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Alpha-methyltryptamine Blocks Facilitation of Lordosis by Progesterone in Spayed, Estrogen-Primed Rats[1]

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ESPINO, C., M. SANO AND G. N. WADE. *Alpha-methyltryptamine blocks facilitation of lordosis by progesterone in spayed, estrogen-primed rats.* PHARMAC. BIOCHEM. BEHAV. 3(4) 557–559, 1975. – Alpha-methyltryptamine, a drug which stimulates serotonergic receptors in the central nervous system, inhibited the induction of sexual receptivity in spayed female rats by estradiol benzoate and progesterone. The drug had no effect on the sexual receptivity induced by estradiol benzoate alone. These data are consistent with the hypothesis that progesterone facilitates lordosis in estrogen-primed rats by inhibiting a serotonergic system in the brain. The data also suggest that estradiol and progesterone act on separate neurochemical systems to induce estrous behavior in rats.

Alpha-methyltryptamine Estradiol Progesterone Serotonin Estrous behavior

SEXUAL receptivity is induced in female rats by the sequential actions of estradiol-17β and progesterone on the neural substrates for lordosis. Intracerebral implantation of minute quantities of estradiol benzoate [6] or progesterone (in estrogen-primed animals [11]) induces estrous behavior in spayed rats. Meyerson and others have amassed a large body of evidence suggesting that there is a serotonergic system in the brain which tonically inhibits lordosis behavior and that progesterone facilitates estrous behavior in estrogen-primed female rats by interfering with serotonergic transmission in this system.

Elevation of brain monoamine levels (including serotonin) with monoamine oxidase (MAO) inhibitors such as pargyline or nialamide suppresses the lordosis induced by estradiol and progesterone. This suppressant effect of MAO inhibitors is intensified by treatment with precursors of serotonin (e.g., 5-hydroxytryptophan) but not with adrenergic precursors (e.g., dihydroxyphenylalanine [7,8]). In addition, drugs which interfere with serotonergic transmission in the brain, such as reserpine, tetrabenazine, parachlorophenylalanine, methysergide, or cinanserin, can substitute for progesterone in facilitating lordosis in estrogen-primed rats [7, 8, 9, 15]. A number of these anti-serotonergic drugs also facilitate lordosis if applied directly to the diencephalon in estrogen-primed rats [14,15].

However, there are some questions remaining in regard to Meyerson's hypothesis. A number of investigators have reported that while serotonergic blockers do facilitate lordosis in ovariectomized, estrogen-primed rats and mice, these drugs are not effective in adrenalectomized animals, suggesting that this effect is due to increased progesterone release by the adrenal glands [2, 3, 5, 10, 12]. However, other investigators have obtained these effects in adrenalectomized rats [7,15].

Another difficulty with this hypothesis is that it has never been demonstrated that the postulated serotonergic inhibitory mechanism is modulated by progesterone and not by estradiol. In the experiments demonstrating an inhibition of lordosis by elevated brain serotonin, estrous behavior was always induced with estradiol and progesterone. The elevated serotonin could be interfering with the estrogen priming rather than with the progesterone facilitation. Conversely, the drugs which inhibit serotonergic transmission could also be acting on estrogen-sensitive neurons to enhance the estrogen priming effects.

In the following experiments we examine the effects of the relatively specific serotonergic agonist [13] alpha-

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methyltryptamine (AMT) on estrogen- and estrogen+ progesterone-induced sexual receptivity in ovariectomized rats. If Meyerson's hypothesis is correct, AMT should block progesterone facilitation of lordosis, but it should have no effect on estrous behavior induced by estradiol benzoate alone.

EXPERIMENT 1

Method

Animals. Ten female Sprague-Dawley rats 90–120 days old were individually housed in wire mesh cages and given ad lib access to Purina Laboratory Chow and tap water. Illumination was provided from 7:00 p.m. to 7:00 a.m. daily, and room temperature was maintained at about 72° F.

Procedure. All animals were ovariectomized under sodium pentobarbital anesthesia (40 mg/kg). Five days following ovariectomy rats were injected subcutaneously with 2 μg estradiol benzoate in sesame oil vehicle followed 36 hr later by 0.5 mg progesterone. Animals were tested for sexual receptivity just prior to and 6 hr following the progesterone injection to assure that progesterone would facilitate lordosis in all rats.

Twelve days later the animals were again injected with 2 μg estradiol benzoate followed 36 hr later by 0.5 mg progesterone. Five-and-a-half hr following the progesterone injection (1/2 hr before testing) half of the rats received intraperitoneal injections of 6 mg/kg AMT, and the other half received the saline vehicle. Both groups were then tested for sexual receptivity. The following week this treatment was repeated, except that the AMT and saline treatments were reversed.

Sexual receptivity was tested in the dark (between 1:00 p.m. and 4:00 p.m.) in a 60 X 60 X 25 cm cardboard mating arena with a sawdust-covered floor. Sexually vigorous male rats were allowed 5 min to adapt to the arena. Females were then introduced and remained in the testing arena until mounted 10 times. Lordoses in response to mounts (neck extension and concave back flexion) were scored as present or absent, and a lordosis quotient was computed (100 X lordoses/mounts).

Results

The order in which the treatments (AMT or saline) were presented had no effect on responsiveness, so the data for the 2 groups were combined. It is clear that 6 mg/kg of AMT significantly inhibited sexual receptivity induced by estradiol and progesterone (paired t-test, $p<0.01$, 2 tails; Table 1).

EXPERIMENT 2

Although AMT inhibited estradiol+progesterone-induced sexual receptivity, this does not necessarily mean that the drug is acting on a progesterone-sensitive system that is inhibiting sexual receptivity. For example, the AMT could inhibit lordosis by interfering with the estrogen conditioning or simply by inducing a general malaise. Experiment 2 tests these possibilities by examining the effect of AMT on estrous behavior induced by estradiol benzoate alone.

Method

Ten female Sprague-Dawley rats 90–120 days old were maintained as in Experiment 1. Animals were ovariectomized under sodium pentobarbital anesthesia (40 mg/kg). Starting 3 days after ovariectomy each animal was given daily injections of 0.4 μg estradiol benzoate at 11:00 a.m. Daily behavioral tests were given at 2:00 p.m. starting 4 days after the first estradiol benzoate injection. Testing was carried out until each subject's lordosis quotient surpassed 70 (approximately the minimum lordosis quotient observed in Experiment 1). When an animal surpassed this level she was injected with 6 mg/kg AMT intraperitoneally and tested for sexual receptivity 30 min later. Behavioral testing was the same as in Experiment 1.

Results

Administration of AMT did not affect sexual receptivity in ovariectomized rats brought into heat with repeated injections of estradiol benzoate (Table 1).

DISCUSSION

Our data are certainly consistent with the hypothesis that progesterone facilitates lordosis in estrogen-primed rats by inhibiting a serotonergic system in the central nervous system. Alpha-methyltryptamine, a relatively specific serotonin receptor stimulator in the brain, inhibited the estrous behavior induced by sequential treatment with estradiol benzoate and progesterone, probably by acting on central serotonergic synapses.

TABLE 1

EFFECT OF ALPHA-METHYLTRYPTAMINE (AMT) ON SEXUAL RECEPTIVITY INDUCED IN OVARIECTOMIZED RATS BY ESTRADIOL BENZOATE (EB) PLUS PROGESTERONE (P) OR BY ESTRADIOL BENZOATE ALONE. (LORDOSIS QUOTIENTS: MEAN ± STANDARD ERROR)

Experiment	Pretreatment	N	Experimental Treatment	
			AMT	Saline
1	EB + P	10	12 ± 1	86 ± 2
2	EB alone	10	83 ± 3	84 ± 2

It has been suggested that in addition to its properties as a serotonergic agonist AMT can also act as a MAO inhibitor [4]. This raises the possibility that AMT could inhibit lordosis by raising neural levels of monoamines other than serotonin. For a number of reasons it is unlikely that any MAO-inhibiting activity of AMT is responsible for its lordosis-suppressing properties: (1) The doses of AMT used to partially inhibit brain MAO activity (45 mg/kg [4]) are far in excess of those we used to completely block the lordosis-facilitating actions of progesterone (6 mg/kg). (2) Vane *et al.* [13] point out that the activity-stimulating effects of AMT in mice are not attenuated by prior treatment with MAO inhibitors, as they should be if AMT acted principally as a MAO inhibitor. (3) Finally, even if the low doses of AMT we used do have any MAO-inhibiting activity, it is likely that brain serotonin levels would be elevated much more than those of other amines. Greig *et al.* [4] have shown that *in vitro* AMT is 10 times as effective in inhibiting oxidation of serotonin as it is in inhibiting catecholamine oxidation by MAO. Thus, it is likely that the lordosis-inhibiting action of AMT we observed is mainly due to its activity as a serotonin receptor stimulator.

Although AMT prevented the facilitation of lordosis by progesterone (Experiment 1), it had no effect on sexual receptivity induced by daily injections of estradiol benzoate alone (Experiment 2). To the best of our knowledge this is the first clear demonstration that a drug affecting central serotonergic transmission selectively influences a progesterone-sensitive system in the brain. These data would seem to indicate that estradiol and progesterone facilitate sexual receptivity in rats by acting on separate neurochemical systems.

Finally, our data do not support the hypothesis that drugs interfering with central serotonergic transmission induce lordosis by stimulating adrenal progesterone release [2, 3, 5, 10, 12]. It is most unlikely that either inhibition or facilitation of adrenal progesterone release could account for the suppression of lordosis by AMT. Zucker [16] has shown that it is extremely difficult to demonstrate inhibitory effects of progesterone on lordosis in rats, particularly in the present experimental paradigm. Thus increased adrenal progesterone release is probably not the source of the inhibition. Conversely, Davidson *et al.* [1] have shown that the adrenal glands are not necessary for the induction of sexual receptivity in rats, so that an inhibition of adrenal progesterone secretion could not explain the actions of AMT.

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