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Male Stimulation of Luteinizing Hormone Surge, Progesterone Secretion and Ovulation in Spontaneously Persistent-Estrous, Aging Rats[1]

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ABSTRACT

In aging, persistently estrous (PE) female rats, there are no estrous cycles or cyclic increases in luteinizing hormone (LH) secretion, but the sexual receptivity to the male is consistently maintained. We recently reported that caging and mating with fertile males elicits an LH surge followed by ovulation in aging PE rats. The present study examined the relationship between the LH surge, the increase in progesterone (P) secretion and ovulation in PE females exposed to males, and assessed whether intromission was essential for the male-induced preovulatory LH surge.

PE rats were implanted with intra-atrial cannulae. Six to eight days later, these females were individually caged with a fertile male and repeatedly sampled (once every 30 or 60 min) between 1400 and 1900 h for assays of plasma LH and P. Sexual behavior of the female was recorded and correlated with the changes in plasma LH and P values. Similar experiments were also performed on cannulated PE rats with their vaginal orifice blocked with adhesive tape during the caging and sampling session. In both experiments, over 90% of the PE females displayed a high degree of lordosis response to mounting by the male, and over 60% of those sexually receptive PE females exhibited an LH surge followed by ovulation. The male-induced preovulatory LH surge occurred in PE females without actual intromission.

Caging with fertile males also elicited a marked increase in plasma P concentrations in PE rats and in PE females prevented from experiencing intromission. A prompt increase in P secretion occurred in all PE females exposed to males, although a significantly larger and sustained rise in plasma P was observed in those females that exhibited an LH surge followed by ovulation. These findings suggested that a specific male factor(s) can activate a neuroendocrine reflex in the PE female, promptly causing the adrenal gland to release P in large quantity. In PE rats that exhibit a preovulatory LH surge in response to the male, high concentrations of plasma LH presumably stimulate the ovaries to further increase P release from the preovulatory follicles. While increased plasma P levels may potentiate the LH surge and/or the ovulatory process, the initiation of the preovulatory LH surge in PE female rats is likely due to stimulation by a specific male factor(s).

INTRODUCTION

The most obvious symptoms of reproductive aging in the female rat are a cessation of estrous cyclicity and a decline in fertility (Ingram et al., 1958; Meites and Huang, 1976; Matt et al., 1986). Beginning at middle age, many multiparous rats become persistently estrous (PE), in which there are no cyclic increases in luteinizing hormone (LH) secretion or spontaneous ovulation (Huang et al., 1978; Lu et al., 1979), despite continued follicular development in the ovary (LaPolt et al., 1985). During this chronic anovulatory state, the ovaries of PE rats typically secrete substantial amounts of estrogen (Lu et al., 1979, 1981) and contain large developing follicles, but are devoid of corpora lutea (Meites and Huang, 1976; Lu et al., 1979). Thus, PE rats exhibit moderately elevated circulating estrogen and low progesterone (P) concentrations persistently (Lu and Kledzik, 1981).

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In spontaneously PE aging rats, the neuroendocrine mechanism for producing an LH surge is unresponsive to the stimulatory feedback action of estrogen (Matt et al., 1988). Previously, we have demonstrated that administration of estrogen followed by P is unable to increase LH release in PE rats under acute ovariectomy conditions, but a similar steroid treatment elicits an LH surge in long-term ovariectomized PE females (Lu et al., 1981). Taken together, these observations suggest that the sustained, intermediate level of circulating estrogen present in PE rats is inhibitory to LH secretion, which may account for the lack of cyclic increases in LH secretion in these aging females (Huang et al., 1978; Lu et al., 1979; Matt et al., 1988).

In the presence of sustained, intermediate levels of circulating estrogen, PE females are consistently sexually receptive to males (Cooper and Linnoila, 1977; Hendricks et al., 1979). Earlier reports also indicate that tubal ova are often found in PE females following caging with males, although no fertile gestations have occurred in these mated PE rats (Meites et al., 1978; Borchardt et al., 1980). Most recently, we have reported that caging and mating with fertile males elicits a prompt LH surge in PE rats (Matt et al., 1988). After mating, however, PE females ovulate few oocytes, and even fewer of these ova develop into normal blastocysts for implantation (Matt et al., 1988). Inasmuch as P has been implicated in facilitating the preovulatory LH surge (Mann and Barraclough, 1973) and the ovulatory process (Takahashi et al., 1974) during the estrous cycle, the present study examined the patterns of both LH and P secretion in PE rats during caging with fertile males, and assessed whether intromission is essential for the male-induced preovulatory LH surge.

MATERIALS AND METHODS

Animals

Multiparous Long-Evans rats (Charles River Lab., Portage, MI) were received at about 8 mo of age, and housed in groups of 5 per cage under standard vivarium conditions. Food and drinking water were available ad libitum, and the rats were maintained in a controlled environment of 25 ± 1°C and 14L:10D (lights on from 0500 h to 1900 h daily) throughout the study. From these females, estrous cycle patterns were determined on the basis of their daily vaginal smears, and the rats were identified as PE if they had exhibited vaginal cytology with cornified epithelial cells for at least 15 consecutive days. For the following experiments, 11- to 12-mo-old PE female rats and 4- to 7-mo-old fertile males were used.

Experimental Procedures

To examine the characteristics of the male-induced LH surge in PE rats, serial blood samples were obtained from the females while they were caged with fertile males. PE rats (n=11) were implanted with intraatrial cannulae while they were under ether anesthesia, according to a previously described technique (Harms and Ojeda, 1974). Since cannulation often disrupts the PE pattern of vaginal smears, cannulated PE rats were allowed to recover prior to the experiment. After 6–8 consecutive days of persistent vaginal cornification, cannulated PE females were ready to be paired with fertile males. On the day of experiment, a male rat was placed into an oval-shaped arena (43.2 cm wide × 61.0 cm long × 61.0 cm high; constructed with transparent vinyl plates) at 1330 h and allowed to acclimate to the arena. At 1400 h, a blood sample (0.4 ml) was taken from the PE rat via the cannula immediately before the female was placed into the same arena. Beginning at 1400 h and continuing to 1900 h, the young male and the PE female were caged together, and the female was briefly removed from the arena for serial blood sampling. Mating behavior, reflected by the lordosis quotient (LQ = number of lordosis postures by the female ÷ number of mounts by the male) was also recorded for 30 min following the first mount by the male. Blood samples (0.4 ml each) were taken once every 30 min for the first 2 h and once every 60 min thereafter. Between samplings, the cannula was flushed with heparinized saline (20 units heparin/ml) in order to maintain its patency. Each blood sample was drawn into a heparinized syringe and centrifuged immediately. Once the plasma had been removed, the blood cells collected from two consecutive samples were pooled and resuspended in sterile saline for subsequent infusion back into the same animal. This regimen of blood cell infusion after every second sample produced no obvious ill effects in these animals. The plasma samples were stored at −20°C until the time of assay, and these samples were used to assess the effects of caging and mating with males on LH and P release.

The next morning after testing with the male, mating was confirmed by the presence of sperm in the vaginal lavage. Mated PE females were killed

by cervical dislocation to check for ovulation. Both oviducts were dissected with the aid of a stereoscopic microscope, and the contents were flushed with Krebs-Ringer saline (pH 7.4; buffered with 20 mM 4-(2-hydroxyethyl)-1-piperazineethane sulfonic acid [HEPES]) into a depression microscopic slide. The total number of ova recovered from each female was recorded and considered as the ovulation rate.

To test whether increases in hormone release and/or ovulation could be induced in PE females caged with males in the absence of intromission, it was necessary to occlude the vaginal orifice so that mounting and all other forms of sexual behavior were possible but that intromission was prevented. PE rats (n=8) were cannulated and allowed to recover as described above. On the day of experiment, the PE female was paired with the fertile male in the arena and repeatedly sampled during 1400–1900 h as described in the first experiment. Immediately after the first blood sample at 1400 h, the vaginal orifice was blocked with adhesive tape, and sexual behavior was recorded during the caging and sampling session. The blood samples were processed as above, and used to reveal the effects of caging with males on LH and P release. On the next morning a vaginal lavage was examined, and the oviducts were flushed to check for ovulation as above.

Hormone Assays and Data Analyses

Plasma concentrations of LH were measured by a double-antibody radioimmunoassay (RIA), as previously described (Lu et al., 1980). This RIA system employed the reagents provided by the National Institute of Diabetes, Digestive and Kidney Diseases and the National Hormone and Pituitary Program, and the LH values are expressed in terms of the reference standard NIDDK rat LH-RP-1. Concentrations of P were measured by specific RIA, as previously described (Lu and Judd, 1982). Prior to the RIA, each sample was extracted with diethyl ether, and the P fraction was separated from other steroids through Celite column chromatography as described by Brenner et al. (1973).

Statistical analyses of plasma concentrations of P over time and between groups were performed by two-way analysis of variance with repeated measures (Winer, 1971). When appropriate, comparisons of means between groups were made by Duncan's multiple-range test, and a confidence level of $p<0.05$ was considered statistically significant.

RESULTS

Effects of Caging and Mating with Fertile Males on LH Release and Ovulation in PE Females

PE female rats caged with fertile males displayed a high degree of lordosis (LQ range: 0.71–1.00) in response to mounting by the male (Table 1). In only one of 11 attempts was there no mounting by the male. The data in Figure 1 depict the pattern of plasma LH levels in PE females during the mating sessions. The top panel of Figure 1 shows the mean ± SEM plasma LH concentrations from 5 females that ovulated after vigorous mating with the male. In these PE rats, a large and sustained increase in plasma LH occurred about 1 h after the caging session had begun, and the peak LH values (1247 ± 189 ng/ml) were found around 1600 h. The male-induced LH surge resulted in ovulation, since tubal ova (8.2 ± 2.0 ova/rat) were found in these females the next morning. Ovulation also occurred in another PE rat (with only one ovum) which exhibited no discernible rise in

TABLE 1. Lordosis quotients and ovulatory responses from persistent-estrous (PE) female rats mated and/or caged with fertile males.

	Ovulating females				Nonovulating females	
Groups	# Rats	Lordosis quotient[a]	Ovulation rate[b]	Incidence of ovulation	# Rats	Lordosis quotient[a]
Control PE females, n = 10	6	1.00 ± 0.00	8.2 ± 2.0	60%	4	0.90 ± 0.07
Females prevented from experiencing intromission, n = 8*	5	0.98 ± 0.01	7.8 ± 1.7	63%	3	1.00 ± 0.00

[a]Lordosis quotient = number of lordosis responses by the female divided by the number of mounts by the male.

[b]Ovulation rate = number of tubal ova recovered.

*The vaginal orifice of the female was blocked with adhesive tape.

plasma LH during the sampling period. The bottom panel of Figure 1 shows the pattern of plasma LH levels in 4 other PE females that displayed active mating behavior, but no ovulation resulted. In this latter group of rats, each female exhibited a transient and small increase in plasma LH at some point after caging had begun, but this increase was followed by a rapid decline. Thus, a lack of ovulation in these 4 PE females was associated with no discernible rise in plasma LH during the sampling period. Perhaps these PE females did not receive adequate male stimulation, or their ability to release LH in response to the male stimulation was diminished. It should be emphasized that there was no difference in the amount of lordosis behavior (Table 1) displayed by those PE females that ovulated (LQ = 1.00 ± 0.00), compared to those that did not (LQ = 0.90 ± 0.07).

Effects of Caging with Fertile Males without Mating on LH Release and Ovulation in PE Females

PE female rats whose vaginal orifice was blocked with adhesive tape also displayed a high degree of lordosis (LQ range: 0.94–1.00) while they were caged with males (Table 1). The data in Figure 2 illustrate the pattern of plasma LH levels in vagina-blocked PE females during the caging sessions. The top panel of Figure 2 shows the mean ± SEM plasma LH concentrations from 5 females that ovulated after mounting by the male without intromission. In these

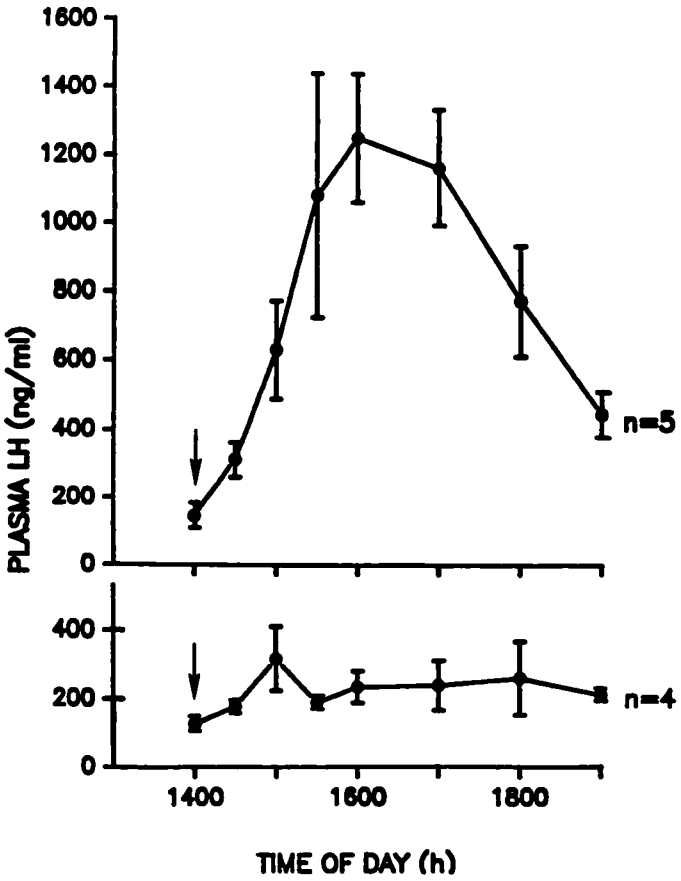

FIG. 1. Effect of caging and mating with fertile males on luteinizing hormone (LH) release in persistent-estrous (PE) female rats. Beginning at 1400 h, each PE female was placed into a transparent arena with a young fertile male (designated by ↓), and serial blood samples were taken via intra-atrial cannulae. The *upper panel* depicts the pattern of plasma LH concentrations (mean ± SEM) in 5 PE females that exhibited an LH surge during the caging/mating session, followed by ovulation. The *lower panel* shows the plasma LH levels in 4 other PE rats that did not exhibit an LH surge or ovulation. Both groups of PE females displayed a high degree of lordosis response to the male, and there was no difference in the lordosis quotient between the two groups.

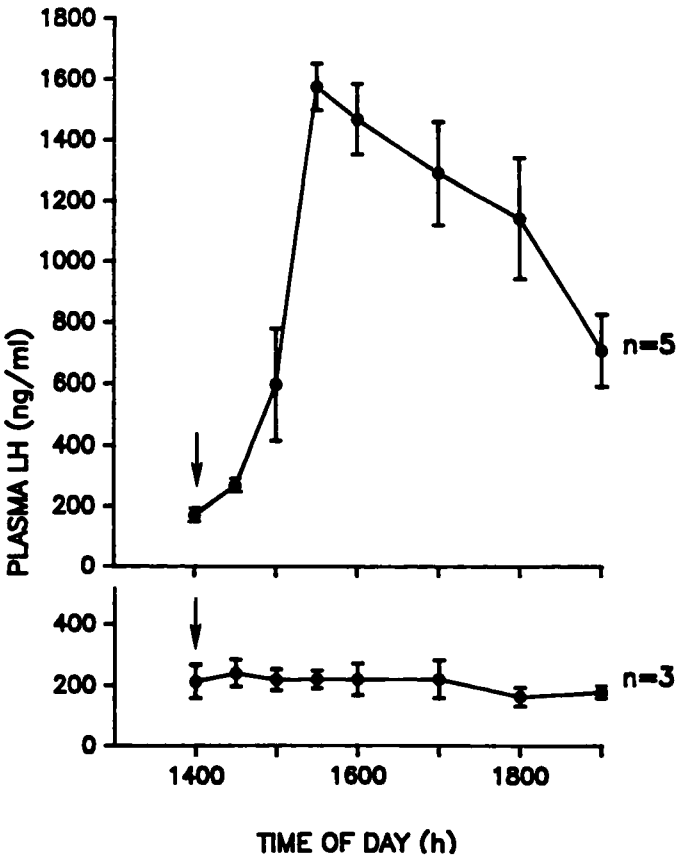

FIG. 2. Effect of caging with fertile males on luteinizing hormone (LH) release in persistent-estrous (PE) female rats that were prevented from experiencing intromission. The vaginal orifice of each PE female was blocked with adhesive tape during the experiment. Beginning at 1400 h, each female was placed into a transparent arena with a young fertile male (designated by ↓), and serial blood samples were taken via intra-atrial cannulae. The *upper panel* depicts the pattern of plasma LH concentrations (mean ± SEM) in 5 PE females that exhibited an LH surge during the caging session, followed by ovulation. The *lower panel* shows the plasma LH levels in 3 other PE rats that did not exhibit an LH surge or ovulation. Both groups of PE female rats displayed a high degree of lordosis response to the male, and there was no difference in the lordosis quotient between the two groups.

PE rats, a large and sustained increase in plasma LH occurred about 1 h after the caging session had begun, and the peak LH values (1573 ± 76 ng/ml) were found around 1530 h. The male-induced LH surge resulted in ovulation, since tubal ova (7.8 ± 1.7 ova/rat) were found in these females the next morning. The bottom panel of Figure 2 shows the pattern of plasma LH levels in 3 other PE females that exhibited no ovulation after being caged with the male. It is evident that caging with males without mating induced neither an LH surge nor ovulation in 3 of 8 PE females tested. Again, there was no difference in the amount of lordosis behavior (Table 1) displayed by those PE rats that ovulated (LQ = 0.98 ± 0.01), compared to those that did not (LQ = 1.00 ± 0.00). Close observation of the male behavior during the caging session indicates that no intromissions occurred in any of these 8 PE females reported. Also, no sperm were found in the vaginal smears of these PE rats.

Correlation between the LH Surge, Progesterone (P) Secretion and Ovulation in PE Females Caged/Mated with the Male

To reveal the relationship between the male-induced LH surge, increases in P secretion, and ovulation in PE rats, the same plasma samples assayed for LH values were also assayed for P. The data in Figure 3 illustrate the correlation of plasma LH and P levels in PE female rats paired with males. The upper panel of Figure 3 depicts the mean ± SEM plasma LH values in these females. Eight PE rats displayed a distinct pattern of LH surges during caging with the male, followed by ovulation. In contrast, 6 other PE females showed no discernible rise in plasma LH and did not ovulate. The lower panel of Figure 3 shows the difference in the patterns of plasma P between the ovulated and nonovulated PE rats. It can be seen that a prompt and marked increase in the plasma P concentration occurred in both groups of female rats within the first 30 min after being exposed to the male. However, a significantly ($p<0.05$) larger and sustained increase in P secretion was observed in those female rats that exhibited an LH surge and ovulation, compared to those that did not. It should be noted that, in those females that ovulated after being exposed to males, the initial marked increase in P secretion preceded the onset of the LH surge. Also, a consistent rise in plasma P was observed in PE females that did not exhibit a discernible increase in LH release after being exposed to males.

DISCUSSION

In previous studies, we have demonstrated that the neuroendocrine mechanism for producing the LH surge remains intact in aging PE female rats, but the

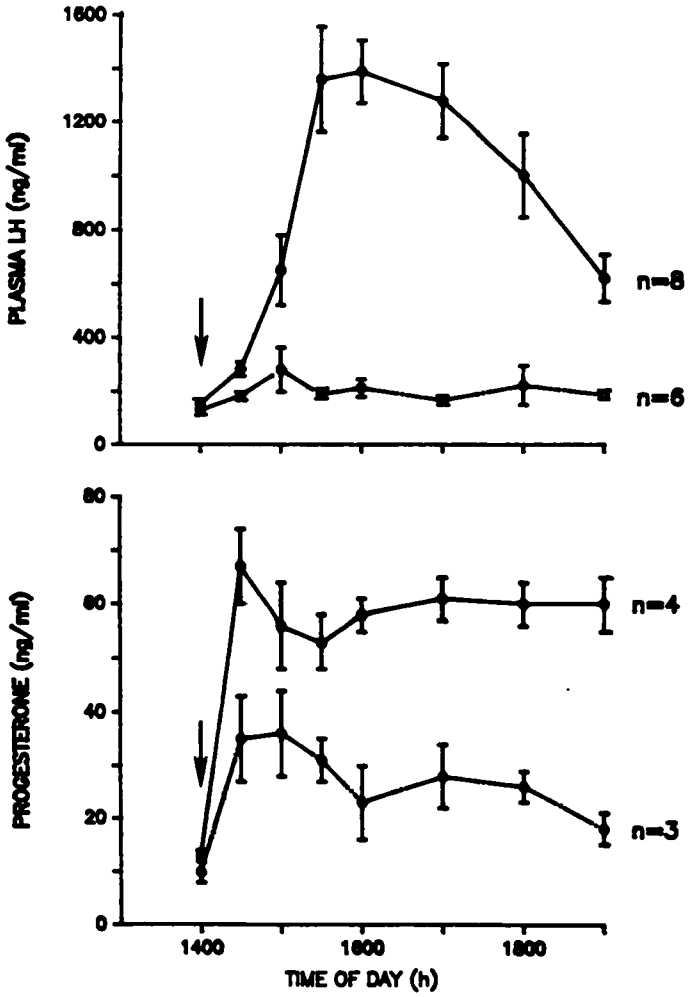

FIG. 3. A comparison of plasma luteinizing hormone (LH) and progesterone (P) concentrations from persistent-estrous (PE) female rats during caging with fertile males. Beginning at 1400 h, each PE female was placed into a transparent arena with a young fertile male (designated by ↓). The *upper panel* depicts the patterns of plasma LH levels (mean ± SEM) in 8 PE females that ovulated and in 6 rats that did not ovulate after being exposed to the male. Of the 8 PE female rats that exhibited an LH surge, 4 females were mated while the other 4 were mounted but not mated. The *lower panel* displays the patterns of plasma P in these same PE rats with their plasma samples measured for LH. From 8 PE females that exhibited an LH surge followed by ovulation, plasma samples were pooled to provide 4 sets of specimens for P RIA, and samples from 6 nonovulating PE rats were pooled to yield 3 sets of specimens for P measurement. Please note that a prompt and marked increase in plasma P levels occurred in all PE females after being exposed to the male, although a significantly ($p<0.05$) larger and sustained rise in plasma P was observed in those females that exhibited an LH surge and ovulation, compared to those that did not.

LH surge mechanism is unresponsive to the stimulatory action of estrogen (Lu et al., 1981; Matt et al., 1988). When PE females are exposed to fertile males, they often exhibit an LH surge followed by ovulation (Matt et al., 1988). The results from the present study confirm these findings, and further demonstrate that the male-induced preovulatory LH surge in PE rats is not dependent on intromission for expression. The latter finding is not unexpected, since a previous report also showed that exposure to male rat urine alone results in ovulation in young female rats rendered PE by exposure to constant light (Johns et al., 1978). The present observation that a preovulatory LH surge can be elicited in aging PE rats by male stimulation explains how contact with fertile males frequently disrupts the PE pattern of vaginal smears or induces pseudopregnancy (Everett, 1964; Meites et al., 1978; Borchardt et al., 1980).

From this study, 5 of 10 PE female rats did not exhibit an LH surge during the caging and mating sessions. Likewise, 3 of 8 females with their vaginal orifice blocked also did not show an increase in LH release during caging with fertile males. Observations of these animals' behavior reveal that all groups of PE rats displayed a high degree of lordosis in response to mounting by the male, and the LQ of these females was the same regardless of whether they exhibited an LH surge. Thus, the lack of a preovulatory LH surge in some of these females after being exposed to males was not due to a lower LQ. Perhaps, in these PE females, there was a functional defect in the LH surge mechanism, while they maintained a normal sexual receptivity. This view seems to be consonant with earlier reports that sexual receptivity in senescent PE female rats is highly comparable to that observed in younger animals (Cooper and Linnoila, 1977; Peng et al., 1977; Hendricks et al., 1979), and that the aging-associated dysfunction of gonadotropin secretion occurs earlier than any aging-related decline of behavioral estrus (Peng et al., 1977; Matt et al., 1988). It is also possible that, in those PE females that exhibit no LH surge, the intensity of the specific stimulus exerted by the male might not have been sufficient to evoke the neuroendocrine reflex leading to an LH surge, despite a high degree of lordosis response. Finally, a review of individual animal's reproductive history does suggest that female rats with a longer history of PE were less likely to exhibit a male-induced preovulatory LH surge, suggesting that extended periods of PE resulting from elevated circulating estrogen may cause a further deterioration of the LH surge mechanism (Lu et al., 1981). This statement requires further studies for confirmation.

The effects of mating on LH release, ovulation, and fertility have been examined in young female rats rendered anovulatory and PE by neonatal androgenization or exposure to constant light. These young rats with experimentally induced PE also display persistent vaginal cornification similar to that of aging, spontaneously PE rats. In androgenized PE rats, mating has been shown to produce irregular estrous cycles, or result in pregnancy (Ericsson and Baker, 1966; Gerall et al., 1980). In rats with constant light-induced PE, Brown-Grant et al. (1973) reported that intromission is the most effective means of inducing ovulation, while mounting alone or nonsexual stress is much less likely to cause ovulation. Davidson et al. (1973) confirmed that nonsexual stimuli can produce ovulation much less frequently than coitus in rats with constant light-induced PE, and demonstrated that nonsexual stimuli do not result in a dramatic increase in LH release. Those findings from previous studies are different from our present observation that the male-induced preovulatory LH surge can occur in aging, spontaneously PE rats without actual intromission. Perhaps, the responsiveness of the LH surge apparatus to male stimulation is not the same between aging, spontaneously PE rats and females with constant light-induced PE. In spontaneously PE rats, mounting by the male alone resulted in the same frequency of ovulation as actual mating. From the previous observations (Brown-Grant et al., 1973; Davidson et al., 1973; Johns et al., 1978) and the present finding, it appears that behavioral, copulatory, and olfactory cues may all be involved in evoking the neuroendocrine reflex responsible for a preovulatory LH surge in PE female rats.

The results from this study reveal that caging with fertile males also elicited a prompt and large increase in circulating P levels in PE rats, and that this rapid increase in plasma P preceded a discernible rise in circulating LH values. Furthermore, such a pronounced increase in plasma P occurred in PE females during the caging session whether the rats exhibited an LH surge or not, although a significantly larger and sustained increase in P secretion was observed in PE females that displayed an LH surge. Interestingly, mounting by the male without actual intromission also evoked a marked increase in P secretion in PE rats. It is well established that the adrenal glands of

the rat are capable of secreting substantial amounts of P, and that stress usually evokes a prompt increase in adrenal release of P (Nequin and Schwartz, 1971). In aging female PE rats, circulating concentrations of P remain relatively low in the absence of functioning corpora lutea associated with a chronic anovulation, while the ovaries show an increased capacity to bind LH (Lu et al., 1979; Lu and Kledzik, 1981). Our present results demonstrate that a significantly greater and sustained increase in P secretion was observed in PE female rats that exhibited an LH surge after being exposed to males. These findings suggest that a specific male factor(s) can activate a neuroendocrine reflex in the PE female, promptly causing the adrenal gland to release P in large quantity. Moreover, in PE rats that exhibited an LH surge after male stimulation, the high concentrations of plasma LH presumably stimulated the ovaries to further produce large amounts of P from the preovulatory follicles. This increase in ovarian P secretion mediated by an LH action perhaps explains the marked difference in plasma P levels between PE females that exhibited an LH surge and those that did not.

Since a pronounced increase in plasma P concentrations occurred in PE rats regardless whether or not the female displayed an LH surge, it can be argued that the initiation of the male-induced preovulatory LH surge was not due to a P-mediated stimulatory action. As early as 1940, Everett reported that a single s.c. injection of P disrupts the persistent vaginal estrus in aging PE rats (Everett, 1940). However, subsequent treatment with P is necessary to induce ovulation and estrous cycles in those animals (Everett, 1940; Huang et al., 1976). It is thought that P administration may interrupt the PE state by decreasing ovarian estrogen secretion (Fortune and Vincent, 1983), but the precise mechanism by which repeated P treatments reinitiate ovulation and estrous cycles in PE rats is not known. On the other hand, it is well recognized that P often exerts a facilitory action to enhance LH secretion under estrogen-primed conditions (Caligaris et al., 1968). Thus, the sustained high levels of plasma LH in PE females after their being exposed to males may be in part due to a facilitory effect produced by the large increase in circulating P. Further experiments are required to assess the functional dynamics between the male-induced LH surge and the increase in P secretion in PE female rats.

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