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Biphasic Effects of the Antiserotonergic Methysergide on Lordosis in Rats

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DAVIS, G. A. AND R. L. KOHL. *Biphasic effects of the antiserotonergic methysergide on lordosis in rats*. PHARMAC. BIOCHEM. BEHAV. 9(4) 487–491, 1978.—As also reported by other workers, the antiserotonergic drug methysergide was found to facilitate lordotic responding in estrogen primed, ovariectomized rats. A second dose of methysergide 24 hr after the first, however, failed to produce any increment in responding. Animals received daily estrogen injections in order to maintain a relatively constant level of priming. After several days of methysergide, a progesterone injection facilitated lordosis to the same degree as in controls receiving only saline and estrogen. When a second injection of progesterone was given 24 hr later, however, the animals failed to respond. In contrast, saline controls with this estrogen paradigm responded equally well to both progesterone injections. These results are discussed in terms of their bearing on possible serotonergic and non-serotonergic mechanisms by which progesterone may control lordosis.

Methysergide Progesterone Sexual behavior

PHARMACOLOGIC studies in recent years have led to the proposal that serotonin (5HT) and other neurotransmitters may be involved in the action of progesterone (P) in facilitating lordosis in estrogen-primed rats [14]. For instance, drugs that inferfere with serotonergic activity facilitate lordosis in the absence of P, while drugs that enhance serotonergic activity suppress lordotic responding elicited by P [5, 6, 14, 21]. Consequently, it has been suggested that a serotonergic system inhibits lordosis and that P blocks this inhibitory influence.

The action of P on lordosis, however, is twofold, and inhibition as well as facilitation can be obtained under some conditions in rats and other rodents [15]. After an initial facilitatory dose of P, for instance, a second injection of the same dose a day later may produce little or no elevation in lordotic responding, though a higher dose of P may still be effective (Wallen, personal communication). This phenomenon has been called the biphasic inhibitory effect of P, but it might also be considered as a kind of tolerance to the steroid. Several explanations for the inhibitory actions of P have been put forth, among them interference with estrogen priming [15] or suppression of P receptors. The first mechanism has been supported by the finding that additional estrogen can block the inhibitory action [16].

Another reasonable possibility is that neurotransmitters may be involved in the inhibitory action of P as they seem to be in its facilitatory action. Some support for this suggestion can be found in experiments by Ladisich [10]. He reported that treatment of rats for several days with amounts of P sufficient to give rise to blood levels comparable to those in the pregnant animal produced an increase in the turnover rate of brain 5HT. This increase could result as a compensation for an initial reduction in serotonergic activity by P and might well be expected to play a role in the suppression of sexual receptivity which is known to occur during pregnancy [17].

We have examined the potential role of 5HT in the inhibitory action of P in 2 ways. Elsewhere we report that the antiserotonergic drug methysergide is able to restore lordosis in rats rendered unresponsive to P by prior treatment with the steroid ([18] Davis and Kohl, manuscript in preparation). This result is consistent with a serotonergic involvement in P inhibition and argues against a role for interference with estrogen priming. In this paper we report the converse approach. If tolerance to the facilitatory action of P on lordosis is due to compensatory change within a serotonergic system, then tolerance might be expected to develop after facilitation of lordosis with an antiserotonergic drug such as methysergide or paracholrophenylalanine (PCPA). Further, if the facilitatory and inhibitory actions of P are mediated through 5HT, then cross-tolerance might be expected and P might not be effective in drug tolerant rats. The present results are also consistent with the involvement of 5HT in P inhibition but imply further that the situation is probably more complicated.

METHODS

Female rats from Holtzman (around 250 g) were bilaterally ovariectomized and housed in pairs under a lighting cycle of 14 hr light and 10 hr dark.

The females were tested with male rats and lordosis quotients (LQ, lordosis/mounts × 100) were determined as previously described [3]. Statistical comparisons were carried out with the Mann-Whitney U Test.

For all experiments animals were primed daily with estradiol benzoate (EB, 2 μg/kg/day SC, Sigma) beginning 5

days before the experiment (which began on Day 1) and continuing for the duration of the experiment.

In one experiment parachlorophenylalanine methylester hydrochloride (PCPA, 300 mg/kg IP in saline, Sigma) was injected on Day 1 at the onset of the dark phase. The animals were tested 4 hr later and for 14 more days at about the same time of day.

For the experiments with methysergide, the drug (15 mg/kg as the maleate in saline, Sandoz) was injected daily at the onset of the dark period for several days as indicated in the text. Testing was done 3–6 hr after injection. In several experiments P (2.0 mg/kg in sesame oil, SC, Prolutin, Schering) was also injected at the onset of dark. The detailed schedules for methysergide and P injections are indicated in the text.

RESULTS

Tolerance to Lordosis Facilitation by Methysergide

The time course of the heat induced by methysergide is shown in Fig. 1. The LQ peaked from 2 to 6 hr after injection of the drug and declined by 8 hr. When these same animals were given a second injection of methysergide 24 hr after the first, they showed no significant increment in LQ at 3 and 6 hr after injection (Fig. 1). Further injections on three more successive days gave similar results (Fig. 1).

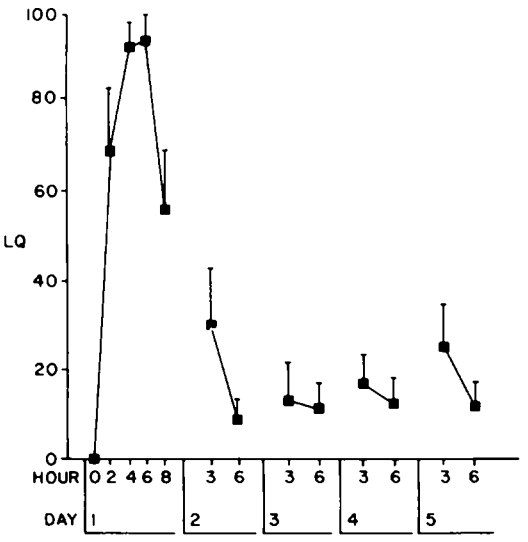

FIG. 1. Lordosis response (LQ) to methysergide treatment on 5 successive days. Ovariectomized rats were given EB on Days -4 through 5 and methysergide on Days 1 through 5 (n - 9). The LQ of saline controls (not shown) remained below 20. The response of methysergide animals was significantly higher ($p < 0.01$) than controls on Hr 2, 4, 6 and 8 of Day 1.

Cross-Tolerance between Methysergide and P

In order to determine if there was any cross tolerance between methysergide and P, animals were given methysergide or saline daily for 7 days. Sexual receptivity was determined in one test on each of the first 5 days, and the results (not shown) were similar to those in the experiment of Fig. 1. On Day 6, P was given at the same time as methysergide and the animals were tested 5 hr later. The response to P in the animals with prior methysergide (Table 1) was high and was not reduced at all in comparison with animals not receiving methysergide. When a second dose of P was given on Day 7, however, the LQ was significantly reduced over that attained on Day 6 (Table 1). With the present estrogen priming regime there was no biphasic effect of P in the saline control group.

Although the LQ after the second injection of P in the methysergide treated animals was reduced, it still was significantly elevated over the pretest value (Table 1). We were interested to see if a stronger biphasic effect of P might be obtained on a third day of P treatment. For this experiment, animals were given methysergide for a total of 5 days, and injections of P simultaneous with the drug were begun on Day 3. A strong response to methysergide was noted in a test on Day 1, testing was omitted on Day 2. On Day 3, the LQ, as expected, was high 6 hr after P injection (Table 2). On Days 4 and 5, however, the test 6 hr after P revealed no significant elevation in LQ over the pretest value. The pretest values were higher in this experiment than in that reported in Table 1.

In both of the experiments with methysergide and P reported above, drug injections were continued on the days the animal received P. It is possible that the continued presence of methysergide might have partially antagonized some compensatory process underlying the methysergide tolerance and rendered any cross-tolerance with P more difficult to detect. Consequently, a further experiment was performed in which methysergide was omitted in one group on the days of P injection (Table 3). Animals in Groups 1 and 2

TABLE 1

FACILITATION AND INHIBITION OF LORDOSIS BY PROGESTERONE (P) IN ESTRADIOL BENZOATE (EB) PRIMED, OVARIECTOMIZED (OVX) RATS PRETREATED WITH METHYSERGIDE

Pretreatment	Day 6 Hr after P	Day 6 LQ ± SE	Day 7 Hr after P	Day 7 LQ ± SE
Methysergide	0	4 ± 4	0	0
	5	91 ± 7*	5	46 ± 17†
Saline	0	7 ± 2	0	10
	5	95 ± 7	5	92 ± 6*

EB was given on Days–4 through 7, methysergide or saline on Days 1 through 7, and P on Days 6 and 7. n 8.

* vs † $p < 0.05$.

TABLE 2

FACICITATION AND INHIBITION OF LORDOSIS BY P (GIVEN FOR THREE DAYS) IN EB PRIMED, OVX RATS PRETREATED WITH METHYSERGIDE

Day 3 Hr after p	LQ ± SE		Day 4 Hr after P	LQ ± SE		Day 5 Hr after P	LQ + SE	
0	19 ± 9		0	35 ± 8		0	38 ± 8	
		$p < 0.005$			n.s.			n.s.
6	100		6	54 + 11*		6	50 ± 12*	

EB was given on Days–4 through 5, methysergide on Days 1 through 5, and P on Days 3, 4 and 5. n = 8.
*significantly different from Hr 6 of Day 3, $p < 0.05$.

TABLE 3

FACILITATION AND INHIBITION OF LORDOSIS BY P IN EB PRIMED, OVX RATS PRETREATED WITH METHYSERGIDE COMPARISON OF GROUP RECEIVING METHYSERGIDE THROUGHOUT EXPERIMENT WITH GROUP RECEIVING THE DRUG ONLY ON DAYS PRIOR TO P INJECTION

Group	Day 4 Hr after P	LQ + SE		Day 5 Hr after P	LQ ± SE	
1						
Methysergide of Days	0	26 ± 10		0	35 + 11	
1-5, P on Days 4,5.			$p < 0.05$			n.s.
n = 9	6	75 · 7		6	42 ± 6	
2						
Methysergide on Days 1-3,	0	30 ± 6		0	25 · 4	
Saline and P on Days 4-5.			$p < 0.005$			n.s.
n = 10	6	92 · 2		6	12 ± 6	
3						
Saline on Days 1-5,	0	28 · 7		0	32 · 8	
P on Days 4-5.			$p < 0.005$			$p < 0.005$
N = 8	6	95 ± 2		6	97 · 2	
4						
EB only on Days 1-5,	—	—		6	31 ± 6	
Oil Control on Day 5.						
n 7						

EB was given on Days –4 through 5.

received 3 daily injections of methysergide without any testing. On Days 4 and 5, Group 1 was given both the drug and P as before while Group 2 received saline and P. Group 3 and 4 animals were saline and EB controls. No difference was seen between Groups 1 and 2 in the Day 4 test 6 hr after injection, and the LQ was high in both cases. On Day 5, P produced no facilitation of lordosis in either Group 1 or 2.

Facilitation of Lordosis by PCPA

When another agent which interferes with serotonergic activity, parachlorophenylalanine (PCPA), was used instead of methysergide there was a long lasting facilitation of lordosis. The LQ reached 89 within 4 hr of injection and remained at this level for 2 more days (Fig. 2). The response declined slowly on subsequent days, but remained significantly higher than controls for at least 9 days after injection.

DISCUSSION

As also reported by other workers [7,21], we found that the 5HT antagonist methysergide facilitates lordosis in estrogen primed rats. In this paper we report 3 new findings: (1) that animals become tolerant to the action of methysergide after a single injection, (2) that P shows no cross-tolerance to the drug with respect to facilitation of lordosis, and (3) that the drug somehow promotes the display of a biphasic inhibitory action of P under conditions where the estrogen level is sufficiently high to block the inhibitory action in control animals.

A number of mechanisms could underlie the tolerance to methysergide. Nothing more than increased clearance of the drug could be at play, for instance, but the fact that the drug alters the inhibitory action of P suggests that it may induce changes, possibly compensatory, in neural circuits controlling lordosis. Further, Klawans and co-workers [8] have reported direct evidence that chronic methysergide leads to an increased response to the 5HT precursor, 5-hydroxytryptophan. Compensatory supersensitivity of 5HT receptors [20] would be a good candidate for the mechanism of these effects but we have been unable to detect any changes in receptor sensitivity in experiments with 5HT agonists [19]. The possibility that non-serotonergic (possibly dopaminer-

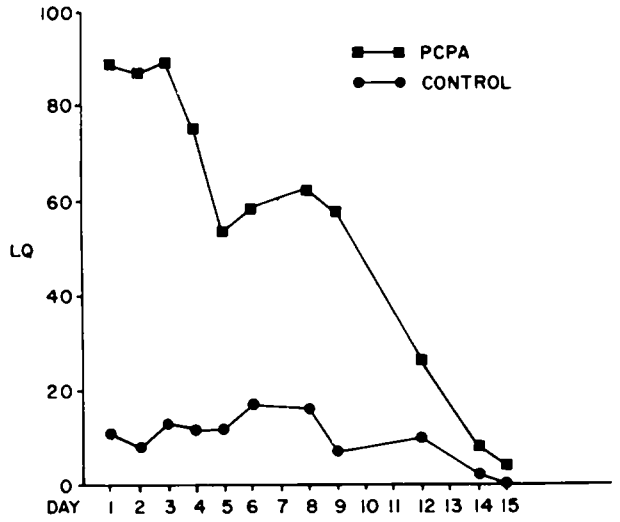

FIG. 2. Lordosis response (LQ) after injection of PCPA. The experimental group (n=8) of ovariectomized rats was given EB on Days 4 through 15 and PCPA on Day 1, with a test 4 hr later. The control group (n=6) was treated similarly but given vehicle on Day 1. The responses of the PCPA and control groups were significantly different ($p<0.01$) for Days 1–9.

gic) effects of methysergide may be involved must be kept in mind [11]. It is of interest that tolerance to the lordosis inhibiting actions of the serotonergic agonist LSD has also been noted, though only after several days of injections [4]. In this case there was no cross-tolerance with a dopaminergic agonist.

No indication of any compensatory change in neural circuits was found when lordosis was facilitated with PCPA rather than methysergide. The LQ after a single dose of the synthesis inhibitor remained high for many days and declined along a curve generally paralleling the reported recovery of 5HT levels after a dose of PCPA [9]. It might have been expected from the methysergide results that the LQ would have fallen off at a more rapid rate. Supersensitivity to the effects of serotonin agonists on lordosis [5] and prolactin release [13] has been reported to occur within a day of PCPA injection and this too might lead one to expect a more rapid decline in LQ. Perhaps, in our experiment the continued inhibition of 5HT synthesis was sufficient to prevent any significant activation of supersensitive receptors.

What light might the present findings with methysergide throw on the mechanisms by which P facilitates and inhibits lordosis? The lack of cross-tolerance to P in methysergide treated animals might seem to argue against the notion that P facilitates lordosis by suppressing serotonergic activity, but, as discussed below, it does not rule it out. On the other hand, the great enhancement of the inhibitory effect on P in drug-tolerant rats is consistent with serotonergic involvement in the actions of the steroid in lordosis. One other way to account for this phenomenon requires comment. If methysergide partially reduced the level of estrogen priming, then a biphasic effect of P could appear. Nadler [16] and Blaustein and Wade [1,2] have shown that the inhibitory action of P is manifested after low doses of EB, but not after higher doses. This explanation seems unlikely to account for the present findings, however, since the continued daily injections of EB would be expected to maintain priming at a high level. That this was so is supported both by the strong responses to the first P injection and by the fairly high estrogen heat noted in several of the experiments in 0 hr tests and in animals receiving only estrogen (Table 2; Table 3, Group 4).

Several mechanisms have been proposed to account for the inhibitory action of P. Interference with estrogen priming has been prominent but is not supported by recent evidence [1, 2, 18]. The suppression by P of its own receptors is another possibility [12,15], but it is difficult to understand in this case why methysergide would facilitate the inhibition.

A third possibility, that neurotransmitters are involved [15], is more in keeping with the present findings. The report that serotonin turnover is increased after inhibitory P treatment [10] provides one means by which the inhibitory action of the steroid could occur, and such an increase could also account for the tolerance to methysergide. The lack of cross tolerance between methysergide and P, however, implies that the situation may be more complicated. For example, P could facilitate lordosis by suppressing a serotonergic system through some mechanism which can overcome or bypass the change underlying methysergide tolerance, possibly acting at a separate site in the system. A dose of P alone might lead to some compensatory change at this site which would be insufficient to produce tolerance, but if the change were combined with that induced by methysergide, lordosis might be blocked.

A similar argument could apply if P works through more than 1 neurotransmitter to facilitate lordosis, perhaps by suppressing both 5HT and dopamine (DA). Certainly there is considerable evidence that both neurotransmitters are involved, and it appears that suppression of either system by itself can lead to lordosis [6]. If the 5HT system becomes ineffective, as occurs after methysergide treatment, the DA system could still maintain the sensitivity of lordosis to P. In order to explain the enhancement of the inhibitory effect of P in methysergide treated rats it could be postulated that tolerance to the action of the steroid develops more easily in the DA than in the 5HT system. The 5HT system, then, could still lead to lordotic responding after a second dose of P even though the DA system had become tolerant to the steroid. If the 5HT system had, on the other hand, previously been rendered ineffective with methysergide, then the characteristics of the DA system would predominate.

These considerations are obviously speculative, but many of the mechanisms suggested are readily open to experimental attack. The phenomenon of tolerance to a lordosis facilitatory drug, then, provides another means for unravelling the mechanisms by which P both facilitates and inhibits lordosis.

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