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Inhibitory Effect of Progesterone on Sexual Receptivity in Female Rats: A Temporal Relationship to Estrogen Administration

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ABSTRACT—The inhibitory effect of progesterone (P) injected at various times on female sexual behavior was investigated in estradiol benzoate (EB) treated ovariectomized rats. Four behavioral tests were carried out at two-week intervals. All females received 5 μg/kg b.w. EB and 0.5 mg P 44 hr after the EB. In the P-control group, an additional 5 mg P was administered at the same time as the injection of EB in four tests. Instead of P, oil was given concurrently with EB in the Oil control group. In the experimental groups, female rats were treated with 5 mg P from 1 to 40 hr before (PB group) or after (PA group) the EB-injection. A sexual behavioral test was started 4 hr after 0.5 mg P. The results show that low levels of lordosis and soliciting behavior were observed in the P group, compared to the Oil-control group. In the PB groups, lordosis quotient (LQ) was low when P was given from 1 to 24 hr before EB. Moreover, animals in which P was given 27-40 hr before EB showed lower LQ than Oil-control animals, but higher LQ than rats in the P-control group. In the PA groups, when P was administered from 1 to 24 hr after EB, low levels of lordosis response were observed, whereas animals which received P 27-40 hr after EB showed a high score of LQ, being comparable to that in the Oil control. These results suggest that the period of 24 hr before and after EB injection is a critical period for inhibitory action of P on female sexual behavior in female rats.

INTRODUCTION

Lordosis and soliciting behavior in female rats are regulated by the interaction between estrogen and progesterone, and the central nervous system (Pfaff *et al.*, 1994). The action of estrogen in the forebrain, such as the ventromedial hypothalamus, is essential to induce female sexual behavior, because direct implantation of estrogen to the area potentiates lordosis behavior (Barfield and Chen, 1977; Rajendren *et al.*, 1991). Progesterone also plays a facilitatory role in regulating lordosis behavior. However, the effect of the facilitation of lordosis by progesterone is synergistic with estrogen, since estrogen without progesterone, but not progesterone without estrogen can enhance lordosis in ovariectomized rats.

On the other hand, low levels of sexual receptivity under the condition of a high level of progesterone in the blood, such as during pregnancy, has been reported (Moralí and Beyer, 1979). Furthermore, progesterone is known to be a factor which terminates the estrus in reflex ovulators, ferrets (Marshall and Hammond, 1945) and rabbits (Beyer *et al.*, 1969). In rodents, besides having a facilitatory role, progesterone also has an inhibitory role in regulating lordosis behavior (see review, Morin, 1977). Injection of progesterone concurrently with estrogen suppresses lordosis behavior in guinea pigs (Zucker, 1966), hamsters (DeBold *et al.*, 1976), and rats (Blaustein and Wade, 1977; Hansen and Södersten, 1979; Marrone *et al.*, 1977; Satou and Yamanouchi, 1994). The inhibition of lordosis by progesterone is thought to be due to an antagonistic effect on estrogen action in the central nervous system (Morin, 1977). The appearance of an inhibitory or a facilitatory effect of progesterone on sexual receptivity is dependent on the timing of the injection of progesterone in relation to the time of estrogen administration in ovariectomized rodents (Blaustein and Wade, 1977). Thus, the effect of progesterone is biphasic in regulating female sexual behavior. As a first step towards clarifying the shift from the inhibitory to the facilitatory period in the timing of the injection of progesterone, progesterone was administered at various times before or after estrogen treatment, and female sexual behavior was observed in ovariectomized rats.

MATERIALS AND METHODS

Female Wistar rats (220-250 g) were housed under a controlled temperature (23-25°C) and photoperiod (14hr:10hr, light:dark). Fifty-four females were ovariectomized under ether anesthesia.

The preliminary test was carried out 2 weeks after ovariectomy. In order to investigate the adequacy of the estrogen-dose to induce

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lordosis in individual animals, females were treated with 5 μg/kg estradiol benzoate (EB; Sigma, 12.5 μg/1 ml sesame oil) and 0.5 mg progesterone (P; Sigma, in 0.1 ml sesame oil) 44 hr after EB. A behavioral test was carried out four hours after the injection of P. In the behavioral test, each female was placed in a plastic observation cage (40×60×50 cm) with two vigorous males, and lordosis quotient (LQ; number of lordosis/10 mounts, ×100) was recorded. The presence or absence of soliciting behavior (ear-wiggling and hopping) in each animal was also recorded. Female rats in which LQ was more than 70 were used in the following experiments.

Two weeks after the preliminary test, the rats were randomly divided into 8 groups, and 4 behavioral tests were performed at two-week intervals. In the experiments, the basic regime for the hormonal treatments was the same as those in the preliminary test, except for the use of additional progesterone. Animals received an additional 5 mg P (dissolved in 0.3 ml sesame oil) before (PB group) or after (PA group) the EB injection at various times. Seven rats were given 5 mg P at the same time as the EB injection in all four tests (P group), according to the modified method of Marrone *et al.* (1977). Instead of P, 0.3 ml oil was injected in 6 rats as a control (Oil group).

In 3 PB groups, 5 mg P was administered from 1 to 40 hr before the injection of EB (see Table 1). Seven rats were given 5 mg P from 1 to 12 hr before the EB in the 4 tests (PB-1 group). In the other PB groups, 5 mg P was given from 15 to 24 hr before EB in 6 rats (PB-2), and from 27 to 40 hr before EB in 6 rats (PB-3). In 3 PA groups, females were given 5 mg P from 1 to 40 hr after the EB. Eight rats were injected with 5 mg P from 1 to 12 hr after the EB (PA-1 group). Two groups of 8 and 6 rats were given 5 mg P from 15 to 24 hr (PA-2), and from 27 to 40 hr (PA-3) respectively.

The differences between the mean LQs among or within groups were analyzed by using the analysis of variance (ANOVA) followed by Fisher's protected least significant difference. The data for the incidences of behavior were evaluated by the chi-square test with Yates' correction.

Table 1. Injection time (hr) of 5 mg progesterone (P) before (PB groups) or after (PA groups) the treatment with estradiol benzoate (EB)

Groups	Injection Time of 5 mg P			
	1st test	2nd test	3rd test	4th test
P	0	0	0	0
PB-1	−1	−3	−7	−12
PB-2	−15	−18	−21	−24
PB-2	−27	−30	−33	−40
PA-1	+1	+3	+7	+12
PA-2	+15	+18	+21	+24
PA-3	+27	+30	+33	+40

EB (5 μg/kg b.w.) was given at the time 0 in each test. In the P group, 5 mg P was given at the same time of EB in all four tests (see text).

RESULTS

All females treated with 5 μg/kg EB and oil displayed lordosis behavior and the LQs were higher than 70. Half of the females showed soliciting behavior over four tests (Fig. 1). On the other hand, in females which received 5 mg P and EB at the same time, the incidence of lordotic response ($p<0.05$, in the 1st and 4th test) and the mean LQs were lower than those of the Oil group ($p<0.01$ in all tests, see F-values in legend of Fig. 1). The incidences of hopping ($p<0.05$) and ear wiggling ($p<0.05$) in the P group were also lower than those in the Oil group. Among the mean LQs in the four tests, there was no statistical difference in the P ($F[3,24]=1.39$, $p<0.01$) or Oil ($F[3,20]=1.25$, $p<0.01$) group.

As well as in the P group, the mean of LQs in the PB-1 group were lower than those of the Oil group ($p<0.01$~0.05, see F-values in the legend of Fig. 2) (Fig. 2). The mean LQs of the PB-2 group were also low, compared to those of P group in each test ($p<0.01$). Incidences of hopping in the PB-1 and the PB-2 were lower than in the Oil group (see Fig. 2). Most rats in the PB-3 group displayed lordotic responses over the four tests. The mean LQs in the PB-3 group were lower than those of the Oil group ($p<0.01$ in the 1st and 2nd tests; $p<0.05$, in the 3rd and 4th tests). When P was given 27 or 40 hr prior

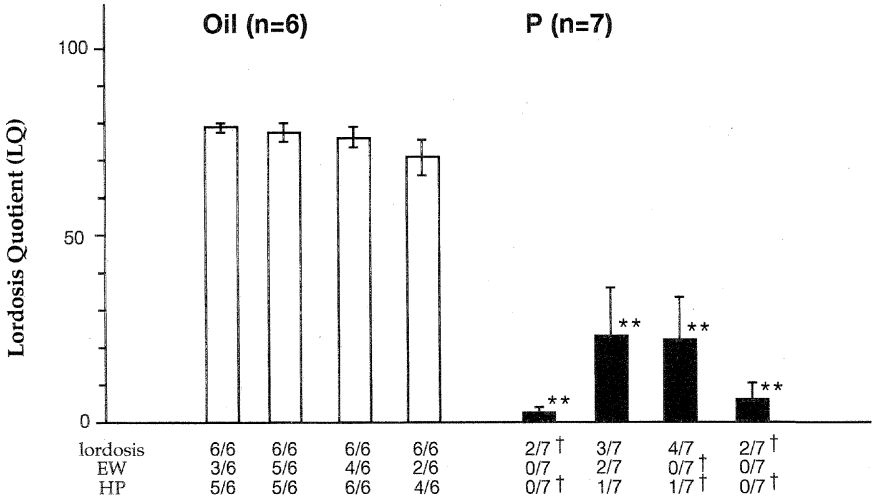

Fig. 1. The mean LQs and incidences of lordosis, ear-wiggling (EW) and hopping (HP) in four tests in the Oil and P groups. In the P group, 5 mg P was injected concurrently with the estradiol benzoate (EB) in all tests. In the Oil group, oil was injected instead of 5 mg of P (see text). **$F(7,46)=17.3$, 10.9, 6.4, 7.2, $p<0.01$ vs Oil group in the 1st to 4th test, respectively. †$p<0.05$ vs Oil group.

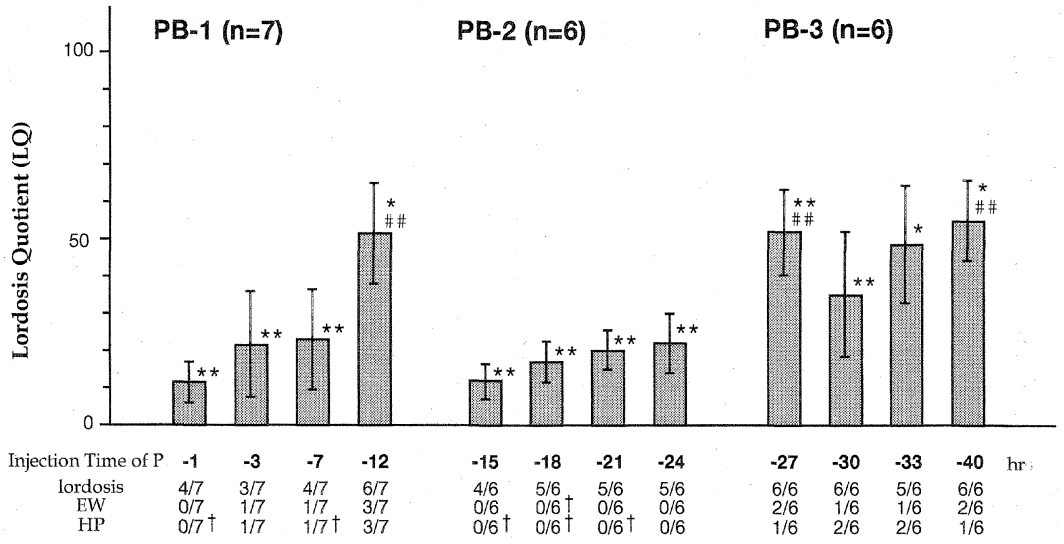

Fig. 2. The mean LQs and incidences of lordosis, ear-wiggling (EW) and hopping (HP) in 3 groups in which P was administered before the estradiol benzoate (EB) (see text). Females were injected with 5 mg P from 1 (-1) to 40 (-40) hr before the injection of EB. F(7,46)=17.3, at -1, -15 and -27 hr; F(7,46)=10.9, at -3, -18 and -30 hr; F(7,46)=6.4, at -7, -21 and -33 hr; F(7,46)=7.2 at -12, -24 and -40 hr, *p<0.05 vs Oil group, **p<0.01 vs Oil group, ##p<0.01 vs P group. †p<0.05 vs Oil group.

to EB, the mean LQs were higher than those of the P group (p<0.01).

In the PA-1 group, the mean LQ was low compared to that of the Oil group (p<0.01, see F-values in the legend of Fig. 3) (Fig. 3). Except for the 4th test in which P was injected 12 hr after the EB, there was no statistically significant difference of LQ between the PA-1 and the P group. The mean LQs of the PA-2 group were lower than those of the Oil group (p<0.01~0.05). Most animals in the PA-1 and the PA-2 groups didn't show soliciting behavior (see Fig. 3). In the PA-3 group, all rats showed lordotic responses. The mean LQs of the PA-3 group were higher than those of the P group (p<0.01), and showed no statistical difference from those of the Oil groups. Half of the PA-3 females displayed soliciting behavior throughout the tests.

DISCUSSION

In the present results, females which received P concurrently with EB-injection showed lower sexual receptivity

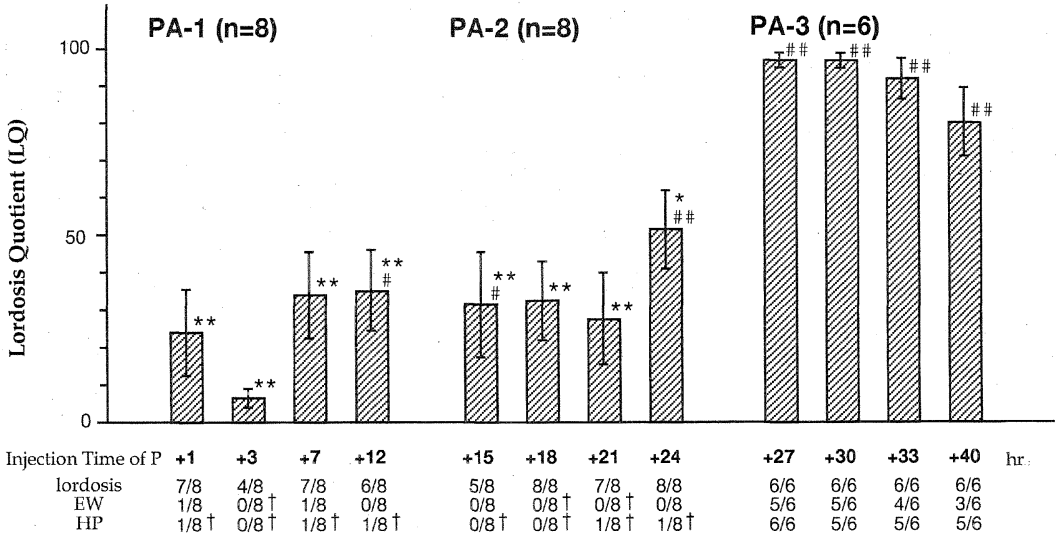

Fig. 3. The mean LQs and incidences of lordosis, ear-wiggling (EW) and hopping (HP) in 3 groups in which P was administered after the estradiol benzoate (EB) (see text). Females were injected with 5 mg P from 1 (+1) to 40 (+40) hr after the injection of EB. F(7,46)=17.3, at +1, +15 and +27 hr; F(7,46)=10.9, at +3, +18 and +30 hr; F(7,46)=6.4, at +7, +21 and +33 hr; F(7,46)=7.2 at +12, +24 and +40 hr, *p<0.05 vs Oil group, **p<0.01 vs Oil group, #p<0.05 vs P group, ##p<0.01 vs P group. †p<0.01~0.05 vs Oil group.

than control females. This result is consistent with previous studies reporting that P has an inhibitory role in regulating female sexual behavior in rats (Blaustein and Wade, 1977; Hansen and Södersten, 1979), guinea pigs (Zucker, 1966) and hamsters (DeBold *et al.*, 1976).

In hamsters, the inhibitory effect of P was seen when P was given 24 hr before estrogen, and the effect decreases according as the injection time separates from the time of estrogen (DeBold *et al.*, 1976). The concurrent injection of P with estrogen is most effective in inhibiting lordosis behavior (DeBold *et al.*, 1976). In rats, lordosis has been reported to be inhibited when P was injected up to 16 hr before estrogen, but not at 48 hr before (Blaustein and Wade, 1977). The present results, however, suggest that a strong inhibitory effect of P can be seen within 24 hr before estrogen treatment. Furthermore, when injected 27 to 40 hr before estrogen, P still suppressed lordosis, but the effect was less than the case of concurrent injection with estrogen. Inhibitory effect of P at 12 hr before estrogen in the fourth test was weak, compared to other time within 24 hr. Because of there being no difference of mean LQs of the control groups among 4 tests, this exception is not thought to be due to the accumulating effect of repeatedly-injected estrogen, or to the accumulated experience. It can not be determined whether this result is produced by the physiological effect of P or by animal and/or by the experimental conditions.

On the other hand, the inhibitory effect of P was observed when administered up to 24 hr after the injection of estrogen, but not 27 to 40 hr after estrogen in the present experiment. Similar results have been reported in hamsters (DeBold *et al.*, 1976). P injected 36 hr after estrogen has a facilitatory effect on lordosis (Zucker, 1967). These reports, together with present results, suggest that the shifting period from the inhibition to the facilitation by P of sexual receptivity is from 24 to 27 hr after estrogen in rats.

The inhibitory effect on female sexual receptivity of anti-estrogens is observed in ovariectomized rats. An estrogen receptor antagonist, CI-628, suppresses lordosis when treated concurrently with, or 24 hr before estrogen (Landau, 1977). CN-55, a non-steroidal anti-estrogen administered at the same time or 24 hr after estrogen was also effective in inhibiting lordosis behavior (Arai and Gorski, 1968). Thus, the effective period for decreasing the sexual receptivity of anti-estrogen seems to be similar to that of P. However, it is possible that the female sexual behavior inhibiting mechanisms are different between P and anti-estrogen, because the subsequent injection of a high dose of P cancels the inhibitory effect of a concurrent injection of P with estrogen, but not the inhibitory effect of CI-628 (Hansen and Södersten, 1979).

P has been reported to act directly on the central nervous system, because direct administrations of P into the ventromedial hypothalamus suppresses (Rubin and Barfield, 1984) or facilitates (Powers, 1972; Rubin and Barfield, 1983; Yanase and Gorski, 1976) lordosis behavior in female rats. The P-receptors were found in the forebrain and lower brainstem (Kato *et al.*, 1978; MacLusky and McEwen, 1978; Turcotte and Blaustein, 1993). Estrogen is known to increase its own receptors (Wise and Parsons, 1984) and P-receptors (Kato *et al.*, 1978; MacLusky and McEwen, 1978). An increase of the P-receptor mRNA in the nerve cells has been observed within 24 hr after the injection of estrogen (Romano *et al.*, 1989). In addition, antisense oligonucleotides to the P-receptor mRNA injected into the ventromedial hypothalamus suppresses lordosis in rats (Ogawa *et al.*, 1994). Furthermore, when P was injected concurrently with estrogen, the amount of P-receptors was less than that when treated with estrogen without P (Moguilewsky and Raynaud, 1979). From these reports, there is a possibility that P affects the receptor-producing mechanisms by estrogen in the nerve cells and inhibits or facilitates female sexual behavior in rats.

Further experiments are necessary to clarify the shifting mechanism from the the inhibition to facilitation of lordosis by progesterone during the period from 24 to 27 hr after estrogen injection.

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