Pharmacology Biochemistry & Behavior, Vol 24, pp 1015–1023, 1986 © Ankho International Inc Printed in the U S A 0091-3057/86 $3 00 + 00

Norepinephrine Infusions Into the Medial Preoptic Area Inhibit Lordosis Behavior

J. D. CALDWELL*[1] AND L. G. CLEMENS

**Department of Psychiatry and Biological Sciences Research Center*
University of North Carolina at Chapel Hill, School of Medicine
Chapel Hill, NC 27514
and Department of Zoology, Michigan State University, East Lansing, MI 48824

Received 20 November 1984

CALDWELL, J D AND L G CLEMENS *Norepinephrine infusions into the medial preoptic area inhibit lordosis behavior* PHARMACOL BIOCHEM BEHAV 24(4) 1015–1023, 1986.—Neurotransmitters, including norepinephrine, have been implicated in the mediation of ovarian steroid induced lordosis behavior in ovariectomized rats. In this study we have found that norepinephrine (NE) infusions into the medial preoptic area (MPOA) reduced lordosis frequencies of estrogen-progesterone treated (0 5 μg estradiol benzoate for three days followed by 500 μg progesterone 4–5 hours before testing) receptive rats Norepinephrine doses of 2 μg or more per animal infused into the MPOA significantly reduced lordosis levels within five minutes Infusions of 10 and 20 μg doses of NE suppressed lordosis levels for 15 minutes after infusion At the lowest inhibitory dose (2 μg/animal) simultaneous infusion of 5 μg/μl of the noradrenergic antagonist yohimbine, but not of phentolamine or propranolol, blocked the reduction in lordosis resulting from NE infusion. Preoptic infusions of epinephrine and clonidine were also effective in reducing lordosis quotients, while methoxamine, phenylephrine and isoproterenol did not alter receptivity These findings are consistent with the conclusion that the direct effect of norepinephrine infusions into the MPOA is inhibition of lordosis responding There is some evidence that this inhibitory influence is mediated by alpha$_2$-noradrenergic receptors

Norepinephrine Lordosis Clonidine Yohimbine Medial preoptic area

SEXUAL receptivity in female rats is controlled by the ovarian steroid hormones, estrogen and progesterone. In the absence of estrogen and progesterone, ovariectomized rats do not become sexually receptive and demonstrate the lordosis posture to the male's mount [7,17]. Estrogen and progesterone are sequestered by brain regions such as the medial preoptic area (MPOA) and medial basal hypothalamus [32, 37, 39, 48]. Implanting estrogen into these areas will enhance female sexual behavior [6, 42, 56] Implanting progesterone into the hypothalamus of estrogen-treated animals also facilitates lordosis behavior [41,56]. These steroids may influence sexual receptivity by altering the activity in these areas of central neurotransmitters such as serotonin, acetylcholine and norepinephrine [2, 10, 13, 16, 33, 35].

After reviewing several studies using peripherally administered agents to alter norepinephrine (NE) activity, we concluded that NE inhibited sexual receptivity Peripheral administration of amphetamines, which release 5-hydroxytryptamine (5-HT) as well as catecholamines [14], resulted in reductions in lordosis levels [10, 18, 34, 36] which were interpreted as due mainly to heightened 5-HT activity. Interestingly when the anterior hypothalamus was lesioned, amphetamines facilitated receptivity [27]. Another study more selectively increased catecholamine levels by giving the precursor L-DOPA along with the monoamine oxidase inhibitor pargyline and reduced sexual receptivity [35]. Norepinephrine was more definitely implicated as the inhibitory agent when Meyerson found that the reduction in lordosis frequencies resulting from L-DOPA treatment was reversed by blocking the conversion of dopamine to NE [33]. Conversely, agents which reduced NE activity increased sexual receptivity in rats. Reducing NE activity either by blocking tyroxine hydroxylase activity with alpha-methyl-para-tyrosine (α-MPT) or by the destruction of catecholamine neurons with 6-hydroxydopamine (6-OHDA) separately or together increased lordosis responding [2, 21, 26]. In contrast, electrolytic destruction of catecholaminergic axonal pathways resulted in the opposite effect on receptivity [25]. Administration of selective alpha-noradrenergic antagonists yohimbine and piperoxane increased lordosis frequency, duration and intensity [21]. The alpha-antagonist yohimbine

[1]Requests for reprints should be addressed to Jack D Caldwell, Ph D , 203 BSRC (220-H), UNC School of Medicine, Chapel Hill, NC 27514

also blocked the significant reductions in lordosis levels after injections of the alpha$_2$-agonist clonidine [15]

Few studies have injected noradrenergic agents centrally and those yielded differing conclusions. In one study, increases in lordosis responding were seen after medial preoptic-anterior hypothalamic infusions of the beta-noradrenergic blocker LB-46 [53] supporting an inhibitory role for NE In another study, infusions of NE or beta-noradrenergic agonists into the same area enhanced lordosis while infusions of the alpha-noradrenergic agonist methoxamine and the beta-blocker propranolol inhibited receptivity [23] These last authors concluded that NE facilitated sexual receptivity However, our preliminary findings that direct infusions of NE into the MPOA inhibited rather than facilitated lordosis behavior [11,12] supported the postulate that noradrenergic input into the MPOA is involved in the inhibition of lordosis behavior

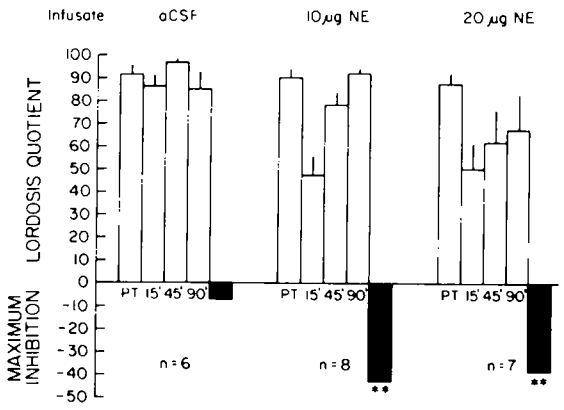

FIG 1 Infusion of 10 or 20 μg doses of norepinephrine (NE) significantly inhibited lordosis in estrogen-progesterone treated ovariectomized rats Maximal inhibition (M I =lowest post-infusion LQ minus pre-infusion LQ) was seen at 15 minutes after NE infusion at both doses The inhibition of lordosis was significant ($p<0$ 01) and transient at both NE doses (Dunnett's $t=6$ 46 and 5 33 for 10 and 20 μg respectively)

METHOD

Animals and Surgery

Sherman strain female rats were bilaterally ovariectomized under ketamine (Vetalar, Parke-Davis, Detroit) anesthesia 3–5 days after they were received from a commercial supplier (Camm, Wayne, NJ) After recovery from ovariectomy, all animals were given a screening test for sexual receptivity after estrogen and progesterone injections (see below). Those animals that were receptive were then implanted under ketamine anesthesia with bilateral cannulae Implant cannulae were made of 23 gauge stainless steel tubing (Small Parts, Inc , Miami, FL). Two of these cannulae were preset 1.6–1 8 mm apart with dental acrylic This cannula assembly was lowered to 1 mm above the target site using a stereotaxic instrument. Medial preoptic area sites were determined to be directly under the bregma, equidistant on either side of the midline suture and 3 4–4 0 mm above vertical zero [29] The two 23 gauge cannulae were then fitted with 27 gauge stainless steel inserts which had been premeasured to extend 1 mm beyond the outer cannulae and into the target site In order to secure the cannula assembly to the skull, four set screws were drilled into the skull and Kadon dental acrylic applied around the screws and assembly

Testing Procedures

In all sexual behavior tests, females received ten mounts by sexually experienced Long-Evans males. Males were allowed to adapt to the testing arena (45×50×58 cm Plexiglas cage floored with 4–7 cm of Sanicel) for several minutes before the introduction of a female. If a male failed to mount a female 10 times, the female was placed with another male in a separate arena A mount was counted when the male palpated the female's flank with his forepaws and exhibited pelvic thrusting The female was recorded as having shown or not shown lordosis during each mount. Lordosis behavior was then measured as a lordosis quotient (LQ) which is defined as the frequency of lordosis postures to ten mounts divided by ten mounts and multiplied by 100 All females were injected intramuscularly with 0.5 μg of estradiol benzoate (EB) in 0.1 cc sesame oil 72, 48, 24 hours before testing and 500 μg of progesterone 4–5 hours before all behavioral tests All animals were subjected to a screening test to these doses of EB and progesterone following ovariectomy. Any animal not achieving a screening test LQ of 70 or more was eliminated from the experiment

Drugs and Infusion

Noradrenergic agents used in these experiments include the agonists: norepinephrine (Arterenol, Sigma, St Louis, MO), epinephrine (Sigma, St Louis, MO), clonidine (Catapres®, Burroughs Wellcome, Research Triangle Park, NC), phenylephrine and isoproterenol (Sigma) There were also three noradrenergic antagonists phenotolamine (CIBA-Geigy, Summit, NJ), yohimbine and propranolol (Sigma).

After determining their pre-infusion receptivity levels, all animals achieving test criteria (LQ not less than 70) were then infused by a microinfusion pump (Harvard Apparatus, Millis, MA). Doses of each agent were premeasured and stored in parafilm-covered culture tubes at appropriate storage temperatures Immediately before infusions, artificial cerebrospinal fluid (aCSF, 16) or isotonic saline vehicle (0 9%) was added with a 1000 μl micropipette to the appropriate culture tube This infusion fluid, or infusate, was then drawn into an infusion cannula (28 gauge) which was attached to a vehicle-filled syringe by polyethylene tubing (20 gauge). The infusion cannula was measured to extend to 1 mm beyond the end of the guide cannula After removing insert cannulae from the animal's cannula assembly, the infusion cannula was inserted and animals were infused for 30 seconds per side at 1 μl/minute during which time animals were allowed freedom of movement After infusion, all insert cannulae were replaced

Histology

Following completion of all tests, animals were perfused with intracardial injections of 0 9% saline followed by 10% phosphate-buffered formalin while they were under pentobarbital anesthesia Brains were then blocked and set in 10% gelatin These blocks were then frozen and sectioned

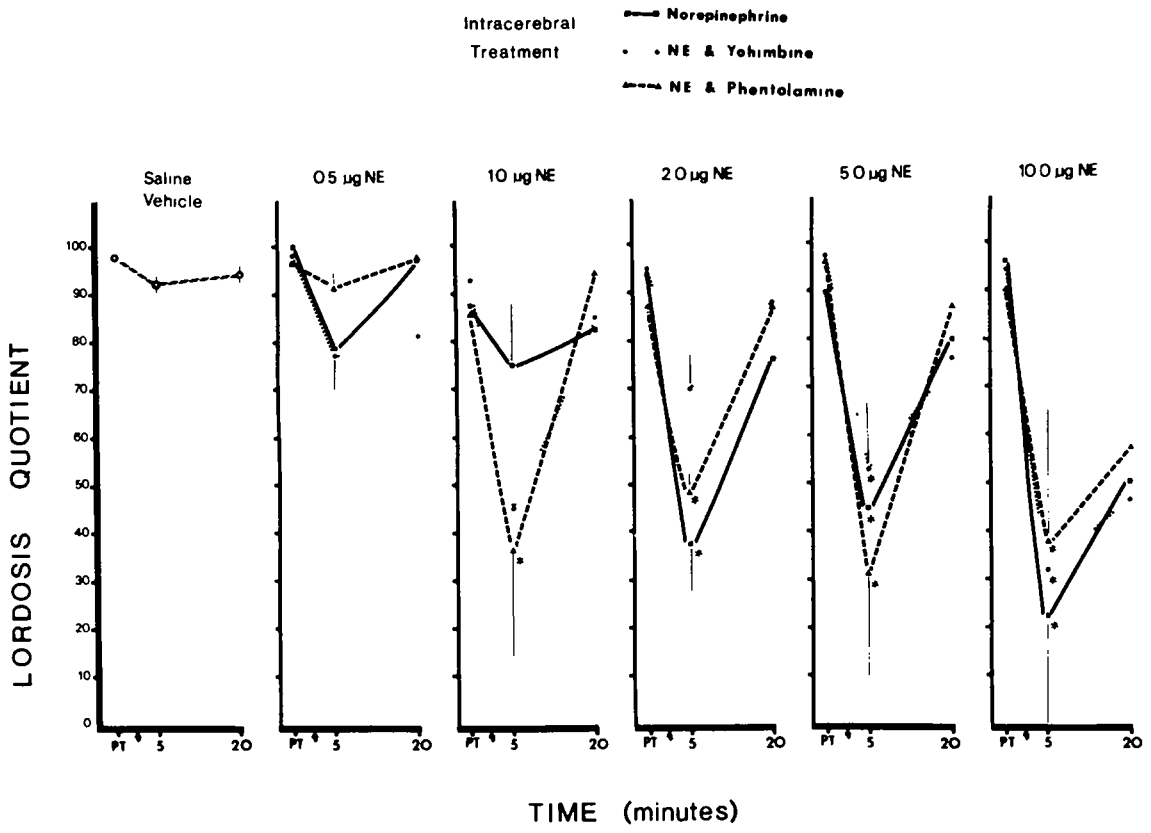

FIG 2 Norepinephrine injected into the medial preoptic area of estrogen-progesterone treated animals resulted in a transient reduction in lordosis behavior Ovariectomized animals were given 0 5 µg EB for three days followed by 500 µg progesterone on the fourth immediately before testing All animals were assigned (see text for design) to receive medial preoptic infusions of a single dose between 0 5 and 10 µg NE with or without 5 µg/µl yohimbine or 5 µg/µl phentolamine. All agents were dissolved in saline vehicle Infusions of 2, 5 and 10 µg but not 0 5 or 1 µg NE resulted in significant ($p<0$ 05) reductions in LQ scores five minutes later (Mann-Whitney U or Dunn's non-parametric comparison) At the 5 and 10 µg NE doses neither alpha-antagonist attenuated the reduction in lordosis frequencies resulting from NE infusion At the lowest effective NE dose (2 µg/animal) there was no significant drop in LQs when yohimbine was added to the infusate Although 1 µg NE in combination with yohimbine resulted in lordosis frequency reductions significantly ($p<0$ 05) below levels for saline-vehicle controls (Dunn's non-parametric comparison), their 5 minute mean LQ scores were not significantly different from those of animals getting 1 µg NE alone (Dunn's non-parametric comparison), their 5 minute mean LQ scores were not significantly different from those of animals getting 1 µg NE alone (t-test on ranks=0 9, $df=13$, $p>0$ 05) Infusion groups contained between 5 and 14 animals each

coronally to a 50 µm thickness. Representative sections were mounted on albuminized slides and stained with neutral red stain. Implant sites were determined by an observer without access to an animal's behavioral performance. Data from animals that did not have implants in the medial preoptic area were eliminated from statistical analysis

EXPERIMENT 1

Experimental Design

A preliminary experiment in our laboratory indicated that infusions of 10 and 20 µg doses of NE into the MPOA would reduce receptivity in estrogen-progesterone treated female rats. Twelve animals were randomly assigned to a three-dose series (one treatment/week) in a Latin Square design. The three doses were 10 µg/animal and 20 µg/animal of NE and an aCSF control dose. Animals were tested before and 15, 45, and 90 minutes after infusion. As in all of these experiments, at least two different doses were infused every day in order not to confound treatment effects with effects of testing days

Statistical comparisons were done on log-transformed data Analysis consisted of a four-factor analysis of variance (time × dose × week × animal). Further analysis was done with Dunnett's t-tests.

Results

Both doses of NE significantly inhibited lordosis behavior 15 minutes after infusion (aCSF versus 10 µg, Dunnett's $t(2,14)=6.36$, $p<0.01$, aCSF versus 20 µg, Dunnett's $t(2,11)=5.33$, $p<0.01$). The inhibitory effect of norepinephrine was transient as lordosis returned to pre-infusion test levels for the 45 and 90 minute tests (see Fig. 1).

Because this effect was transient and only appeared during the first post-infusion test there was no significant effect

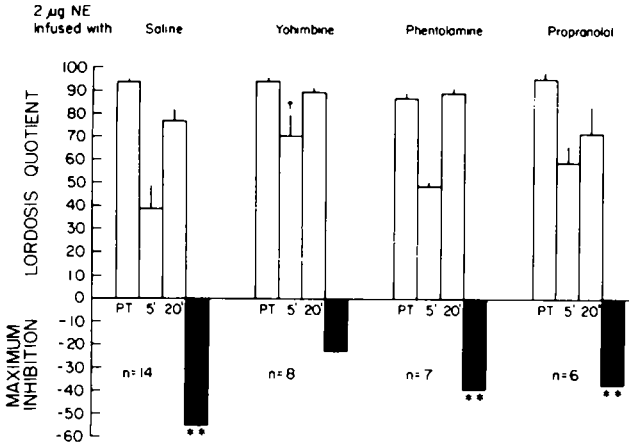

FIG 3 Yohimbine was the only noradrenergic antagonist used that blocked the inhibitory effect of norepinephrine All antagonists were infused simultaneously with 2 μg NE into the MPOA Maximum inhibition was the mean 5 minute LQ minus the mean pretest (PT) LQ Maximum inhibition of lordosis was significant (**=$p<0$ 01) after 2 μg NE alone or with 5 μg/μl phentolamine or propranolol (paired $t=5$ 4, 3 4 and 4 5, $df=13$ 6 and 5 respectively) Only the addition of yohimbine significantly (†=$p<0$ 05) blocked the drop in LQs five minutes after 2 μg NE infusions (t test on ranked five minute LQs for animals receiving yohimbine versus those getting 2 μg NE alone=2 5, $df=20$)

of NE dose across three post-infusion tests, $F(2,4)=5$ 34, $p>0.10$ While a few animals moved slowly after receiving NE, most of the animals showed no locomotor dysfunction

EXPERIMENT 2

Although animals did not demonstrate any behavioral debilitation in Experiment 1, the possibility existed that 10 and 20 μg doses had pharmacological rather than physiological effects. In an attempt to avoid this, Experiment 2 was conducted with four lower doses of NE (0 5, 1, 2 and 5 μg) as well as with a 10 μg dose Because the 15 minute observation was the only post-infusion test where a significant decrease in receptivity was seen, the possibility existed that the point of maximal inhibition occurred at some time earlier than 15 minutes after infusion. In order to test this possibility animals were first tested 3–5 minutes after infusion and then again 20 minutes after infusion

Norepinephrine is believed to act on several different classes of receptors [4] If the inhibitory effect of norepinephrine is exclusive to one set of these receptors then it ought to be possible to block the effect with the appropriate noradrenergic antagonist Different noradrenergic antagonists were given simultaneously with NE to determine which receptors NE might be acting on to inhibit lordosis The antagonists used were phentolamine, yohimbine, and propranolol; alpha$_1$, alpha$_2$ and beta-antagonists respectively [8, 24, 49, 50].

Experimental Design

Eighteen female rats were randomly assigned to three groups Each group was infused with a different dose of NE (2, 5 and 10 μg per animal) Within each group each animal was assigned to a three-treatment series (one treatment/week) as used in Experiment 1. These three treatments consisted of an infusate solution of 5 μg/μl/animal yohimbine, 5 μg/μl/animal phentolamine or saline vehicle added to the NE dose just prior to infusion Saline was used as a vehicle because of difficulty in dissolving yohimbine in the aCSF solution This design was repeated for three lower doses (0 5, 1 and 2 μg/animal) of NE using eighteen different animals Using a simple two-week crossover design, eight other animals were treated with 2 μg NE with either aCSF alone or with 5 μg/μl/animal propranolol added Receptivity tests were conducted before infusion and 5 and 20 minutes after infusion Inhibition was measured as a change in lordosis quotient between the pre-infusion and the 5 minute post-infusion tests Paired t-tests were done on these differences for each group Kruskal-Wallis tests were used to compare lordosis quotients both across the various NE doses and to compare NE treatment alone with NE plus antagonists at all doses Further comparisons were done, where appropriate, with a non-parametric Dunn's multiple comparisons procedure [28] and Mann-Whitney U tests [45] Student's t-tests on ranked data were used to compare LQs of animals receiving a NE dose to animals getting the same NE dose plus an antagonist

Results

Norepinephrine significantly reduced lordosis responding five minutes after its infusion at three doses, 2, 5 and 10 μg (see Fig 2; test of NE versus saline vehicle-induced reductions with the Mann-Whitney $U=29$, 4 and 11 for 2, 5 and 10 μg doses respectively, $p<0$ 01 for 2 and 5 μg and $p<0$ 05 for 10 μg dose)

For all norepinephrine doses, lordosis levels returned to pre-infusion levels by the 20 minute test The two lowest doses of NE (0 5 and 1 μg) did not significantly attenuate receptivity. Although 1 μg NE in combination with yohimbine resulted in lordosis frequency reductions significantly ($p<0.05$) below levels for saline-vehicle controls (Dunn's non-parametric comparison), their 5 minute mean LQ scores were not significantly different from those of animals getting 1 μg NE alone (t-test on rank=0.9, $df=13$, $p>0$ 05) At the 5 and 10 μg doses none of the noradrenergic antagonists blocked the inhibition of lordosis (see Fig. 2) For the 5 and 10 μg doses of NE all infusate combinations showed significant inhibition of lordosis at 5 minutes At 2 μg NE, the lowest effective inhibitory dose, the alpha$_2$-antagonist yohimbine appeared to block the reduction in receptivity effected by norepinephrine (see Fig 3) There was no significant reduction in lordosis after 5 minutes when yohimbine was added to the infusate (saline vehicle alone versus 2 μg NE with yohimbine, Dunn's comparison $q=7$ 06, $p>0$ 05) Lordosis levels, were significantly ($p<0$ 05) higher 5 minutes after infusion of 2 μg NE and yohimbine than in animals receiving 2 μg NE alone (t-test on ranked LQs=2 52, $df=20$) The addition of 5 μg/μl propranolol or phentolamine had no effect on the 2 μg norepinephrine-induced decrease in lordosis (Fig 3, saline vehicle alone versus 2 μg NE with phentolamine, $q=14.7$, $p<0.01$, versus 2 μg NE with propranolol $q=13.5$, $p<0.05$) because LQs of animals 5 minutes after receiving 2 μg NE were not significantly different from LQs of those receiving 2 μg NE with either phenotolamine or propranolol (t-tests ranks=1.39 and 1 89, $df=19$ and 18 respectively) Thus only the alpha$_2$-antagonist yohimbine showed any capacity to block the reduction in lordosis seen at 5 minutes after NE infusion and this was restricted to the 2 μg NE dose

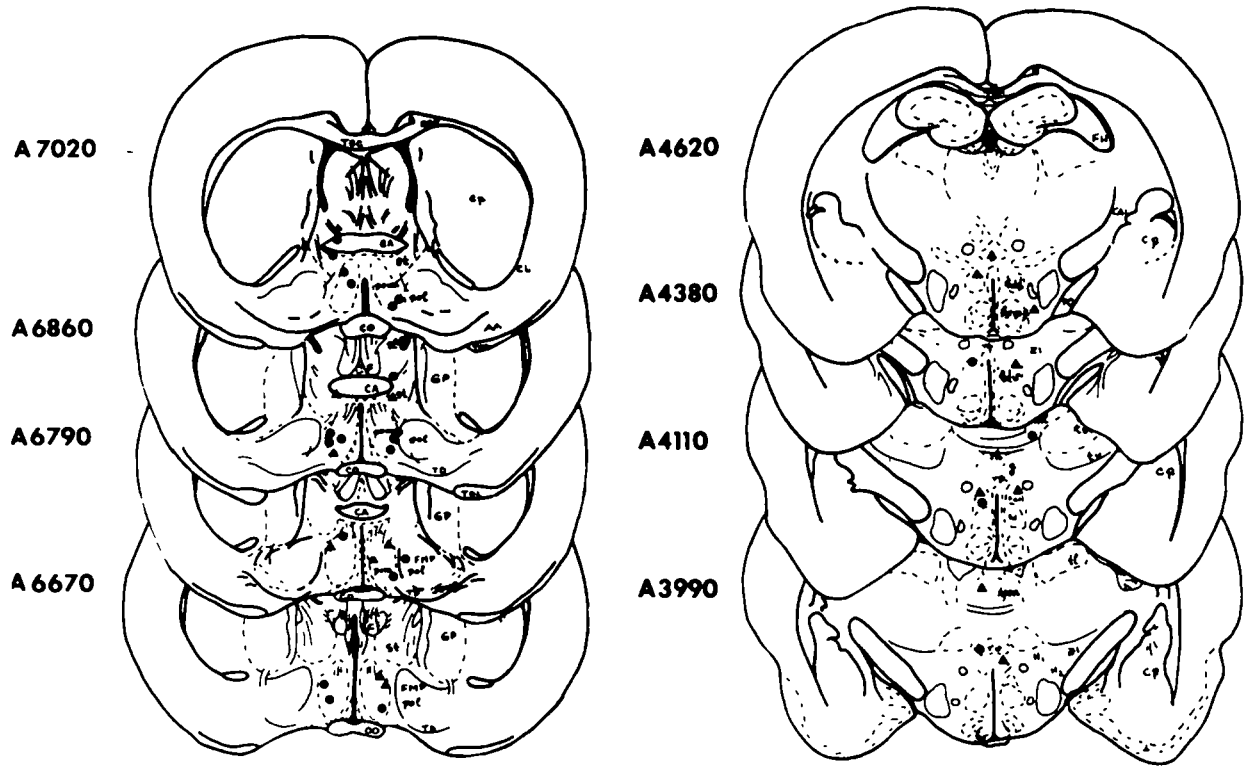

FIG 4 Analysis of these representative implant sites demonstrates that the inhibitory effect of NE infusion is localized in the medial preoptic area (pom above, coronal sections adapted from [29] by J Kaminski) Receptive females from experiments 1 and 2 were used in this histological analysis Animals in which NE infusions resulted in a reduction of lordosis quotient of 40 or more are designated as responders (solid circles) All others were non-responders (solid triangles)

Location of implant	#responders	#non-responders
MPOA sites	30*	9#
Non-MPOA sites	12	22

*Fisher's test $F=11.24$, $p<0.001$
#Chi-square versus responders for MPOA, $\chi^2=11.3$, $p<0.001$

Analysis of the implant sites of the animals in Experiments 1 and 2 indicated that animals with implants located in the MPOA showed inhibition of lordosis after NE infusion significantly more often than those with implants in other brain sites (Fig 4)

EXPERIMENT 3

In Experiment 2 norepinephrine induced a short-latency, transient reduction in lordosis frequency. In this next experiment, noradrenergic agonists were used in an attempt to mimic the inhibitory effect of NE and determine which receptors mediated the inhibitory influence of NE. Three agents with high affinity for alpha-receptors and one with higher affinity for beta-receptors were used. The first two alpha-agonists, methoxamine and phenylephrine, have reportedly greater affinity for $alpha_1$-receptors than for $alpha_2$-receptors [8,51]. The third agent, clonidine, is widely used as an agonist of $alpha_2$-receptors [8, 24, 31, 51]. The beta-agonist, isoproterenol, was used to test if the inhibitory effect of norepinephrine might be mediated via beta-receptors. The fourth agent was epinephrine, a metabolite of norepinephrine. Like norepinephrine, epinephrine acts on both alpha- and beta-receptors. There is some evidence that epinephrine has a slightly greater affinity for $alpha_2$ than $alpha_1$-receptors [8, 49, 50]

Experimental Design

Sixty animals were randomly assigned to agonist infusate groups All animals were infused with vehicle and two doses of an agonist. Due to difficulties in dissolving epinephrine in

TABLE 1

MEAN LORDOSIS QUOTIENTS (±SE) OF ESTROGEN-PROGESTERONE TREATED OVARIECTOMIZED RATS BEFORE AND AFTER INFUSIONS OF NORADRENERGIC AGENTS

Agent	Dose μg	N=	Pre-infusion test	5 minute post-infusion	20 minute post-infusion
Saline vehicle	—	9	94 4 ± 0 4	73 3 ± 8 8	87 5 ± 1 9
Clonidine	0 5	19	92 5 ± 0 4	72 1 ± 3 9	38 0 ± 5 6†
	1	9	94 4 ± 1 0	66 0 ± 4 0	20 0 ± 5 4†
aCSF vehicle	—	15	94 5 ± 1 6	75 8 ± 9 5	83 7 ± 5 0
Epinephrine	2	13	91 4 ± 0 6	48 3 ± 11*	74 0 ± 5 1
	5	12	91 6 ± 0 6	40 0 ± 7 9†	57 5 ± 13
Phenylephrine	2	9	96 6 ± 0 5	67 7 ± 10	85 5 ± 3 5
	5	11	90 6 ± 0 9	79 0 ± 8 8	93 5 ± 1 2
Methoxamine	2	6	91 6 ± 0 8	75 0 ± 7 1	95 0 ± 0 4
	5	5	92 0 ± 1 1	72 0 ± 27	86 0 ± 11
Isoproterenol	2	9	96 6 ± 0 5	77 4 ± 6 4	85 4 ± 1 0
	5	8	96 2 ± 0 6	87 2 ± 2 4	87 2 ± 2 5

Dunn's non-parametric test *=$p<0$ 05, †=$p<0$ 01

aCSF, 1 ml of 0.25 M ascorbic acid was added to the epinephrine dose and then diluted with 3 ml aCSF. The control aCSF for this group contained equimolar ascorbic acid levels. Agonist doses were 2 and 5 μg for all agents except clonidine which, because of its potency, was infused at 0 5 and 1 μg doses. The vehicle for clonidine infusions was 0.9% normal saline. The two agonist doses and vehicle treatments were counter-balanced across three weeks of testing All animals were estrogen and progesterone treated as in Experiment 1 to show receptivity. Kruskal-Wallis tests were used to compare test scores of vehicle-infused animals with LQs for agonist-infused animals Further testing was done where appropriate with Dunn's non-parametric comparisons and Mann-Whitney U tests

Results

Clonidine and epinephrine exerted significant reductions on lordosis responding (see Table 1). Of the five agents given these two have the greatest affinity for alpha$_2$-receptors Clonidine and epinephrine infusions resulted in significantly greater reductions in LQ than did their vehicles (Kruskal-Wallis H=22 9, $p<0.001$ for clonidine, H=9.35, $p<0.01$ for epinephrine), whereas none of the other agonists exerted such reductions (for methoxamine, phenylephrine and isoproterenol, H=1 76, 3 91, 0 92 respectively, not significant) Epinephrine infusions, at 2 and 5 μg, resulted in significant ($p<0.05$) reductions in lordosis at 5 minutes (Dunn's comparison vs. aCSF=10.6 and 12 9 for 2 and 5 μg respectively) This reduction was transient, with receptivity returning to control levels by the 20 minute test. This effect replicated the results seen with norepinephrine. Clonidine's inhibitory effect was greatest at the 20 minute test (Dunn's comparison=13 8 and 18 2, $p<0.01$, for 0.5 and 1 0 μg respectively). The beta-noradrenergic agonist isoproterenol and the alpha$_1$-noradrenergic agonists methoxamine and phenylephrine failed to reduce receptivity in estrogen-progesterone treated animals (see Table 1)

GENERAL DISCUSSION

Norepinephrine infused into the medial preoptic area inhibited lordosis behavior within five minutes of its infusion. This short-latency reduction in lordosis frequency resulted after infusion of doses as low as 1 μg per cannula (2 μg/animal) Estrogen-progesterone treated receptive animals recovered from this inhibition in twenty minutes Higher doses of NE (10 and 20 μg/animal) inhibited lordosis up to 15 minutes after bilateral infusion Attempts to counteract the inhibitory effect of NE with specific receptor antagonists were frustrated by the transient nature of the NE inhibition Only the alpha-antagonist yohimbine blocked the inhibitory action of the lowest effective dose of norepinephrine (2 μg/animal) Epinephrine infused at 2 or 5 μg doses resulted in a reduction in receptivity similar to that seen with norepinephrine with a similar rapid recovery The alpha-agonist clonidine inhibited lordosis to the 20 minute post-infusion test In contrast, the alpha$_1$-noradrenergic agonists, methoxamine and phenylephrine, did not reduce lordosis nor did the beta-agonist isoproterenol

The present findings confirm the results of Davis and Kohl [15] who inhibited lordosis in rats by giving them the alpha$_2$-agonist clonidine systemically Our central infusions of clonidine, as well as norepinephrine and epinephrine, resulted in reductions in lordosis responding In the Davis and Kohl study peripheral administration of yohimbine blocked the clonidine-induced inhibition, whereas in our study central yohimbine showed a capacity to block the inhibitory effects of a 2 μg infusion of NE. The lowest effective dose of clonidine reported by Davis and Kohl was 33 μg/kg, considerably more than the 0.5 μg dose effective with intracerebral infusions. It may be that animals are more sensitive to the inhibitory effects of clonidine when it is infused intrahypothalamically because such injections are closer to the site of drug action

Several researchers have demonstrated that manipulations that reduce noradrenergic activity increase receptivity,

and treatments that increase noradrenergic activity reduce lordosis behavior When tetrabenazine or alpha-methyl-para-tyrosine was used to decrease noradrenergic activity, increased lordosis levels were seen in estrogen-pretreated ovariectomized animals [2,3]. Use of 6-hydroxydopamine to decrease catecholamine activity resulted in increased lordosis behavior [26] Intracerebral infusions of the alpha-blockers yohimbine and piperoxane [21] or the beta-blocker LB-46 [53] were followed by increases in lordosis behavior.

Treatments that increased noradrenergic activity by various methods resulted in reduced receptivity Amphetamines, which release monoamines in general, reduced lordosis responding [10, 34, 36]. More specifically implicating NE, administration of the catecholamine precursor, L-DOPA, decreased lordosis levels; an effect which was enhanced by the addition of the monoamine oxidase inhibitor, pargyline [35], and reversed by blocking conversion of dopamine to norepinephrine [33]. The above studies along with the data reported in this study are consistent with the conclusion that norepinephrine inhibits lordosis behavior

In contrast to the present findings, some studies report a facilitative role for norepinephrine on receptivity in rats Decreases in receptivity were seen in animals with electrolytic lesions aimed at midbrain dorsal and ventral noradrenergic pathways [25] These findings are somewhat difficult to interpret because of indications of some behavioral debilitation in those animals and later evidence from these same authors that 6-OHDA lesioned animals demonstrated enhanced lordosis responding [26] Others found enhanced receptivity after amphetamines were given to animals with medial preoptic-anterior hypothalamic lesions [27] This may indicate that the medial preoptic-anterior hypothalamic area must be intact in order for catecholamine release to inhibit sexual receptivity This is consistent with evidence that electrical stimulation of the MPOA inhibited lordosis behavior [40] and combined with the present study may suggest that noradrenergic input into the MPOA partially mediates this inhibitory effect

Foreman and Moss reported facilitative effects on lordosis levels of NE and beta-noradrenergic agonists infused into the MPOA [23]. The apparent contradiction of their results and ours may be explained by any of three differences in procedure and results· (1) the time of maximal effect, (2) differences in steroid treatment, and (3) the doses of NE and noradrenergic agents infused Our inhibitory effect of NE occurred five minutes after infusions and was gone by 20 minutes, while their facilitative effects were maximal 105 minutes after infusion. These seeming contradictions may actually represent a temporally biphasic effect of NE on lordosis behavior They gave animals doses of estrone titrated so that animals demonstrated low pretest receptivity levels, while our animals were treated with estrogen and progesterone and demonstrated higher initial lordosis quotients The importance of the progesterone-norepinephrine interaction was recently demonstrated when the dopamine-beta-hydroxylase inhibitor U-14624 increased hypothalamic nuclear progestin receptors in estrogen-treated guinea pigs [9] Whereas their facilitative doses of NE and beta-agonists were 200 and 800 ng, we saw no inhibition of lordosis levels at doses below 2 μg NE and no inhibition after infusion of the beta-agonist isoproterenol. The opposite effects on lordosis responding at differing doses may suggest that lower doses of NE act on different noradrenergic receptors (possibly beta-receptors) while higher doses act more immediately on other receptors (possibly alpha$_2$-receptors)

Feder and Ruf [22] were the first to suggest that peripheral administration of ACTH would enhance lordosis levels by releasing adrenal progestins Later others [19,38] explained changes in sexual receptivity after the catecholaminergic treatments as due to their effects on the pituitary-adrenal axis. Certainly, norepinephrine is involved in the control of ACTH release (see [52]). However, adrenal progestins took several hours to affect receptivity [22], whereas our inhibitory effects of NE occurred five minutes after its administration Furthermore, evidence that central administration of CRF which releases ACTH inhibited lordosis with a short latency [47], while various ACTH fragments had both facilitative and inhibitory effects on receptivity [55] suggested a complex interaction of the hypothalamic-hypophyseal-adrenal axis with sexual receptivity.

Norepinephrine, epinephrine and clonidine significantly reduced lordosis behavior whereas methoxamine, phenylephrine and isoproterenol did not alter receptivity. These first three agents show much higher affinity for alpha$_2$-noradrenergic receptors than do the last three agonists [8, 30, 50, 51]. Yohimbine, which is believed to be specific for alpha$_2$-receptors [20,51], blocked the reduction in lordosis induced by the lowest effective NE dose (2 μg). Phentolamine, which was not effective in antagonizing NE inhibition, is not as specific to tritiated-clonidine binding sites as is yohimbine [24, 50, 51]. From the present findings we concluded that the inhibitory effects of NE infusions into the MPOA are mediated, at least in part, by alpha$_2$-noradrenergic receptors.

Our evidence that NE acts on alpha$_2$-receptors to inhibit lordosis frequencies raises questions as to the nature and location of these receptors Alpha$_2$-noradrenergic receptors are found in the MPOA [57] but within this brain area their location with respect to the synapse is unknown. In the CNS and in peripheral vascular tissue, alpha$_2$-receptors have been demonstrated both pre- and post-synaptically [8, 43, 49] On noradrenergic cell bodies in the locus coeruleus alpha$_2$-receptors act as autoreceptors inhibiting NE release [1] Clonidine infusions into the MPOA potently inhibited lordosis which might suggest an effect on preoptic autoreceptors reducing release of endogenous NE. However, infusion of NE itself also inhibited lordosis behavior. Postulating an inhibitory effect of exogenous NE on presynaptic autoreceptors requires that such receptors be either more accessible to exogenous NE or have a higher affinity for infused NE than would post-synaptic noradrenergic receptors. There is no evidence that either one of these conditions apply in medial preoptic alpha-receptors. Thus the question of the cellular location of the alpha$_2$-noradrenergic receptors which are responsible for the inhibition of lordosis remains unanswered.

Extensive work has been done on the involvement of medial preoptic NE innervation on LHRH release There is evidence that increased medial preoptic NE turnover is associated with elevated LHRH release (see [54]). Since central administration of LHRH resulted in increased receptivity levels [23, 44, 46], mechanisms which control its release are of interest in the study of sexual receptivity The question of how preoptic LHRH might induce lordosis has, however, been little studied. There is recent evidence that LHRH administration will decrease NE turnover in the medial preoptic nucleus [5] It may be that as part of its own short negative feedback loop, LHRH decreases medial preoptic NE turnover thus helping to disinhibit lordosis behavior. This possible peptide-monoaminergic interaction needs further examination.

ACKNOWLEDGEMENTS

We thank Dr David Brigham for his invaluable technical assistance We also gratefully acknowledge the statistical aid of Drs John Gill and Clyde Anderson and the clerical assistance of Ms Dori Yarbrough, Ms Debbie Lowery, and Ms Ruth Ann Reynolds This work was completed with funds provided by NIH HD-07670

REFERENCES

1 Aghajanian, G K and C P VanderMaelen A_2-adrenoceptor-mediated hyperpolarization of locus coeruleus neurons Intracellular studies *in vivo* *Science* **215**: 1394–1396, 1982
2 Ahlenius, S , J Engel, H Eriksson and P Sodersten Importance of central catecholamines in the mediation of lordosis behavior in ovariectomized rats treated with estrogen and inhibitors of monoamine synthesis *J Neural Transm* **33**: 247–255, 1972
3 Ahlenius, S , J Engel, H Eriksson and P Sodersten Effects of tetrabenazine on lordosis behaviour and on brain monoamines in the female rat *J Neural Transm* **33**: 155–162, 1972
4 Ahlquist, R P A study of the adrenotropic receptors *Am J Physiol* **153**: 586–600, 1948
5 Andersson, K , K Fuxe, L F Agnati, P Eneroth and M Camuri Luteinizing hormone-releasing hormone increases dopamine turnover in the lateral palisade zone of the median eminence and reduces noradrenaline turnover in the nuc preopticus medialis of hypophysectomized male rat *Neurosci Lett* **45**: 253–258, 1984
6 Barfield, R J and J J Chen Activation of estrous behavior on ovariectomized rats by intracerebral implants of estradiol benzoate *Endocrinology* **101**: 1716–1725, 1977
7 Beach, F A Importance of progesterone to induction of sexual receptivity in spayed female rats *Proc Soc Exp Biol Med* **51**: 369–371, 1942
8 Berthelsen, S and W A Pettinger A functional basis for classification of α-adrenergic receptors *Life Sci* **21**: 595–606, 1977
9 Blaustein, J D Noradrenergic inhibitors cause accumulation of nuclear progestin receptors in guinea pig hypothalamus *Brain Res* **325**: 89–98, 1985
10 Caggiula, A R , J G Herndon, R Scanlon, D Greenstone, W Bradshaw and D Sharp Dissociation of active from immobility components of sexual behavior in female rats by central 6-hydroxydopamine Implications for responsiveness *Brain Res* **172**: 505–520, 1979
11 Caldwell, J D Noradrenergic influence on the sexual receptivity of female rats (*Rattus norvegicus*) Doctoral Dissertion, Michigan State University, 1983
12 Caldwell, J D and L G Clemens Norepinephrine infused into the medial preoptic area inhibits lordosis behavior in female rats *Soc Neurosci Abstr* 253 3, 1982
13 Clemens, L G , G P. Dohanich and P J Barr Cholinergic regulation of feminine sexual behaviour in laboratory rats In *Hormones and Behavior in Higher Vertebrates*, edited by J Balthazart, E Prove and R Gilles Berlin Springer-Verlag, 1983
14 Costa, E Pharmacological implications of brain monoamine turnover rates elicited by (+)-amphetamine and some chemically related compounds In *Psychopharmacology, Sexual Disorders and Drug Abuse*, edited by T A Ban, J R Boisier, G J Gessa, H Heimann, L Hollister, H E Lehmen, I Munkvad, H Steinberg, F Salser, A Sundwall and O. Vinar Amsterdam North Holland, pp 637–658
15 Davis, G A and R Kohl The influence of alpha-receptors on lordosis in the female rat *Pharmacol Biochem Behavior* **6**: 47–53, 1977
16 Dohanich, G P and L G Clemens Brain areas implicated in cholinergic regulation of sexual behavior *Horm Behav* **15**: 157–161, 1981
17 Edwards, D A , R E Whalen and R D Nadler Induction of estrus Estrogen-progesterone interactions *Physiol Behav* **3**: 29–33, 1968
18 Eliasson, M , A Michanek and B J Meyerson A differential inhibitory action of LSD and amphetamine on copulatory behavior in the female rat *Acta Physiol Toxicol* **33**: Suppl 1, Abstract 22, 1972
19 Eriksson, E and P Sodersten A failure to facilitate lordosis behavior in adrenalectomized and gonadectomized estrogen-primed with monoamine-synthesis inhibitors *Horm Behav* **4**: 23–30, 1971
20 Eriksson, E , S Eden and K Modigh Importance of norepinephrine A_2-receptor activation for morphine-induced rat growth hormone secretion *Neuroendocrinology* **33**: 91–96, 1981
21 Everitt, B J , K Fuxe, T Hokfelt and G Jonsson Role of monoamines in the control by hormones of sexual receptivity *Pharmacol Biochem Behav* **3**: 557–559, 1975
22 Feder, H H and K B Ruf Stimulation of progesterone release and estrous behavior by ACTH in ovariectomized rodents *Endocrinology* **84**: 171–174, 1969
23 Foreman, M M and R L Moss Role of hypothalamic alpha- and beta-adrenergic receptors in the control of lordosis behavior in the ovariectomized rat *Pharmacol Biochem Behav* **9**: 235–241, 1978
24 Greenberg, D A , D C U'Prichard and S H Snyder Alpha-noradrenergic receptor binding in mammalian brain Differential labeling of agonist and antagonist states *Life Sci* **19**: 69–76, 1976
25 Herndon, J G , Jr Effects of midbrain lesions on female sexual behavior in the rat *Physiol Behav* **17**: 143–145, 1976
26 Herndon, J G , A R Caggiula, D Sharp, D Ellis, E Redgate and M. J Zigmond Selective enhancement of the lordotic component of female sexual behavior in rats following destruction of central catecholamine-containing systems *Brain Res* **141**: 137–151, 1978
27 Herndon, J G and D B Neill Amphetamine reversal of sexual impairment following anterior hypothalamic lesions in female rats *Pharmacol Biochem Behav* **1**: 285–288, 1973
28 Hollander, M and D A Wolfe *Nonparametric Statistical Methods* New York Wiley, 1973
29 Konig, J H R and R A Klippel *The Rat Brain A Stereotaxic Atlas of the Forebrain and Lower Parts of the Brain Stem* Huntington, NY Robert A Krieger Inc , 1963
30 Leibowitz, S F , M Jhanwar-Uniyal, B Dvorkin and M H Maynard Distribution of α-adrenergic and β-adrenergic and dopaminergic receptors in discrete hypothalamic areas of rat *Brain Res* **233**: 97–114, 1982
31 Leung, P C K , G W Arendash, D I Whitmoyer, R A Gorski and C H Sawyer Differential effect of central adrenoceptor agonists on luteinizing hormone release *Neuroendocrinology* **34**: 207–214, 1982
32 MacLusky, N J and B S McEwen Oestrogen modulates progestin receptor concentrations in some rat brain regions but not in others *Nature* **274**: 276–278, 1978
33 Meyerson, B J Central nervous monoamines and hormone induced estrus behaviour in the spayed rat *Acta Physiol Scand* **63**: Suppl 241, 1964

34 Meyerson, B J Amphetamine and 5-HT inhibition of copulatory behaviour in the female rat *Ann Med Exp Fenn* **46**: 394–398, 1968
35 Meyerson, B J., M Eliasson, L Lindstrom, A. Michanek and A Ch. Soderland Monoamines and female sexual behaviour. In *Psychopharmacology, Sexual Disorders and Drug Abuse*, edited by T A Ban, J R. Boisier, G. Gessa, H Hessmann, L Hollister, H Lehmen, I Munkvad, J Steinberg, F Salser, A Sundwall and O Vinar Amsterdam North Holland, 1973, pp 463–472
36 Michanek, A and B J Meyerson A comparative study of different amphetamines on copulatory behavior and stereotype activity in the female rat *Psychopharmacology (Berlin)* **53**: 175–183, 1977
37 Moguilewsky, M and J P Raynaud Estrogen-sensitive progestin-binding sites in the female rat brain and pituitary *Biol Reprod* **17**: 165–175, 1977
38 Paris, C A , J A Resko and R W Cory A possible mechanism for the induction of lordosis by reserpine in spayed rats *Biol Reprod* **4**: 23–30, 1971
39 Pfaff, D W Uptake of *H-estradiol by the female rat brain *Endocrinology* **82**: 1149–1155, 1968
40 Pfaff, D W and Y Sakuma Facilitation of the lordosis reflex of female rats from the ventromedial nucleus of the hypothalamus **228**: 189–202, 1979
41 Powers, J B Facilitation of lordosis in ovariectomized rats by intracerebral progesterone implants *Brain Res* **48**: 311–336, 1972
42 Ross, J , C Clayburgh, L G Clemens and R A Gorski. Short latency induction of estrous behavior with intracerebral gonadal hormones in ovariectomized rats *Endocrinology* **89**: 32–38, 1971
43 Ruffolo, R R , Jr α-Adrenoceptors In *Monographs in the Neural Sciences*, vol 10 Basel Karger, 1984, pp 224–253
44 Sakuma, Y and D W Pfaff LHRH in the mesencephalic central gray can potentiate lordosis reflex of female rats *Nature* **283**: 566–567, 1980
45 Siegel, S *Nonparametric Statistics for the Behavioral Sciences* New York McGraw-Hill, 1956
46 Sirinathsinghji, D J S. GnRH in the spinal subarachnoid space potentiates lordosis behavior in the female rat *Physiol Behav* **31**: 717–723, 1983
47 Sirinathsinghji, D J S , L H Rees, J Rivier and W Vale Corticotropin-releasing factor is a potent inhibitor of sexual receptivity in the female rat *Nature* **305**: 232–235, 1983
48 Stumpf, W E Estradiol-concentrating neurons Topography in the hypothalamus by dry-mount autoradiography *Science* **162**: 1001–1003, 1968
49 U'Prichard, D C., W D Bechtel, B. M. Rouot and S H. Snyder Multiple apparent alpha-noradrenergic receptor binding sites in rat brain Effect of 6-hydroxydopamine *Mol Pharmacol* **13**: 454–473, 1976
50 U'Prichard, D C , D A Greenberg and S H Snyder Binding characteristics of a radiolabelled agonist and antagonist at central nervous system alpha-noradrenergic receptors *Mol Pharmacol* **13**: 454–473, 1976
51 U'Prichard, D C and S H Snyder Distinct alpha-noradrenergic receptors differentiated by binding and physiological relationships *Life Sci* **24**: 79–88, 1979
52 van Loon, G R Brain catecholamines and ACTH secretion In *Frontiers in Neuroendocrinology*, vol 8, edited by W F Ganong and L Martini New York Oxford Univ Press, 1973, pp 209–247
53 Ward, I L , W R Crowley, H P Zemlan and D L Margules Monoaminergic mediation of female sexual behavior *J Comp Physiol Psychol* **88**: 53–61, 1975
54 Weiner, R. I and W F Ganong Role of brain monoamines and histamine in regulation of anterior pituitary secretion *Physiol Rev* **58**: 905–976, 1978
55 Wilson, C A , A J Thody and D. Everard Effect of various ACTH analogs on lordosis behavior in the female rat *Horm Behav* **13**: 293–300, 1979
56 Yanase, M and R A Gorski Sites of estrogen and progesterone facilitation of lordosis behavior with intracerebral gonadal hormones in the spayed rat *Biol Reprod* **15**: 536–543, 1976
57 Young, W S , III and M J Kuhar Noradrenergic alpha$_1$ and alpha$_2$ receptors Autoradiographic visualization *Eur J Pharmacol* **59**: 317–319, 1979