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BRIEF COMMUNICATION

Facilitation of Lordosis Behavior by Clonidine in Female Guinea Pigs

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CROWLEY, W. R., B. L. NOCK AND H. H. FEDER. *Facilitation of lordosis behavior by clonidine in female guinea pigs* PHARMAC. BIOCHEM. BEHAV. 8(2) 207–209, 1978. — Lordosis behavior was induced in previously unreceptive, ovariectomized estrogen-primed female guinea pigs by administration of the noradrenergic agonist clonidine. Clonidine also enhanced lordosis responding in females that were weakly receptive after estrogen priming. Unlike progesterone, the lordosis-facilitating effect of clonidine was not accompanied by a subsequent refractory period. Clonidine had a weak lordosis facilitatory effect when administered during the decline in receptivity to estrogen plus progesterone-primed animals and failed to induce lordosis when administered during the refractory period.

Clonidine Estrogen Lordosis Progesterone

IN ovariectomized guinea pigs, treatment with estrogen, followed 24–48 hr later by progesterone reliably induces sexual receptivity, characterized by the assumption of the lordosis posture. Monoamine neurotransmitters have also been implicated in the control of female receptivity in several rodent species [2, 3, 4]. In a recent study [2], we reported that ovariectomized guinea pigs, rendered sexually receptive by sequential administration of estrogen plus progesterone, showed an increased duration of individual lordosis responses after treatment with the noradrenergic agonist clonidine [1]. Blockade of noradrenergic receptors with phenoxybenzamine abolished lordosis responding within several hours. These experiments suggest that in the guinea pig, noradrenergic mechanisms may underlie, in part, the induction of sexual receptivity by ovarian hormones. As a further test of this hypothesis, the present experiments investigated whether clonidine mimics the facilitatory effect of progesterone on lordosis responding in estrogen-primed guinea pigs not already showing lordosis. Besides facilitating the display of lordosis, progesterone also induces a subsequent refractory period during which lordosis cannot be induced by further administration of ovarian hormones [7, 8, 11, 12]. This study also tested whether clonidine induces a comparable refractory period. Two further experiments tested whether clonidine enhances lordosis responding when given during the decline in receptivity or during the refractory period.

GENERAL METHOD

Animals

Female Hartley strain guinea pigs, 60–70 days of age, were obtained from Camm Research Labs (Wayne, NJ), housed 8–10 per cage in a room with lights on from 0500 to 1900 hr, and given Purina guinea pig chow and water ad lib, supplemented by lettuce once weekly.

Behavioral Tests

All animals were ovariectomized bilaterally shortly after arrival. Two weeks after surgery, all animals received a subcutaneous injection of estradiol benzoate (EB; 1.5 μg/animal in 0.1 ml sesame oil) at hr 0, followed at 40 hr by 0.5 mg progesterone in 0.1 ml sesame oil. Tests for lordosis used the manual stimulation technique of Young *et al.* [9], and were conducted at hourly intervals, starting at 40 hr and continuing until 10 hr after progesterone. Lordosis duration was measured in seconds, and an animal was considered sexually receptive if it displayed lordosis of at least 1 sec duration on two consecutive hourly tests. Only females fulfilling this cirterion were used for further experiments.

Experiment 1: Facilitation of Lordosis by Clonidine

Procedure. Two weeks after the initial screening test, all selected animals received an injection of 1.5 μg EB at Hour 0. Commencing at Hour 40, the animals received three hourly tests for lordosis. This predrug screening served to identify animals showing lordosis to EB treatment alone. At 42.5 hr, animals were given an injection of either clonidine HCl (0.1, 0.5 or 1.0 mg/kg of salt, IP, in saline) or saline alone. Tests for lordosis were conducted at 30 min intervals until 3 hr post-drug and thereafter at hourly intervals until 6 hr postdrug. Sixty-four hr after EB, 0.5 mg

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of progesterone was administered to all animals, and tests for lordosis were conducted until 8 hr postprogesterone.

Results. Neither the 0.1 nor the 0.5 mg/kg dosages of clonidine induced lordosis in animals not showing lordosis after EB alone (non EB heats) or enhanced lordosis in those animals showing lordosis after EB alone (EB heats). It should be noted that 0.1 mg/kg of clonidine enhanced lordosis in our previous study, which used EB plus progesterone-primed females [2]. However, Fig. 1 shows that 1 mg/kg of clonidine significantly increased the proportion of non-EB heat animals showing lordosis during the first two hr post-drug. Cumulatively, 7/8 clonidine-treated animals showed lordosis as compared to 2/8 controls ($p<0.025$, one-tailed Fisher's Exact Test). Individual lordosis responses ranged from 4–11 sec in duration for animals that received clonidine. These responses were generally less intense than typically observed after progesterone and usually consisted of a weak flattening of the back, brief display of the vaginal area, and occasional rumbling vocalization. Figure 1 also shows that 1 mg/kg of clonidine also tended to enhance the display of lordosis in animals already responding to EB alone. The maximal drug effect occurred at one hr, as mean lordosis duration in the clonidine-treated group was 14 ± 4 sec, compared to 6 ± 1 sec in the saline-treated controls ($p<0.01$, t-test). Most of the clonidine-treated animals persisted in showing lordosis to palpation 3–4 hr after receiving the drug, while the responsivity of the saline-treated controls had declined by this time. Sedation was noted only at the highest dose of clonidine.

Within several hr after receiving progesterone at 64 hr after EB, all saline and clonidine-treated animals showed lordosis. Thus, clonidine did not induce a refractory period as does a comparably timed injection of progesterone ([6,11], and below).

Experiment 2: Administration of Clonidine During the Decline in Receptivity or During the Refractory Period

Procedure. Two weeks after an initial screening test, each animal received the standard EB plus progesterone treatment, and, commencing 8 hr after progesterone, was tested at 30 min intervals until it showed a weak lordosis of 5 sec or less in duration. Saline, clonidine (1 mg/kg), or progesterone (0.5 mg/animal) was administered. Testing continued at 30 min intervals until 8 hr post drug. In a second part of this experiment, previously screened guinea pigs received the standard EB plus progesterone treatment and were tested until all showed lordosis. Clonidine, progesterone (in the same dosages as above), or saline was administered 24 hr postprogesterone during the refractory period. Tests were conducted hourly until 8 hr post-drug.

Results. Figure 2 shows that the gradual decline in receptivity (see saline animals) was only briefly delayed by clonidine treatment. Fisher's Exact Tests showed that the proportion of clonidine-treated animals showing lordosis was significantly higher than saline only at 2 hr post-drug. Furthermore, Kruskall-Wallis analyses of variance performed at each time interval did not reveal any statistically significant differences among the three groups in mean individual lordosis durations.

In the second experiment, all animals that were receptive after the initial progesterone injection failed to show lordosis when tested 24 hr later. Neither progesterone nor

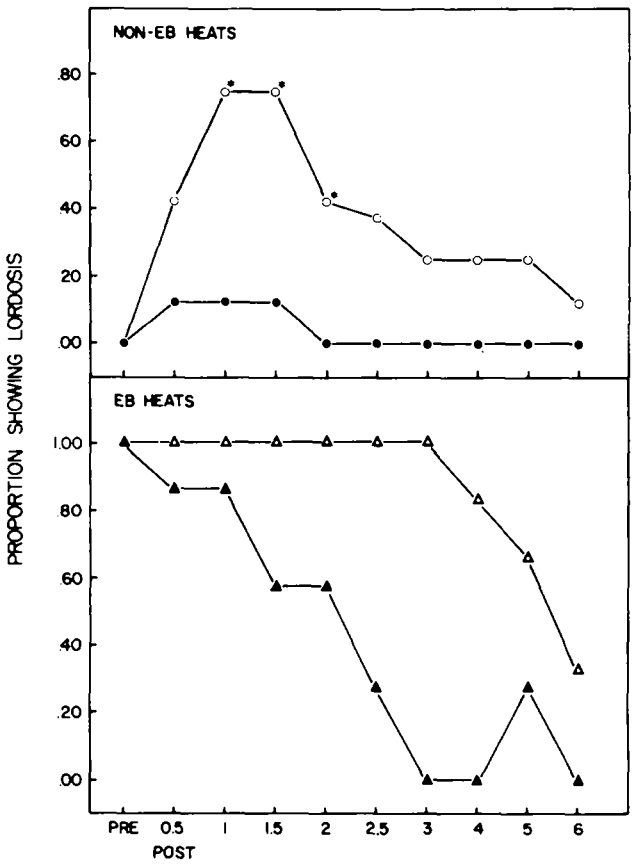

FIG. 1. Proportion of estrogen-primed female guinea pigs showing lordosis after clonidine HCl (1 mg/kg) or saline. Non EB heats: ●———● denotes saline (n = 8); ○———○ denotes clonidine (n = 8). * denotes $p<0.025$, one-tailed Fisher's Exact Test. EB heats: ▲———▲ denotes saline (n = 7). △———△ denotes clonidine (n = 6).

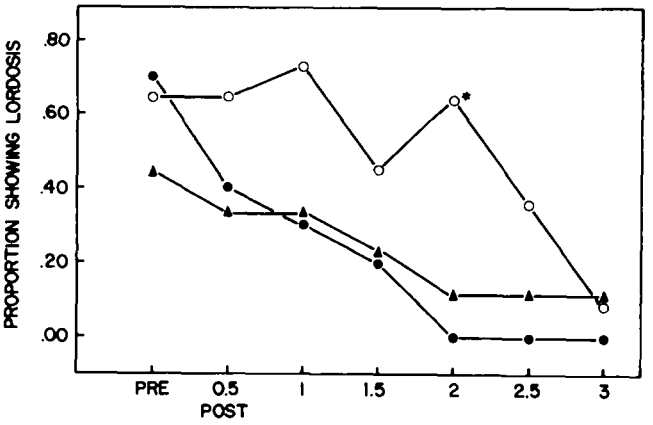

FIG. 2. Proportion of estrogen plus progesterone-primed female guinea pigs showing lordosis after clonidine HCl (○———○, n = 11), saline ●———●, n = 10), or progesterone (▲———▲, n = 9) during the decline in receptivity, * denotes $p<0.01$ vs saline, one-tailed Fisher's Exact Test.

clonidine induced lordosis responding when given during the refractory period.

DISCUSSION

It is not clear at this time whether clonidine mimics some aspect of ovarian hormone action or whether clonidine facilitates behavior by a process different from that induced by ovarian hormones. Thus, even though clonidine, like progesterone, facilitated lordosis in estrogen-primed guinea pigs, its actions appear to differ from progesterone's in at least three respects. First, the quality of lordosis induced by clonidine was lower than typically observed after progesterone. Second, clonidine, unlike progesterone, did not induce behavioral refractoriness to a subsequent progesterone injection. These dissimilarities may be due to differences in bioavailability, disposition, or metabolism of the compounds as well as the likelihood that ovarian hormones affect systems other than the noradrenergic. Third, clonidine enhanced lordosis responding in guinea pigs already rendered sexually receptive by combined estrogen plus progesterone treatment [2], an effect not shared by progesterone. For these same reasons, it is unlikely that clonidine induced lordosis by causing release of adrenal progesterone. Clonidine had only a weak lordosis-facilitating effect when administered during the decline in receptivity and was completely without effect when given during the refractory period. The reason for this is unknown at present, but it is possible that one mechanism underlying the progesterone-induced refractory period is a loss of sensitivity of noradrenergic receptors to stimulation, perhaps as a result of heightened noradrenergic stimulation during receptivity.

Further work is required to more clearly delineate the role of noradrenergic system in mediating sexual behavior in the female guinea pig, and especially to determine whether the facilitation of lordosis responding by clonidine is specific to this drug or is a property of adrenergic agonists in general. Another potential problem with the use of clonidine is that it has been shown to reduce central serotonergic activity [1]. This effect may contribute to the facilitation of lordosis as decreases in serotonergic transmission have been reported to enhance lordosis responding in rats [4, 5, 10].

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