PROCEEDINGS OF THE SOCIETY FOR EXPERIMENTAL BIOLOGY AND MEDICINE 179, 173–179 (1985)

Differences in the Response Pattern of Aged Female Rats to Treatment with Lergotrile Mesylate (42081)

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Abstract. Old female rats exhibiting either the constant vaginal estrous (CE) or the prolonged diestrous (PD) state were given lergotrile mesylate (LM). Treatment with 4.5 mg/kg daily significantly reduced prolactin (PRL) levels in both groups. The basal luteinizing hormone (LH) levels were not altered. In the CE group, LM treatment produced a single prolonged cycle, with the reemergence of the CE state. However, in the PD group, the rats exhibited several regular cycles during the treatment period. Several of these rats also showed evidence of having an LH surge during the reinduced cycle. These results suggest that high PRL levels may be a causative factor with respect to acyclicity in the PD rats, but PRL does not seem to be a major contributor to acyclicity in the aged CE rat.

Studies of female rats have shown that as age increases, the incidence of normal 4- or 5-day ovarian cycles decreases and the animals enter noncyclic reproductive states. These aged rats show a high incidence of the constant vaginal estrous (CE) or the prolonged diestrous (PD) state. In the CE state, the animals show persistent vaginal cornification with the presence of large ovarian follicles (1). Ovulatory failure in these animals is evident by the lack of corpora lutea (2) and the absence of cyclic LH surges (3). In the PD rat, 8–20 days of leukocytic smears are seen with the ovaries containing large numbers of corpora lutea (4). These diestrous periods are interrupted with the occurrence of ovulatory cycles (3).

Hormonal analysis indicates that the CE rats exhibit increased estradiol levels while the PD rats have elevated levels of estradiol and progesterone (5–7). Elevated PRL levels are seen in both groups of aged rats (8, 9). Luteinizing hormone levels are equivocal in that they have been reported to be higher than (10), lower than (11), or the same as in young cycling rats (12).

The differences in vaginal cytology, ovarian structure, and hormonal profile suggest that hypothalamic–pituitary–ovarian function may be quite different in the two states of aging. Since both CE and PD rats exhibit hyperprolactinemia, it is thought that this may contribute to the disruption of the cycle. Reports suggest that elevated levels of serum PRL may be a contributing factor responsible for the acyclic states. Some degree of cyclicity has been induced in CE rats with L-Dopa (13–15). Lergotrile mesylate (LM) and 2-bromo-α-ergocryptine (CB-154), dopamine agonists, have also been shown to reinitiate cycles in aged PD rats (15–17).

In this study, we attempted to assess the relative contribution of hyperprolactinemia to the maintenance of the CE and PD states. The experiments were designed to evaluate the effects of LM treatment on the vaginal smear pattern in both CE and PD rats, and its additional effects upon the LH surge and upon basal LH and PRL levels.

Materials and Methods. Sprague–Dawley rats (Simonson Company, Gilroy, Calif.) were housed individually in air-conditioned quarters (24 ± 2°C). The rats were maintained on a 14:10 light:dark cycle (lights on 0500 hr). All animals were given food (Wayne Lab Blox) and tap water *ad libitum*. After each experiment, the sella turcica of the aged rats was carefully examined for the possible presence of a pituitary tumor. None of the rats used in the study showed any evidence of having a pituitary tumor.

Aged rats exhibiting a PD condition or exhibiting a CE condition were given daily

[1] To whom reprint requests should be addressed.

0037-9727/85 $1.50

subcutaneous injections of LM (4.5 mg/kg body wt) or an equal volume of 0.9% saline. We have shown that this dose of LM suppresses PRL secretion for up to 48 hr (Wiggins and Ratner, unpublished observation). A criterion for the PD state was the appearance of a diestrous smear for at least 7 consecutive days; for the CE state, it was the appearance of an estrous smear for at least 20 consecutive days. When these criteria were met, injections were initiated and continued for 3 weeks. During the treatment period, the following parameters were measured.

(1) Reinitiation of cyclicity. A daily record of vaginal smear patterns was maintained on all rats which received LM or saline treatment. The occurrence of cyclicity, the duration of a reinduced cycle, and the number of days taken to reinduce a cycle were recorded.

(2) Serum levels of PRL and LH. Blood was collected by orbital sinus puncture (OSP) from animals just before and on the morning of diestrus at 7- to 9-day intervals after LM injections were begun. Blood was drawn by OSP, with the rat rendered unconscious by ether. A pasteur pipet was inserted medially to the orbital sinus and gently pressed downward with a twisting motion. The pipet was of sufficient size so that no anticoagulant was needed. While this collecting procedure is known to induce a stress-related increase in PRL values (18), it was the only suitable method available that would allow us to collect samples over a 3- to 4-week period. Preliminary experiments had also shown that LM could substantially reduce the effect of OSP on PRL release (Wiggins and Ratner, unpublished observation). In addition, in order to get basal levels of PRL, some animals in each group were decapitated before treatment was begun and blood was collected for assay. All blood samples were allowed to clot overnight at 4°C and centrifuged at 2200*g* for 20 min. The serum was withdrawn and stored at −20°C until the appropriate assays were performed.

(3) Existence of an LH surge. After the reinitiation of cycles following LM treatment, blood was collected by OSP in the morning (0900–1000 hr) and in the evening (1800–1900 hr) on the day of proestrus in rats exhibiting 4-day cycles.

Serum PRL and LH were measured by radioimmunoassay with reagents provided by the Hormone Distribution Program of the NIAMDD. Prolactin and LH were expressed in nanograms per milliliter of serum based on NIAMDD rat PRL-RP-1 and NIAMDD rat LH-RP-1, respectively, as reference standards.

The data were analyzed using the Student–Newman–Keuls procedure with analysis of variance. A P value of less than 0.05 was considered significant.

Results. *(1) Reinitiation of cyclicity.* Table I shows the effects of LM injections on the reinitiation of vaginal cyclicity in PD rats of different ages (14–16 months, 19–20 months, 24–25 months) and aged CE rats (17–20 months). All rats exhibiting the PD state resumed vaginal cyclicity shortly after the LM injections were begun. There was some variation in the time taken to reinduce the cycle, the youngest group responding most rapidly. The cycle length varied slightly for each group of PD rats, ranging from 5.3 $\pm$ 0.3 days for the 14- to 16-month group to 5.9 $\pm$ 0.5 days for the 24- to 25-month group. After injections were discontinued, most rats completed the current cycle and then reentered the PD state. However, a few animals showed an additional cycle before the PD state reoccurred. In contrast, the treated CE rats did not resume normal vaginal cyclicity, but all showed a deviation from the CE state shortly after injections were begun. This deviation consisted of 2 or 3 days of leukocytic smears and then the reemergence of the CE state. Even though injections were continued for 3 weeks, they remained in the CE state. When a similar dose of LM was injected into young cycling rats over a 3-week period, no change in the cycle was seen (Wiggins and Ratner, unpublished observation).

The youngest group of PD rats treated with saline also showed a deviation from the PD state starting 8.0 $\pm$ 0.6 days after the injections were begun. This deviation consisted of 1 or 2 days of cornified cells. Upon continuing saline treatment, the PD state reemerged. Similar findings were seen in the 19- to 20-month PD rats given saline except that a deviation from the PD state started 13.5 $\pm$ 6.7 days after saline injections were

TABLE I. EFFECT OF LERGOTRILE MESYLATE (LM) ON THE REINITIATION OF CYCLES IN AGED PROLONGED DIESTROUS (PD) AND CONSTANT ESTROUS (CE) RATS[a]

Type of rat	Treatment	Days to induce deviation from CE or PD state	Duration of induced cycle in days	Comments
PD 14–16 month (n = 8)	Saline	8.0 ± 0.6[b]	No cycle	All rats showed deviation PD state; PD state reemerged during treatment
PD 14–16 month (n = 13)	LM	2.7 ± 0.3	5.3 ± 0.3	The cycle was maintained during treatment
PD 19–20 month (n = 8)	Saline	13.5 ± 6.7	No cycle	All but one rat showed deviation from PD state; PD state reemerged during treatment
PD 19–20 month (n = 10)	LM	4.1 ± 0.4	5.5 ± 0.2	The cycle was maintained during treatment
PD 24–25 month (n = 8)	Saline	No deviation	No cycle	No rats showed deviations from PD state
PD 24–25 month (n = 8)	LM	3.8 ± 0.4	5.9 ± 0.5	The cycle was maintained during treatment
CE 17–20 month (n = 8)	Saline	No deviation		No rats showed deviations from CE state
CE 17–20 month (n = 12)	LM	2.3 ± 0.2	No cycle	All rats showed deviation from CE state; CE state reemerged during treatment

[a] Rats were injected daily with LM (4.5 mg/kg) or saline.
[b] Values are expressed as means ± SEM.

begun. The oldest group of PD rats treated with saline showed continuous leukocytic smears. None of the saline-treated CE rats showed a deviation from the CE state.

(2) Serum levels of PRL and LH. In Fig. 1, the effect of LM treatment on PRL and LH levels in 17- to 20-mo old CE rats is shown. The PRL level in blood collected by decapitation from CE rats before injection was 35.87 ± 11.4 ng/ml. The PRL level in serum obtained by OSP was much higher (188.0 ± 37.0 ng/ml). This increase is the result of the stress of the OSP collection procedure. Treatment with LM was able to significantly depress PRL levels ($P < 0.001$). During the treatment period, PRL values were below those found in samples taken by decapitation. LH levels were not affected. No changes in PRL or LH levels occurred in rats injected with saline.

Fig. 2 shows PRL and LH levels before and during LM treatment in PD rats of different ages. In both PD groups, serum PRL levels were significantly lowered from initial values measured by OSP ($P < 0.001$ and $P < 0.005$, respectively), while serum LH levels did not significantly change. In the 14- to 16-month group, a serum sample collected by decapitation was measured before LM treatment; this value (33.16 ± 7.0 ng/ml) is significantly lower than that found in serum obtained by OSP under the same conditions ($P < 0.02$). A preinjection level of serum PRL was also obtained by decapitation in the 24- to 25-month group. In this instance a value of 86.7 ± 7.1 ng/ml was

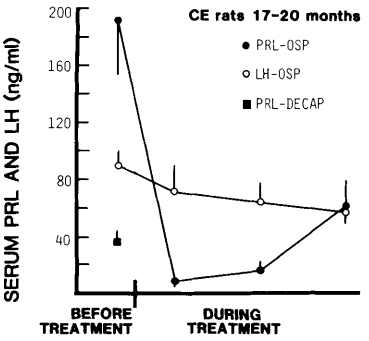

FIG. 1. Serum levels of prolactin (PRL) and luteinizing hormone (LH) before and during treatment with lergotrile mesylate in aged constant estrous (CE) rats (17–20 months of age). Blood was collected by orbital sinus puncture (OSP) just before and on the morning of diestrus at 7- to 9-day intervals after LM injections were begun. Additional samples were also collected from untreated rats by decapitation (DECAP). Values are expressed as means ± SEM of each group.

obtained, which was not different from the preinjection value of 84.5 ± 16.8 ng/ml obtained by OSP. In both PD groups, the basal PRL levels from decapitated rats were significantly higher than found in decapitated young cycling rats on the day of diestrus. After LM treatment PRL values in all groups of PD rats dropped below those from untreated decapitated rats. Saline injections produced no significant changes in serum PRL or LH throughout the treatment interval in any of the groups studied.

(3) Existence of an LH surge. Table II shows the levels of LH on the morning (0900–1000 hr) and evening (1800–1900 hr) of proestrus in both the CE and PD rats which received LM treatment. Only 1 of 5 treated CE rats showed an LH surge. However, 5 of 6 14- to 16-month PD rats showed an LH surge, the mean value being 4382 ± 1202 ng/ml and 5 of 10 19- to 20-month PD rats showed an LH surge, the mean value being 6485 ± 1577 ng/ml. In both groups of PD rats, the mean surge value was greater than that seen in the young cycling rats (1542 ± 277 ng/ml).

Discussion. In these studies we have shown that aged PD rats responded differently to LM when compared to CE rats. While LM induced resumption of vaginal cyclicity in PD rats at all ages, CE rats showed only a slight deviation in the cycle with reemergence into the CE state. Even though cyclicity was not induced in the CE rats, LM treatment did suppress PRL levels below those found in samples from untreated decapitated rats, suggesting that high PRL levels are not the causative factor with respect to the acyclicity in these rats.

While LM treatment did not maintain normal cyclicity in CE rats, a clear deviation in the usual estrous smear pattern was seen shortly after the LM treatment was begun. This deviation consisted of 2 or 3 days of leukocytic smears and infrequently a characteristic proestrous smear pattern was seen. The characteristic estrous smear pattern reemerged after this short period and, thereafter, the CE state was maintained even though LM treatment continued. Everett (17) reported results similar to these using a different stimulus. He found a leukocytic smear pattern 2 days after the administration of an ovulatory dose of LH to CE rats. Most of these rats resumed short cycles, some became

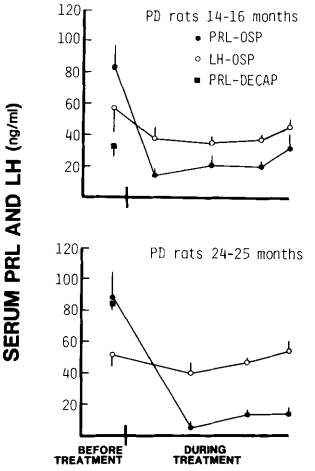

FIG. 2. Serum levels of prolactin (PRL) and luteinizing hormone (LH) before and during treatment with lergotrile mesylate in aged prolonged diestrous (PD) rats of different ages. Blood was collected by orbital sinus puncture (OSP) just before and on the morning of diestrus at 7- to 9-day intervals after LM injections were begun. Additional samples were also collected from untreated rats by decapitation (DECAP). Values are expressed as means ± SEM of each group.

TABLE II. EFFECT OF LERGOTRILE MESYLATE (LM) ON THE REINITIATION OF A LH SURGE PATTERN IN AGED PROLONGED DIESTROUS (PD) AND CONSTANT ESTROUS (CE) RATS[a]

Type of rat	Treatment	Total no. of rats	No. of rats showing an LH surge	LH (ng/ml)[b]	
				0900 hr	1800 hr
CE 17–20 month	LM	5	1	85	700
PD 14–16 month	LM	6	5	58.8 ± 8.6	4382 ± 1202[c]
PD 19–20 month	LM	9	5	44.6 ± 6.0	6485 ± 1577[c]
Young cycling 4–5 month	Saline	5	5	69.5 ± 8.7	1542 ± 277[c]

[a] Rats were injected daily with LM (4.5 mg/kg or saline).

[b] LH was measured in the morning (0900 hr) and evening (1800 hr) of proestrus. Blood was collected by orbital sinus puncture. Values are expressed as means ± LM. Values represent only the rats which showed an LH surge.

[c] Indicates statistical significance of $P < 0.001$ by the Student-Newman-Keuls test when compared to 0900 samples.

pseudopregnant, and in others, the CE state reemerged after a short period of diestrus. He postulated that these events were determined by the serum PRL levels that were present after LH treatment. He tested this hypothesis by giving injections of LM during the first diestrus after the ovulatory dose of LH and found that the CE state reemerged in 81% of the rats. He felt that the reemergence of the CE state occurred because the very low PRL levels produced by the LM injection were unable to maintain the luteal progesterone secretion in order to promote another LH surge. However, progesterone levels were not measured by Everett nor in our studies.

While LM treatment lowered PRL levels equally well in both CE and PD rats, it did not have any significant effect on basal LH levels in either group. However, a number of PD rats which showed a reinitiation of cyclicity did show an LH surge pattern on the day of proestrus. The surge amplitude was considerably greater in the LM-treated PD rats than was seen in young cycling rats. This enhanced pattern could possibly be the result of an increased sensitivity to LM in the anovulatory PD rat. Whether this surge pattern is dependent upon the decrease in PRL levels has yet to be shown. We have also looked for additional evidence of ovulation in both CE and PD rats after LM treatment. Interestingly, we have not found the presence of ova in the oviduct on the morning following LH surges. However, when the ovaries of these rats were examined, they were hypertrophied and contained an increased number of corpora lutea (Wiggins and Ratner, unpublished observation). Thus, we have evidence that luteinization occurs, but we have no direct evidence that ovulation has occurred during the reinduced cycles. Whether this is as a result of an ovarian deficit remains to be determined. The formation of corpora lutea in the absence of any detectable ova could also be the result of an inappropriate timing of the LH surge. Even though we did measure an increase in LH in the evening, only two time periods were examined. Since the specific LH surge pattern was not determined, it is possible that the timing of the surge was different in the treated PD rats than was seen in the young cycling control animals.

Estes *et al.* (16) have also provided data which suggest that hyperprolactinemia rather than impaired LH secretory mechanisms is primarily responsible for the PD state in old rats. These workers showed that an inability of ovariectomy to decrease PRL is in marked contrast to the reduction seen in the CE rat (9). They suggest that a hypothalamo-hypophyseal defect rather than an ovarian mechanism is responsible for the hyperprolactinemic state of the PD rat. It has been hypothesized that age-related increases in PRL may be due to decreases in hypothalamic dopamine content and/or turnover (19). In a recent report, Wise (20) found that baseline PRL concentrations were higher in middle-

aged cycling rats, further suggesting that such a change may be important in age-related transition to an acyclic state. However, Lu *et al.* (21) found serum PRL levels were normal in CE rats during the earlier phase, suggesting that PRL was not a causitive factor. The levels of PRL in the early phases of PD have not been reported. However, we found that while PRL values were only moderately increased before treatment in the younger group of PD rats (14–16 month), they were substantially elevated in the older PD group (24–25 month). These findings provide additional evidence that there is a progressive change with increasing age.

Clemens and Bennett (15) were the first to report reinitiation of cyclicity in aged PD rats using lergotrile treatment. They also showed that PRL levels were lowered 1 hour after the rats were given an injection of LM. We were able to show that PRL levels remain suppressed during the weeks of LM treatment. Since the PD rats also showed a reinitiation of cyclicity, it is possible that the high PRL levels may contribute to the reproductive dysfunction seen in these animals. Increased PRL levels have been reported to suppress the estrous cycle (22), produce pseudopregnancy in mature rats (23), and inhibit gonadotropic hormone secretion (24).

Gilman *et al.* (25) reported that the aged PD rats show a nocturnal PRL surge, but not the diurnal surge as seen in young pseudopregnant rats. There is also recent evidence to suggest that the surges differ in the aged CE and PD rat. Damassa *et al.* (8) demonstrated that predominantly diurnal and nocturnal PRL surges occur, respectively, in CE and PD rats. They suggest that distinctly different steroid hormonal patterns may be involved in the control of early PRL patterns in aging rats. We recently found evidence of a nocturnal PRL surge in rats made pseudopregnant with pimozide treatment (Wiggins and Ratner, unpublished observation). This suggests that a decrease in dopamine input may produce a nocturnal surge in the pseudopregnant rat. Whether PRL surges in the PD rats are under similar dopaminergic control and whether they contribute to the dysfunction have yet to be determined.

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Received December 26, 1984. P.S.E.B.M. 1985, Vol. 179.
Accepted February 1, 1985.