-3. Choline chloride doses 12–50 times those of HC-3 are required to prevent significant reduction in ACh level [4,31]. A second control in Experiment 3 was infusion of an active dose of HC-3 into cortical brain areas of females receiving estrogen and progesterone to determine if HC-3 action was specific to particular brain sites. No alteration in lordotic behavior was expected from cortical infusion of HC-3 since this area does not concentrate ovarian hormones [26].

Method

Bilateral implants in the medial preoptic area were performed as previously described in Experiment 2. A single test session was conducted. Animals were primed with 0.5 μg EB 72, 48, and 24 hr before behavioral testing. Approximately 4–5 hr after receiving 500 μg P, each female was pretested and afterward bilaterally infused with either HC-3 (5 μg/cannula), or HC-3 (5 μg/cannula) plus choline chloride (120 μg/cannula). The effects of these intracerebral treatments were tested 30 and 60 min after infusion.

Bilateral implants were also placed in the neocortex (Fig. 1) of ovariectomized females at coordinates: anterior 10.0 mm, lateral 1.0 mm, horizontal 9.0 mm. During the two weeks following implantation, animals were primed with 0.5 μg EB 72, 48, and 24 hr before behavioral testing. Approximately 4–5 hr after receiving 500 μg P, each female was pretested and afterward bilaterally infused with either HC-3 (5 μg/cannula), or HC-3 (5 μg/cannula) plus choline chloride (120 μg/cannula). The effects of these intracerebral treatments were tested 30 and 60 min after infusion.

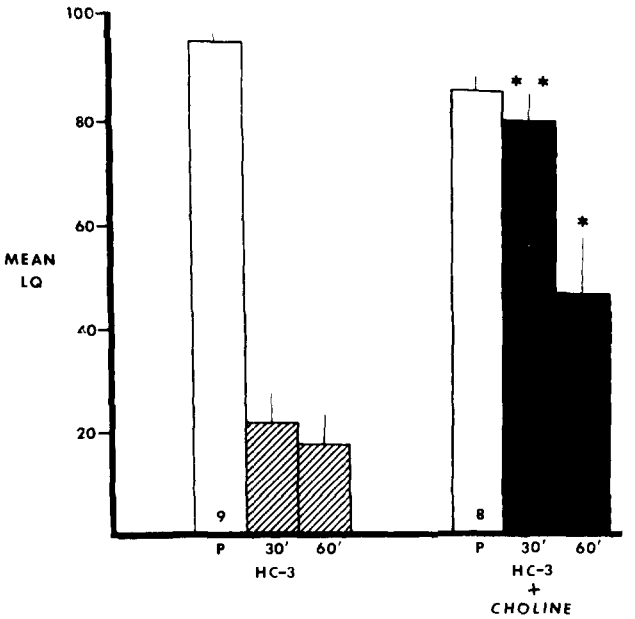

FIG. 4. Mean lordosis quotients (LQ) with standard errors recorded from female rats treated intracerebrally with either HC-3 (5 μg/cannula) or HC-3 (5 μg/cannula) plus choline chloride (120 μg/cannula). All females were primed with 0.5 μg EB for three days and 500 μg P 4–5 hr before testing. Unshaded bars represent preinfusion scores. The effects of intracerebral treatment were tested 30 and 60 min after infusion. The change in LQ following HC-3 was compared to the change observed following HC-3 plus choline (Mann-Whitney U; $*p<0.05$, $**p<0.01$).

Results

When HC-3 (5 μg/cannula) was infused in combination with choline chloride (120 μg/cannula) the inhibitory effect of HC-3 on lordotic behavior was arrested (Fig. 4). The reductions in LQ observed at 30 and 60 min following HC-3 infusion were significantly greater than reductions seen following infusion of HC-3 plus choline chloride (at 30 min, $p<0.01$; at 60 min, $p<0.05$). The disruption of the inhibitory effect of HC-3 achieved by choline chloride began to wane by 60 min. There was no evidence of behavioral inhibition following infusion of HC-3 (5 μg/cannula) into the neocortex of EB and P treated females (Table 1).

EXPERIMENT 4

Atropine, a native alkaloid, has been shown to antagonize the action of ACh at cholinergic synapses by interfering with muscarinic receptors [19]. We predicted that hypothalamic infusion of atropine, in the sulfate form, would therefore inhibit the lordotic behavior of females primed with estrogen and progesterone.

Method

Bilateral implants in the medial preoptic area were per-

TABLE 1

MEAN LORDOSIS QUOTIENTS (± SE) OF FEMALE RATS TREATED NEOCORTICALLY WITH HC-3 FOLLOWING ADMINISTRATION OF EB AND P

	Week 1				Week 2		
Intracerebral treatment*	n	Pretest	30 min	60 min	n	Pretest	30 min	60 min
HC-3 (5 μg/cannula)	8	88 ± 4	90 ± 3	89 ± 5	7	90 ± 4	93 ± 3	89 ± 3

*All females were primed with 0.5 μg EB for three days and 500 μg P 4–5 hr before testing. The effect of intracerebral treatment was tested 30 and 60 min after infusion.

formed as previously described in Experiment 2. A single test session was conducted. Animals were primed with 0.5 μg EB 72, 48, and 24 hr before behavioral testing. Approximately 4–5 hr after receiving 500 μg P, each female was pretested and afterward bilaterally infused with one of three solutions: 10 μg atropine sulfate/cannula, 20 μg atropine sulfate/cannula, CSF vehicle. The effects of intracerebral treatments were tested 15, 45, and 90 min after infusion.

Results

Atropine sulfate reduced the lordotic behavior of females when infused into the preoptic area at a dose of 20 μg/cannula (Fig. 5). Significant inhibition was observed in females primed with EB (0.5 μg) plus P (500 μg) at 15 min ($p<0.05$), 45 min ($p<0.05$), and 90 min ($p<0.05$) following infusion. A lower dose of atropine sulfate (10 μg/cannula) failed to alter lordotic behavior following preoptic infusion.

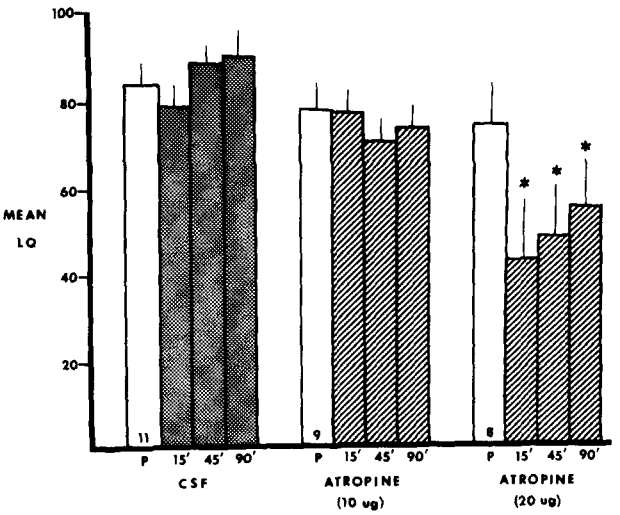

FIG. 5. Mean lordosis quotients (LQ) with standard errors recorded from female rats treated intracerebrally with either atropine sulfate or CSF vehicle. All females were primed with 0.5 μg EB for three days and 500 μg P 4–5 hr before testing. Unshaded bars represent preinfusion scores. The effects of intracerebral treatments were tested 15, 45, and 90 min after infusion. The change in LQ following atropine sulfate was compared to the change observed following CSF at corresponding test times (Mann-Whitney U; *$p<0.05$).

GENERAL DISCUSSION

This series of experiments confirms and extends recent findings which indicate that central cholinergic tone may influence hormonally-induced sexual behavior. Substantial increases in lordotic behavior have been observed following systemic administration of nicotine to estrogen-primed females [15]. This facilitative action of nicotine was prevented by prior treatment with a nicotinic receptor blocker but unaltered by pretreatment with dopaminergic antagonists. In the preceding study [6], muscarinic agonists increased lordosis in estrogen-primed females when implanted into either the mesencephalic reticular formation or the medial preoptic area. The facilitation of lordosis by central stimulation of muscarinic receptors was blocked by pretreatment with an appropriate cholinergic antagonist.

In the present study, a significant inhibition of lordotic behavior was observed following intracerebral treatment with agents known to disrupt cholinergic function. Infusion of HC-3, a compound which retards ACh synthesis [16, 17, 23], reduced the incidence of lordosis in ovariectomized females receiving EB and P. Similarly, atropine sulfate, a muscarinic receptor blocker [19], inhibited lordotic behavior in EB and P primed females. The latter result contradicts some previous reports which failed to alter lordotic behavior by systemic administration of atropine sulfate [21,22]. However, the high dose of atropine sulfate required to elicit a significant reduction in lordosis (20 μg/cannula) makes interpretation of this inhibitory effect difficult to assess. Similar reductions in lordotic behavior have been observed following systemic treatment with high doses (10 mg) of atropine sulfate [30]. High concentrations of atropine sulfate may be necessary to occlude a significant percentage of muscarinic receptor sites [33].

Earlier reports by Lindstrom described both facilitative and inhibitory roles of cholinergic stimulants in the mediation of lordosis. Cholinergic agonists pilocarpine, oxotremorine, and arecoline were initially found to reduce lordotic behavior in females treated with estrogen and progesterone [22]. A serotonergic mechanism appeared to be implicated, however, since pretreatment with parachlorophenylalanine blocked the inhibitory effect of pilocarpine [20]. Pilocarpine and oxotremorine were later shown to increase lordotic behavior in estrogen-primed females 4 hr after systemic injection [21]. The facilitation was blocked by atropine sulfate, reinforcing a cholinergic interpretation. However, the enhancement of lordosis was attributed to a drug-induced release of adrenal progestins since adrenalectomy abolished the increase. The discrep-

ancy between data reported by Lindstrom and observations recorded in our laboratory may be related to the mode of drug delivery or testing procedures. Direct cholinergic stimulation of appropriate brain areas induced a prompt lordotic response which persisted in the absence of the adrenal glands [6].

The action of HC-3 reported in the present study was both localized and specific. The inhibition of lordosis was observed only after HC-3 infusion into sites known to concentrate estrogen [26]. Further, HC-3 failed to significantly reduce the incidence of lordosis when choline chloride was added to the infusate. Decline in ACh levels following intraventricular infusion of HC-3 [13] can be counteracted by simultaneous infusion of choline chloride [31]. Evidence from *in vitro* preparations indicates that HC-3 inhibits the high affinity uptake of choline necessary for the intraneuronal synthesis of ACh [16, 17, 23]. However, the mechanism by which HC-3 alters *in vivo* ACh synthesis is not completely understood. Although intraventricular infusions of low doses of HC-3 (0.3–10 μg) effectively deplete ACh levels, concomitant inhibition of central ^{14}C-choline uptake has not been demonstrated [13,27]. Choline chloride does prevent both depletion of ACh levels [31] and high affinity choline uptake [27] when infused with higher intraventricular doses of HC-3 (10–300 μg). A recent report by Freeman and coworkers [14], however, indicates that lower *in vivo* doses of HC-3 probably reduce endogenous ACh levels by inhibiting high affinity uptake of choline. We were able to counteract the inhibitory effects of HC-3 (5 μg/cannula) on lordosis by simultaneous administration of choline chloride (120 μg/cannula). This dose of HC-3 may have been effective in arresting choline uptake within a localized area around the cannula tip. Presumably the functional integrity of cholinergic processes within this region is vital to the full display of lordotic behavior.

Behavioral inhibition was observed within 15 min after HC-3 infusion in Experiment 1 and within 30 min after HC-3 infusion in Experiments 2 and 3. Similarly, whole brain ACh levels have been reported to be significantly depleted within 10 min after intraventricular infusion of HC-3 [31], while lowest ACh levels were detected after several hours [13,14]. In Experiment 3, the inhibition of lordosis by HC-3 was completely prevented 30 min after infusion of HC-3 with choline chloride. However, by 60 min after infusion, the behavioral inhibition by HC-3 was only partially arrested. This waning effect may have been the result of a rapid transport of excess choline from the brain [2].

A tolerance to the inhibitory effects of HC-3 was observed in some groups during the second week of behavioral testing. This tolerance to HC-3 was prevented when the dose of HC-3 was increased (Experiment 1) or when the priming dose of EB was decreased (Experiment 2). Consequently, it seems likely that repeated steroid treatment induced a behavioral tolerance to HC-3 by the second week of testing. This explanation of our results seems most tenable since the occurrence of tolerance to HC-3 was clearly dependent upon the relative amount of EB administered and the period of exposure. Selye [28,29] has demonstrated the protection afforded by steroids against toxic levels of neurotropic agents. "Catatoxic" steroids, such as estradiol, may heighten the activity of various enzyme systems which degrade and inactivate the neurotropic drug [29]. Alternatively, repeated steroid exposure may have accelerated ACh synthesis such that the lower dose of HC-3 (5 μg/cannula) was less effective on the second week.

Our results demonstrate that manipulations which disrupt the normal cholinergic activity of certain brain regions inhibit the display of behaviors appropriate to mating. This finding, combined with data presented in the preceding study [6], clearly indicate a facilitative role of acetylcholine in the mediation of hormonally-induced lordosis. As in the cases of the other transmitters shown to influence sexual behavior, the mechanism by which cholinergic events facilitate lordosis is unknown. Neuropharmacological agents which alter lordotic behavior have been traditionally interpreted as acting in a manner analogous, if not identical, to progesterone. This suggestion has yet to be adequately substantiated. Consequently, any conclusion which depicts the actions of neurotransmitters as interacting with either estrogen or progesterone processes may be premature.

ACKNOWLEDGEMENTS

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