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Physostigmine Facilitation of Lordosis in Naturally Cycling Female Rats

CHERYL S. MENARD AND GARY P. DOHANICH[1]

Department of Psychology and Neuroscience Program, Tulane University, New Orleans, LA 70118

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MENARD, C. S. AND G. P. DOHANICH. *Physostigmine facilitation of lordosis in naturally cycling female rats.* PHARMACOL BIOCHEM BEHAV 36(4) 853–858, 1990. — Both systemic and intracerebral administrations of the cholinergic muscarinic antagonist, scopolamine, have been shown to inhibit naturally occurring sexual behavior in intact, cycling female rats. The present study examined the facilitative effects of the acetylcholinesterase inhibitor, physostigmine (eserine), on sexual behavior in intact, cycling female rats. Cycling was determined by daily monitoring of sexual behavior and vaginal cytology. When administered during either early proestrus or proestrus, physostigmine activated lordosis 15 min and 1 hr after intraventricular infusion (10 μg bilaterally). However, infusion of physostigmine failed to facilitate lordosis 15 min after administration during either diestrus I, mid-diestrus, or diestrus II. The administration of this cholinergic agent did not interrupt cyclicity patterns. Because estrogen levels are highest during proestrus and cholinergic facilitation appears to be limited to this time, it is suggested that estrogen priming of central cholinergic systems is necessary for the cholinergic regulation of sexual behavior in intact, cycling female rats.

Physostigmine Eserine Estrous cycle Acetylcholine Lordosis Female sexual behavior

SEXUAL behavior in female rats is characterized by soliciting behaviors, such as hopping and darting in the presence of a male rat, and lordosis, a ventral arching of the spine with the elevation of the perineum during mounting by a male rat. Sexual receptivity usually occurs within an 8–10-hour period during the proestrous-estrous stage of the estrous cycle which is 4 to 5 days in length. The occurrence of lordosis in female rats is activated and maintained by steroid hormones released from the ovaries (1). It has been further suggested that central cholinergic activity may contribute to the regulation of sexual behavior by steroids (4).

Intracerebral administration of the cholinergic agents, carbachol, bethanechol, and oxotremorine facilitates lordosis in ovariectomized rats primed with low levels of hormones (6, 7, 12). Pretreatment with the cholinergic muscarinic receptor blockers, atropine and scopolamine, prevents the facilitative effects of cholinergic agonists (6,7). In addition, facilitation of lordosis in ovariectomized rats primed with low levels of hormone has been reported with intracerebral infusion of physostigmine, an acetylcholinesterase inhibitor that elevates endogenous levels of acetylcholine (3). Again, these facilitative effects on lordosis were prevented by prior administration of atropine (3).

Intracerebral and systemic administrations of scopolamine alone have been found to be inhibitory to lordotic responding in ovariectomized, hormonally primed female rats (3, 8, 12, 16). In addition, intracerebral and systemic administrations of scopolamine recently were found to inhibit lordosis in intact, naturally cycling female rats (15). These results suggest that cholinergic regulation is a neural component of natural receptivity in female rats.

The present study extended the investigation of the cholinergic regulation of sexual behavior in female rats by attempting to activate lordosis in intact, cycling female rats by administration of physostigmine. If it is possible to inhibit natural receptivity with cholinergic antagonists such as scopolamine (15), then it may be possible to facilitate lordosis with a cholinergic agent such as physostigmine during stages of the estrous cycle when sexual receptivity normally does not occur. The ability of physostigmine to activate lordosis was compared at different stages of the estrous cycle which are characterized by varied hormonal levels.

GENERAL METHOD

Animals

Subjects were 42 Long-Evans hooded female rats, 175–200 g in weight, purchased from Harlan Sprague Dawley, Co. (Indianapolis, IN). Throughout the experiments, female rats were individually housed and maintained on a 12/12 light/dark cycle (lights off at 0800 hr) in a temperature-controlled vivarium. Twelve Long-Evans hooded male rats, used in behavior testing, were housed in pairs in the same vivarium as the females.

To avoid interruptions in the estrous cycle due to irregularities in the light/dark cycle, all manipulations and testing, with the exception of stereotaxic surgery, were performed within the vivarium.

Cycling

Reproductive cycling of female rats was determined by daily

[1]Requests for reprints should be addressed to Dr. Gary P. Dohanich, Department of Psychology, Tulane University, New Orleans, LA 70118.

examination of vaginal cytology and sexual behavior. Vaginal smears were collected daily at the beginning and end of each dark cycle. Smears were stained with toluidine blue and examined microscopically. Sexual behavior was observed once daily during the afternoon of the dark cycle. Animals were considered to be successfully cycling when vaginal cytology and sexual behavior, corresponding to normal 4-day or 5-day cycles, occurred for at least 2 cycles. This criterion was maintained throughout both experiments.

Stereotaxic Surgery

Surgery was performed during the dark phase of the light/dark cycle outside of the vivarium. Before surgery, animals were visually masked to reduce exposure to light during the procedure.

On the first day of diestrus after successfully cycling twice, female rats were anesthetized with Ketaset (100 mg/kg: Bristol Laboratories, Syracuse, NY) and Rompun (7.4 mg/kg: Miles Laboratories, Shawnee, KS). Double-barrel cannulae were placed bilaterally into the lateral ventricles of each female rat by stereotaxic procedure. Each cannula consisted of a guide constructed from 23-gauge stainless steel tubing, fitted with an insert constructed from 28-gauge tubing. The guides were anchored to the skull with machine screws and dental acrylic. Inserts extended into the ventricles 1 mm beyond the guide tips and were removed only during infusion.

Behavior Testing

A standard 10-gal glass aquarium with a Plexiglas lid served as a behavior testing chamber. All behavior testing was conducted within the vivarium in which the animals were housed. Before all behavior testing, females were vaginally masked with duct tape to avoid pregnancy.

During daily behavior testing to monitor cycling, females were introduced to the chamber occupied by a male rat and allowed 3 mounts. If lordosis was observed for at least 2 of the 3 mounts, then the female was considered to be receptive. Females failing to receive 3 mounts within 10 min were transferred to another chamber and testing was completed with a different male.

During behavior testing to determine drug effects, females received 10 mounts. Each response to a mount by a female was recorded as either no lordosis or lordosis. The lordosis quotient [(number of lordoses/number of mounts) × 100] was computed. Again, females failing to receive the 10 mounts within 10 min were transferred to another chamber for the completion of the test.

Drug Administration

Prior to infusion, physostigmine hemisulfate (Sigma Chemical Co., St. Louis, MO) was dissolved in saline solution vehicle. A Hamilton microsyringe, driven by a syringe infusion pump (Model 22: Harvard Apparatus, Southnatick, MA) was connected to a 28-gauge stainless steel infusion insert by PE 20 polyethylene tubing. Saline vehicle or physostigmine solution was delivered at 0.5 μl/30 sec into the lateral ventricle. The solution was allowed to diffuse for 30 sec before the infusion insert was removed from the guide and replaced with the temporary insert. This procedure was repeated contralaterally.

EXPERIMENT 1

In the first experiment, the incidence of lordosis in intact, cycling female rats was examined 15 minutes after bilateral infusion of saline vehicle or physostigmine (10 μg/cannula) into the lateral ventricles during diestrus I, mid-diestrus, and diestrus II. Physostigmine has been shown to facilitate the incidence of lordosis following intraventricular administration at this dose level in ovariectomized rats that have been primed with low levels of estrogen (3).

METHOD

After stereotaxic surgery, the vaginal cytology and sexual behavior of 16 female rats were monitored daily. The initial drug testing occurred during the afternoon of the first mid-diestrous period (the second day after displaying natural receptivity) immediately following 2 complete cycles.

Prior to drug treatment, each female rat was introduced into the behavior testing chamber where she received 10 mounts by a male rat. The female was then randomly assigned to receive bilateral intraventricular infusions of either saline vehicle (0.5 μl/cannula) or physostigmine (10 μg/cannula). After infusions were completed, the animal was placed in her home cage for 15 min. She was then returned to the testing chamber where she received 10 mounts by a male. Cyclic activity was monitored daily for 2 complete cycles after drug testing.

During diestrus II (the third day after the display of natural receptivity) of the next cycle, animals were again pretested, infused with either saline vehicle or physostigmine solution, and posttested. Daily monitoring occurred for another 2 cycles. Drug testing was repeated again during diestrus I (the first day after the display of natural receptivity) of the next cycle and followed by daily monitoring for another 2 cycles. Using this paradigm, each female rat was tested using the same drug treatment at 3 different stages of the estrous cycle.

RESULTS

Cycling

Cycling before and after surgery were compared to determine if surgery and anesthesia disrupted cyclicity. Cycling percentage (number of cycles/number of days) was used to estimate cycling efficiency. Therefore, a cycling percentage of 25% would be characteristic of a 4-day cycling animal, while a cycling percentage of 20% would be characteristic of a 5-day cycling animal. Comparisons of cycling percentages before surgery (mean = 24.0%, SD = 2.2%) and after surgery (mean = 21.7%, SD = 2.0%) indicated that surgery performed under anesthetic administration of Ketaset/Rompun did not significantly interrupt normal cycling in female Long-Evans hooded rats.

All 16 female rats used in Experiment 1 were found to be cycling normally at the time of the initial drug testing which occurred during mid-diestrus. Eight animals were assigned to receive saline infusion, while 8 animals were assigned to receive physostigmine infusion on the first day of drug testing. On the second day of drug testing, which occurred during diestrus II, 13 animals were cycling normally, with 6 remaining in the saline group and 7 in the physostigmine group. At time of the third drug testing which occurred during diestrus I, 9 animals were cycling normally, with 4 remaining in the saline group and 5 in the physostigmine group. The number of animals remaining in the experiment was decreased on the second and third days of testing because of infection in the colony. In order to insure that only healthy animals were included in the experiment, females showing symptoms of physical stress and abnormally high leucocytic activity in their vaginal smears were dropped from the experiment. Therefore, cycling percentages and lordosis quotients reported in this study are based only on healthy animals.

Cycling before and after infusion performed during mid-

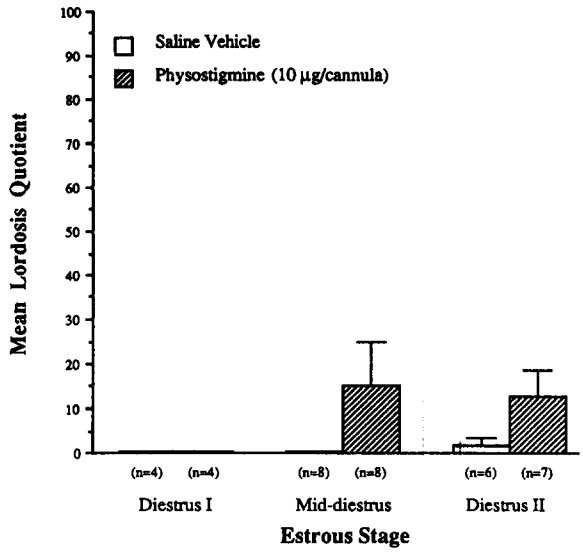

FIG. 1. Physostigmine failed to activate lordosis (lordosis quotient ±SEM) 15 min after intraventricular infusion (10 µg/cannula) during diestrus I, mid-diestrus, and diestrus II.

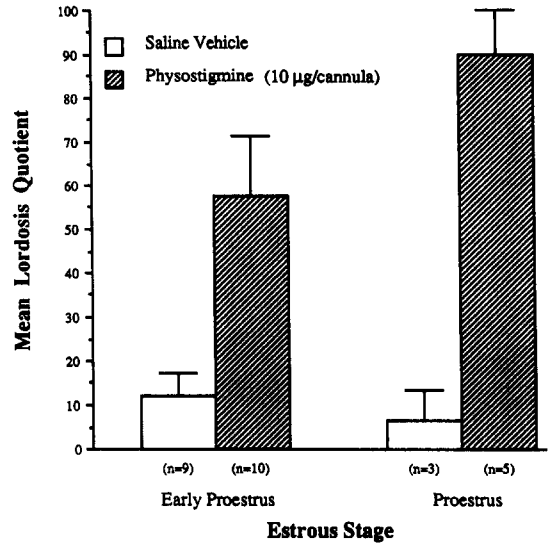

FIG. 2. Physostigmine activated lordosis (lordosis quotient ±SEM) 15 min after intraventricular infusion (10 µg/cannula) during early proestrus ($p<0.0065$) and proestrus ($p<0.0011$).

diestrus, diestrus II, and diestrus I were compared to determine if infusion disrupted cycling. Comparison of cycling percentages before infusion and after infusion during mid-diestrus (before: mean = 21.7%, SD = 2.0%; after: mean = 22.4%, SD = 1.8%), diestrus II (before: mean = 22.4%, SD = 1.8%; after: mean = 24.0%, SD = 1.9%), and diestrus I (before: mean = 24.0%, SD = 1.9%; after: mean = 22.6%, SD = 4.0%) indicated that infusion of either saline or physostigmine did not interrupt normal cycling activity. Furthermore, comparisons of cycling percentages between groups after receiving bilateral infusion of saline or physostigmine at mid-diestrus (saline: mean = 22.7%, SD = 1.8%; physostigmine: mean = 22.1%, SD = 1.9%), diestrus II (saline: mean = 24.4%, SD = 0.9%; physostigmine: mean = 23.6%, SD = 2.6%), and diestrus I (saline: mean = 25.0%, SD = 0.0%; physostigmine: mean = 20.6%, SD = 4.6%) indicated no significant differences in cycling activity.

Behavior

For the purpose of analysis, lordosis quotients (LQ) were calculated as described before. Intraventricular administration of physostigmine (10 µg/cannula) during mid-diestrus (mean LQ = 15.0%, SD = 27.8%), diestrus II (mean LQ = 12.9%, SD = 15.0%), or diestrus I (mean LQ = 0.0%, SD = 0.0%) did not significantly increase the incidence of lordosis as compared to intraventricular infusion of saline during mid-diestrus (mean LQ = 0.0%, SD = 0.0%), diestrus II (mean LQ = 1.7%, SD = 4.1%), and diestrus I (mean LQ = 0.0%, SD = 0.0%) (see Fig. 1). These results differ from facilitative effects previously reported with intraventricular administration of physostigmine in ovariectomized female rats primed with estrogen (3).

EXPERIMENT 2

In the second experiment, the incidence of lordosis in 26 intact, cycling female rats was examined 15 minutes and 1 hour after bilateral infusion of either saline vehicle or physostigmine into the lateral ventricles during early proestrus or proestrus when endogenous estrogen levels are highest (2,18). It has been suggested that estrogen priming of the cholinergic system may be necessary for the regulation of sexual behavior (4).

METHOD

After stereotaxic surgery, the vaginal cytology and sexual behavior of 26 female rats were monitored daily. During proestrus (the morning of the fourth day after displaying natural receptivity), each female was pretested for sexual receptivity. If the animal was found to be receptive (LQ >= 50%), then she received no further manipulations at that time and was allowed to cycle again. However, if the animal was not receptive (LQ<50%), then she was randomly assigned to receive intraventricular infusion of either saline vehicle (0.5 µl/cannula) or physostigmine (10 µg/cannula). After receiving bilateral infusion, the female was placed in her home cage. She was returned to the behavior testing chamber at 15 min and 1 hr after the drug administration and allowed 10 mounts by a male. Behavior testing was also repeated during proestrus-estrus (the afternoon of the 4th day) in order to compare facilitative effects obtained with the administration of physostigmine during early proestrus and proestrus to natural receptivity which usually occurs at this time. Daily monitoring of cyclic activity continued for 2 cycles following drug testing to determine the effects of physostigmine infusions on cyclicity.

RESULTS

Cycling

Cycling percentages before and after surgery were compared as in Experiment 1 to determine if surgery and anesthesia interrupted cycling. Comparisons of cycling percentages before surgery (mean = 23.2%, SD = 2.2%) and after surgery (mean = 22.9%, SD = 1.7%) indicated that, as in Experiment 1, surgery performed under anesthetic administration of Ketaset/Rompun did not significantly interrupt normal cycling in female Long-Evans hooded rats.

Cycling percentages before and after infusions were also compared to determine if infusion during proestrus or early

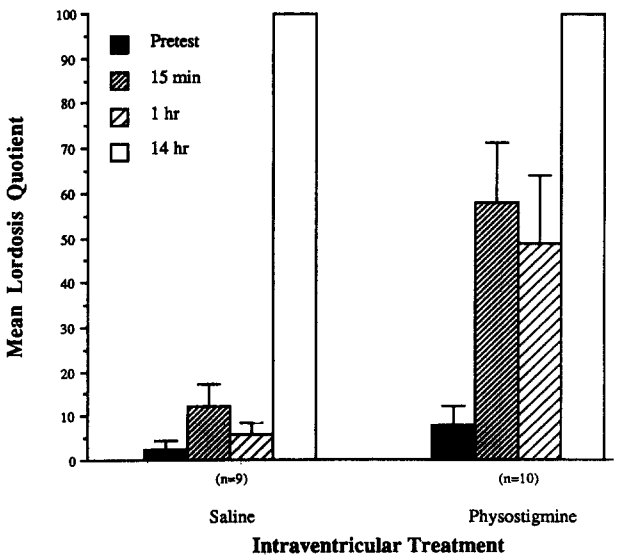

FIG. 3. Physostigmine activated lordosis (lordosis quotient ± SEM) 15 min ($p<0.0065$) and 1 hr ($p<0.0121$) after intraventricular infusion (10 μg/cannula) during early proestrus. This activation of lordosis was significantly different from natural receptivity 14 hr later ($p<0.0072$, $p<0.0037$).

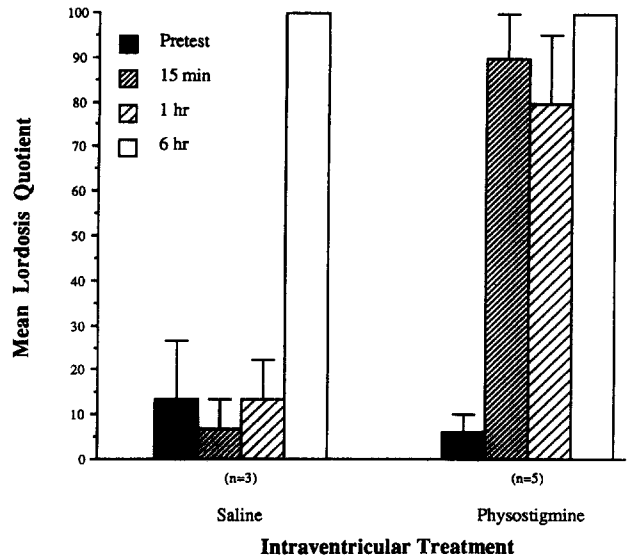

FIG. 4. Physostigmine activation of lordosis (lordosis quotient ± SEM) 15 min ($p<0.0011$) and 1 hr ($p<0.0200$) after intraventricular infusion (10 μg/cannula) during proestrus. This activation of lordosis was not significantly different from natural receptivity 6 hr later.

proestrus disrupted cycling. Comparison of cycling percentages before infusion and after infusion during Proestrus (before: mean = 23.8%, SD = 1.1%; after: mean = 24.2%, SD = 6.7%) or early proestrus (before: mean = 22.1%, SD = 2.4%; after: mean = 23.5%, SD = 1.9%) indicated that infusion of either saline or physostigmine did not interrupt normal cyclic activity. Furthermore, comparisons of cycling percentages between groups after receiving infusion of saline or physostigmine at proestrus (saline: mean = 22.7%, SD = 2.5%; physostigmine: mean = 24.0%, SD = 1.4%) and early proestrus (saline: mean = 24.8%, SD = 0.7%; physostigmine: mean = 24.7%, SD = 0.7%) indicated no significant differences in cycling activity.

Behavior

All 26 female rats that received surgery in Experiment 2 were found to be cycling normally at the time of the first pretest during proestrus: 8 were unreceptive (LQ<50%) and 18 were receptive (LQ> = 50%). Of the 8 unreceptive females, 5 were assigned to receive physostigmine infusion and 3 were assigned to receive saline infusion. The 18 receptive females were allowed to cycle again and then pretested during early proestrus of the next cycle. All 18 females were unreceptive (LQ<50%) at this time. Nine were assigned to receive physostigmine infusion and 9 were assigned to receive saline infusion.

Lordosis was found to be significantly facilitated ($p<0.0001$) 15 min after intraventricular administration of physostigmine (10 μg/cannula) during early proestrus (mean LQ = 57.78%, SD = 41.16%) and proestrus (mean LQ = 90.00%, SD = 22.36%) as compared to infusion of saline vehicle during early proestrus (mean LQ = 12.22%, SD = 14.81%) and proestrus (mean LQ = 6.67%, SD = 11.55%). Independent comparisons of lordosis quotients during either early proestrus ($p<0.0065$) or proestrus ($p<0.0011$) further indicated that drug effects at 15 min after infusion of physostigmine did not differ across these two phases (see Fig. 2). Furthermore, the facilitative effects obtained after the administration of physostigmine during early proestrus and proestrus was significantly greater ($p<0.0001$) than responding after administration of physostigmine during all diestrous phases in Experiment 1 (see Figs. 1 and 2).

Significant facilitative effects ($p<0.0006$) were also found 1 hr after intraventricular administration of physostigmine during both early proestrus (mean LQ = 48.89%, SD = 45.12%) and proestrus (mean LQ = 80.00%, SD = 33.91%) as compared to saline infusion during early proestrus (mean LQ = 5.56%, SD = 8.82%) and proestrus (mean LQ = 13.33%, SD = 15.28%) (see Figs. 3 and 4). Independent analyses of lordosis quotients 1 hr after intraventricular administration of physostigmine during either early proestrus ($p<0.0121$) or proestrus ($p<0.0200$) were also found to be significant. This indicates that the facilitative effect on receptivity with the administration of this cholinergic agent is maintained for at least one hr.

All animals were found to be 100% receptive during estrus-proestrus regardless of whether they received administration of physostigmine or saline 14 hr earlier during early proestrus or 6 hr earlier during proestrus (see Figs. 3 and 4). This suggests that the administration of physostigmine at either early proestrus or proestrus does not affect natural receptivity when administered at these times.

Post hoc comparisons of lordosis quotients were also conducted to determine if facilitation of lordosis at 15 min and 1 hr after physostigmine administration differed significantly from natural receptivity 14 or 6 hr later (see Figs. 3 and 4). In animals receiving physostigmine infusion during early proestrus, lordosis at both 15 min ($p<0.0072$) and 1 hr ($p<0.0037$) after administration was significantly less than natural receptivity 14 hr later. However, in animals receiving physostigmine infusion at proestrus, lordosis quotients at 15 min and 1 hr were not significantly different to natural receptivity 6 hr later. This suggests that physostigmine facilitation of receptivity during proestrus may be comparable to natural receptivity, whereas receptivity after the administration of physostigmine during early proestrus may not. These results may further suggest that endogenous conditions, that allow for the cholinergic facilitation of sexual receptivity, exist during proestrus but are not fully developed at early proestrus.

During behavior testing, the presence of solicitation, in the form of hopping and darting, was also noted. Facilitation of lordosis 15 min after the administration of physostigmine during either early proestrus or proestrus was not accompanied by solicitation. However, facilitation of lordosis at 1 hr after administration of physostigmine was found to be accompanied by solicitative responding in 3 of the 9 animals receiving physostigmine during early proestrus and 4 of the 5 animals receiving physostigmine during proestrus. No solicitation was observed in any animals receiving saline. Chi-Squared Analyses of solicitation frequencies 1 hr after administration of physostigmine or saline during proestrus ($p<0.01$) or early proestrus ($p<0.05$) were both significant. These results suggest that the cholinergic regulation of solicitation may also occur.

GENERAL DISCUSSION

Intraventricular infusion of physostigmine (10 μg/cannula), an acetylcholinesterase inhibitor, significantly facilitated lordosis behavior in intact, cycling female rats at early proestrus and proestrus, but not at diestrus I, mid-diestrus, or diestrus II. These results are consistent with previous reports of physostigmine facilitation of lordosis in ovariectomized female rats primed with low levels of estrogen (3), offering further support for the cholinergic regulation of sexual behavior. Based on these results, it is also suggested that the cholinergic facilitation of sexual behavior is possible only at specific times during the estrous cycle. Because this facilitation appears to be limited to shortly before and during proestrus, when endogenous estrogen plasma levels are highest (2,18), it is possible that an increase in estrogen titers above diestrous levels is necessary for cholinergic facilitation of sexual behavior.

Estrogen has been shown to alter cholinergic receptor binding (9–11, 19, 20) and related enzymatic activity (13,14) in a number of brain regions implicated in the regulation of sexual behavior. Furthermore, muscarinic receptor binding in certain brain regions is highest at proestrus when estrogen levels are also highest (19). Facilitation of sexual behavior appears to occur only at this time. Consequently, it is possible that estrogen priming of central cholinergic systems is necessary for cholinergic activation of sexual behavior in intact female rats. The inability to obtain facilitative effects with intraventricular administration of physostigmine during diestrus further suggests that diestrous levels of estrogen may be too low to sufficiently prime central cholinergic systems that regulate lordosis.

Consistent with findings previously reported with administration of scopolamine in intact, cycling female rats (15), intracerebral administration of physostigmine also did not significantly alter cyclicity as measured in this study. While it appears that cholinergic mechanisms regulate specific cyclic events, such as sexual receptivity, not all components of the estrous cycle are regulated by cholinergic systems. Although the administration of the muscarinic agonist, pilocarpine, has been shown to advance ovulation during normal 5-day cycling (17), this evidence does not suggest an interruption or resetting of the estrous cycle. Instead, the general regulation of cyclicity is controlled by circadian clock mechanisms (21), that may function without cholinergic input.

During behavior testing of sexual receptivity in this study, it was observed that a significant proportion of the female rats were exhibiting components of proceptivity 1 hr after the administration of physostigmine during either early proestrus or proestrus. However, no proceptive behaviors were observed at 15 min after physostigmine administration. Although female rats displayed lordosis at 15 min after physostigmine infusions, a moderate slowing of movement induced by the drug may have prevented the occurrence of active behaviors like soliciting. No such slowing in behavior was evident at 1 hr after administration of physostigmine when females displayed facilitation of both lordosis and soliciting.

Proceptivity in intact, cycling female rats has been observed to persist when natural receptivity was inhibited by the administration of scopolamine (15). In addition, cholinergic agonists have been shown to activate lordosis, but not proceptivity in ovariectomized, estrogen-primed female rats (5–7). Previously, these results have supported the independent regulation of proceptivity and receptivity. The present observation of proceptivity 1 hr after administration of physostigmine suggests that cholinergic regulation of both behaviors may occur. However, because the onset and magnitude of these behaviors differed, the cholinergic system may have differential regulatory effects on each.

Although much evidence supports the role of acetylcholine in the regulation of sexual behavior, the localization and description of central cholinergic mechanisms involved in this regulation are still unclear. Furthermore, the hormonal dependency of these cholinergic mechanisms is not yet defined. However, because the cholinergic regulation of sexual behavior is limited to stages of the estrous cycle when estrogen is highest, the dependency of this cholinergic mechanism on sufficient estrogen stimulation is indicated. The serum estrogen levels necessary to support cholinergic systems that regulate lordosis currently is under investigation in both gonadally intact and ovariectomized preparations.

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