Activation of Lordotic Responding in Female Rats by Suppression of Serotonergic Activity

Abstract. *After systemic administration of several serotonergic antagonists, female rats that had been ovariectomized, adrenalectomized, and estrogen-primed showed lordotic responding. Lordosis could also be elicited after direct placement of serotonergic receptor blockers into hypothalamic sites known to contain serotonergic terminals. None of the treatments activated the soliciting component of the estrous behavior pattern of the female rat. It is postulated that the hypothalamus contains serotonergic terminals which suppress lordotic responding.*

It has been postulated that the activity of monoamine neurons controls the occurrence of estrous behavior in female rats (*1–3*). Systemic administration of drugs that decrease overall monoamine concentrations, such as reserpine, tetrabenazine, or parachlorophenylalanine (PCPA), activates lordotic behavior in estrogen-primed, ovariectomized female rats as effectively as the ovarian hormone progesterone. Conversely, drugs that increase brain monoamine concentrations, such as monoamine oxidase (MAO) inhibitors, suppress ongoing lordotic behavior in females treated with estrogen and progesterone. Because the suppressant effect of MAO inhibitors is intensified by the addition of the metabolic precursors of serotonin but not by the precursors of norepinephrine or dopamine, Meyerson has suggested that a serotonergic neural system inhibits estrous behavior. Decreased concentrations of serotonin would release estrous behavior, whereas increased concentrations would prevent or terminate the behavior.

The results of two studies do not support Meyerson's hypothesis. One study found that systemic administration of reserpine activated lordosis in estrogen-primed, ovariectomized female mice but failed to elicit the behavior if the animals were also adrenalectomized (*4*). The authors suggested that reserpine activated lordosis by the release of adrenal progesterone rather than by the depletion of monoamines. However, Meyerson (*2*), using a repeated testing procedure, found that estrous behavior occurred 5 to 8 hours after reserpine administration to adrenalectomized female rats. The failure to obtain lordosis in mice (*4*) may be due to the use of a single test given 2 to 3 hours after drug administration, a time prior to the reported onset of the effect of reserpine on lordosis in adrenalectomized females (*2*).

Failure to activate lordosis with estrogen and PCPA (*5*) has also been reported. This negative result may have been due to the use of low doses of estrogen and an amount of PCPA less than that reported to produce maximum depletion of serotonin (*6*). Meyerson has demonstrated an inverse relation between degree of serotonin depletion and amount of estrogen priming needed to elicit estrous behavior (*3*).

We conducted experiments to provide more information about the controversial hypothesis that serotonin suppresses estrous behavior. All female rats were adrenalectomized and drugs were used that interfere with serotonergic activity by several different mechanisms. We selected tetrabenazine methane sulfonate, an inhibitor of vesicular uptake (*7*); PCPA, an inhibitor of tryptophan hydroxylase activity, the rate-limiting step in the synthesis of serotonin (*6*); and methysergide maleate and cinanserin hydrochloride, potent blockers of serotonergic receptors (*8*).

Female Sprague-Dawley rats, 70 days old with a mean weight of 201 g (±23 g), were maintained with free access to food and water and a reversed 12-hour-light–12-hour-dark cycle. The animals were bilaterally ovariectomized and adrenalectomized. Ovariectomized female rats do not show maximum receptivity on the first administration of estradiol benzoate (EB) and progesterone. Therefore, EB (10 μg) was injected immediately after the operation, followed 42 hours later by progesterone (1.0 mg). Beginning 1 week after the operation, daily vaginal smears were taken to determine if all endogenous estrogen sources were removed. Thirty-seven healthy animals had no sign of endogenous estrogen as indicated by five consecutive diestrous smears.

Table 1. The effects of serotonergic antagonists, systemically administered, on the estrous behavior of estrogen-primed female rats; LQ, lordosis quotient.

Treatment	*N*	Mean LQ	Mean quality
Carrier	8	.16	1.8
PCPA	8	.64	3.0
Methysergide	8	.61	3.0
Progesterone	7	.94	5.6

Prior to the test for estrous behavior, all animals were injected with EB (10 μg intramuscularly) dissolved in 0.1 ml of cottonseed oil. Forty-two hours later, they were given a control test prior to drug administration for receptivity to vigorous males. Females showing minimum receptivity, as indicated by lordosis responses on less than 20 percent of the mounts by the male, were randomly assigned to one of the following treatment groups: methysergide maleate (3 mg/kg, intraperitoneally), tetrabenazine methane sulfonate (1.5 mg/kg, intraperitoneally), progesterone (1.0 mg, intramuscularly), or Tween 80, the drug carrier solution (0.6 ml of a 5 percent solution, intraperitoneally). All of these animals were tested again 2, 4, 6, and 8 hours after drug administration. Maximum depletion of serotonin occurs 66 to 72 hours after PCPA administration (*6*), therefore animals in this group were given PCPA (316 mg/kg, intraperitoneally) 24 hours before EB priming. Tests were repeated 66, 68, 70, and 74 hours after PCPA administration.

Each female rat was tested with a vigorous male rat for 5 minutes in a standard test chamber described in (*9*). If the male did not mount the female a minimum of four times, he was replaced. Mounts and lordotic responses were recorded by an experimenter who was unaware of the drug treatment of the animal. Responses were converted to a lordosis-to-mount ratio and expressed as a lordosis quotient (LQ). In addition, each interaction was rated on a seven-point qualitative scale (*9*); that is, 1 indicates the absence of lordosis and 7 indicates the full estrous pattern, including vigorous soliciting behavior (darting, hopping, and ear wiggling) and intense lordotic arching.

The administration of tetrabenazine caused heavy sedation, ptosis, and diarrhea in six rats. Three of these animals died within 12 hours. While one rat showed the full lordotic pattern, the animals were generally too debilitated to allow evaluation of their behavioral potentials. The data from this group has therefore been deleted.

Methysergide, PCPA, and progesterone were all effective in inducing lordotic responding. The reaction to methysergide was optimum at 2 to 4 hours, progesterone at 6 hours, and PCPA at 66 to 70 hours. Table 1 summarizes the mean LQ and highest qual-

Table 2. The effects of central monoamine receptor blockers on the estrous behavior of estrogen-primed female rats.

Treatment	Medial preoptic–anterior hypothalamus $N=3$		Posterior hypothalamus $N=5$		Caudate nucleus $N=5$	
	Mean LQ	Median quality	Mean LQ	Median quality	Mean LQ	Median quality
Blank	.09	1	.09	1	.02	1
Methysergide	.82	4	.57	4	.10	1
Cinanserin	.44	4	.73	4		
Phentolamine	.02	1	.13	1		

ity rating taken for each animal at the time of optimum drug action. For the carrier group the maximum score for each animal regardless of the time of testing was also used. As indicated in Table 1, lordotic behavior was activated by the administration of the ovarian hormone progesterone, by blockade of the synthesis of serotonin with PCPA, or by blockade of serotonergic receptors with methysergide. Kruskal-Wallis tests indicated that the treatment groups were significantly different in both LQ ($P<.001$) and quality scores ($P<.01$). Subsequent Mann-Whitney U tests showed that the LQ ($P<.01$) and quality scores ($P<.001$) of all three experimental groups were significantly larger than those of the carrier control group. The groups treated with PCPA and methysergide had significantly lower LQ ($P<.001$) and quality scores ($P<.002$) than did the progesterone group. There were no significant differences between the PCPA and methysergide groups on either measure. The difference in the quality rating between the progesterone and the PCPA and methysergide groups was due largely to a total absence of soliciting behavior in the latter two groups. While all generally displayed high quality lordotic postures, characterized by an arching of the neck and rump which was often held after the male had dismounted, the active component of the estrous behavior pattern of the female rat was seen only with progesterone treatment.

We also have administered methysergide or cinanserin hydrochloride directly into the brain of EB-primed, ovariectomized female rats. Dual wall cannulas were chronically implanted into sites in the medial preoptic area, the anterior hypothalamus, or the posterior hypothalamic nucleus. These sites are known to contain serotonergic terminals (*10*) or to have the capacity to take up estrogen from the bloodstream (*11*), or both. We have some evidence (Table 2) which indicates that unilateral treatment of any of these three sites with 10 μg of either methysergide or cinanserin significantly increased the LQ and quality scores (*12*). Soliciting behavior was not activated by either of these manipulations. Administration of methysergide to control sites in the caudate nucleus did not produce lordotic behavior. Moreover, placement of empty cannulas or application of phentolamine hydrochloride, an alpha noradrenergic receptor blocker (*13*), failed to elicit lordosis at any of the above-mentioned hypothalamic sites where serotonergic blockers had succeeded.

Our results indicate that lordotic behavior can be elicited in EB-primed female rats by the administration of drugs that interfere with serotonergic activity in the brain. Because all animals had been adrenalectomized, the effects cannot be attributed to the liberation of adrenal steroids. Whereas the passive lordotic reflex was induced by the inhibition of serotonergic activity, the active soliciting component of the female estrous pattern was not released. Soliciting behavior may be under the control of nonserotonergic neurons. The absence of soliciting cannot be attributed to an impairment in general activity since neither methysergide, PCPA, nor cinanserin caused sedation, general debilitation, or fatality.

In summary, the finding that lordotic behavior can be elicited by a variety of antiserotonergic manipulations supports Meyerson's hypothesis of an inhibitory serotonergic system for female sexual behavior. Moreover, we have some evidence that serotonin exerts this action at receptors in the hypothalamus and the preoptic area. However, since the soliciting pattern was not evoked by either systemic or central administration of these drugs, activation of the total estrous behavior pattern must also involve transmitters other than serotonin.

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Opiate Receptor: Demonstration in Nervous Tissue

Abstract. *Tritiated naloxone, a powerful opiate antagonist, specifically binds to an opiate receptor of mammalian brain and guinea pig intestine. Competition for the opiate receptor by various opiates and their antagonists closely parallels their pharmacological potency. The opiate receptor is confined to nervous tissue.*

Pharmacological evidence for the existence of a specific opiate receptor is compelling, but heretofore it has not been directly demonstrated biochemically. We report here a direct demonstration of opiate receptor binding, its