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Time Course of the Luteolytic Action of LH in 4-Day Estrous Cyclic Rats

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In 4-day estrous cyclic rats the neutralization of postovulatory biological activity of LH (by means of a single 0.5 ml sc injection of an anti-LH serum) (LHAS) at any time between 12.00 h on estrus and 12.00 h on metestrus prolongs the estrous cycle corpus luteum (CL) progesterone secretion for almost 24 hours. Injection of LHAS later on during the estrous cycle has no effect on CL progesterone secretion. It is concluded that postovulatory LH secreted up to time of CL maximum capacity to produce progesterone (metestrus afternoon) accelerates the intrinsic luteolytic mechanism, and that once the intrinsic luteolytic process has been swithched on (shortly after noon of metestrus), it will lead to the CL functional demise regardless of the luteolytic action of LH.

Key words: Estrous cycle, Corpus luteum, Progesterone, LH, Luteolysis.

During the diestrous phase of the rat estrous cycle, the pituitary secretes LH in a pulsatile form (5, 6, 7). This «basal» or «tonic» secretion of LH is known to exert an important role in both estrogen production by the maturing follicle (4, 16) and the length of the rat reproductive cycle (12).

Another important factor in the determination of the length of the rat estrous cycle is the duration of the corpus luteum (CL) progesterone secretion (14). Although the CL progesterone secretion of the rat cycle is autonomous, it has been shown that the neutralization of the biological activity of the tonic secretion of LH (by means of a single 0.5 ml sc injection early in the diestrous period) produces a prolongation of CL progesterone secretion (10, 18). However, while this injection of anti-LH serum affects the follicular estrogens production for approximately five days (13), it acts on CL progesterone secretion for about 24 hours only (10, 18).

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Besides the preferentially made steroid, CL activity differs from that of the follicle in the secretion kinetics of the main steroid produced. While the latter shows a typical linear increase, the former presents a wave shaped form (rising, plateau and regression phases) (11, 14). The present experiment was undertaken to determine whether the functional stage of the CL in the rat estrous reproductive cycle at which the luteolytic effects of LH are neutralized determines the duration of the progesterone secretion.

Materials and Methods

Mature virgin Holtzman (Sprague-Davies) female rats were used. They were maintained in a light (14 h light: 10 h darkness, light on at 5.00 h) and temperature controlled (24° C) room and fed with Purina rat chow and water *ad libitum*. Vaginal smears were recorded daily and those rats showing at least two consecutive 4-day estrous cycle were selected for this experiment. The day of vaginal estrous was designated as day 1 of the cycle.

Experimental plan. — Experiment 1 was set up with eight different groups of rats (4-5 each) injected sc with 0.5 ml of an anti-bovine LH serum (LHAS) at the following times of the rat estrous cycle: 12.00, 18.00 or 24.00 h on the day of vaginal estrous (day 1), or 06.00, 12.00, 18.00 or 24.00 h on the day of vaginal metestrus (day 2) or 06.00 h on the day of diestrus (day 3). The immunological characteristics and biological effects of this antiserum have been described previously (13). Control rats were injected with 0.5 ml of a normal horse serum (NHS).

Experiment 2 tested the participation of diestrous secreted PRL on CL progesterone secretion in rats deprived of the biological activity of LH. This was done in rats injected with two sc injections of 0.4 mg of bromocriptine (CB-154) dissolved in 0.25 ml of ethanol at 06.00 and 18.00 h on day 2, and with LHAS at 06.00 on day 2. Control injections consisted of 0.25 ml of 70 % ethanol and 0.5 ml of NHS.

Blood collection and progesterone assay. — Under light ether anesthesia less than 0.5 ml of blood was collected by direct jugular venipuncture at 08.00 and 16.00 h during days 2 and 3 and at 08.00 h on day 4 in experiment 1. Only one blood sample at 08.00 on day 3 was collected in experiment 2. Blood was kept at 4° C to clot overnight. After that the serum was removed and stored at −20° C until assayed for progesterone as previously described (8).

Statistic. — All values of both the length of the diestrous phase and the peripheral serum progesterone concentration are shown as mean ± SE. Its statistical significance was assessed by analysis of variance (ANOVA) and when differences were found they were compared with the Newman-Keuls multiple range test.

Results and Discussion

The effects of an anti-LH serum at different times of the estrous cycle on the peripheral serum concentration of progesterone throughout the diestrous period of 4-day cycle rats are show in fig. 1.

The length of the diestrous period was 2 days in NHS treated rats and from 4 to 10 in LHAS injected ones (table I), a good expression of LHAS ability to neutralize the biological activity of LH.

Serum progesterone levels at 08.00 h on day 3 in rats injected with 0.5 ml of LHAS on day 2 (metestrus) are not affected by the administration of bromocriptine (table II).

The results show that in 4-day estrous

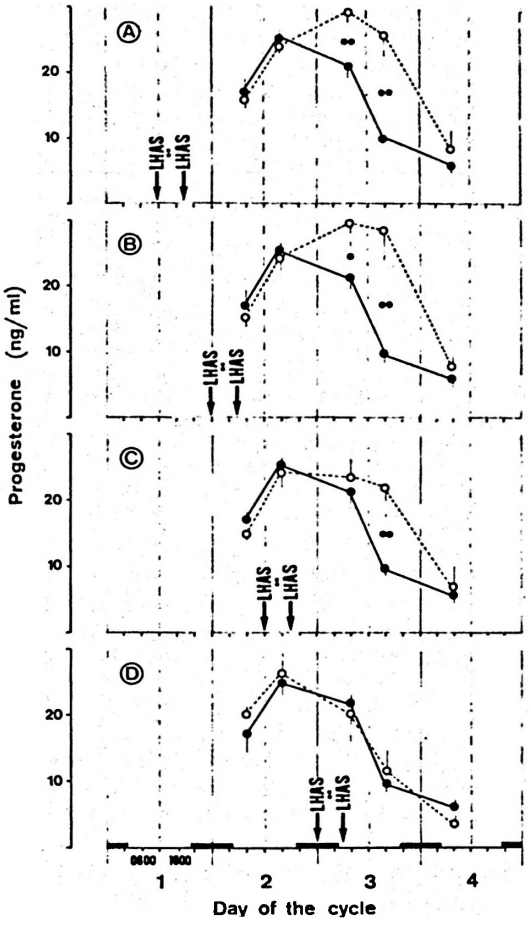

Fig. 1. *Peripheral serum concentration of progesterone (means ± SE) throughout the diestrous period of 4-day cyclic rats injected with 0.5 ml of an anti-LH serum (LHAS) at different times of the estrous cycle.*

Day 1, day of vaginal estrous. Since no differences in the progesterone levels were found at different times in normal horse serum (NHS) injected rats, the solid line includes all the rats given NHS (n = 38). Broken lines represent the time course of the progesterone levels in LHAS injected rats. (A) Rats injected shortly before the begining of the rising phase: including 5 rats injected at 12.00 h day 1 and 4 rats injected at 18.00 h day 1. (B) Rats injected during the rising phase of CL progesterone secretion: including 5 rats injected at 24.00 day 1 and 5 rats injected at 06.00 h day 2. (C) Rats injected around the maximum capacity of progesterone secretion: including 4 rats injected at 12.00 day 2 and 4 rats injected at 18.00 h day 2. (D) Rats injected during the regression phase: including 5 rats injected at 24.00 day 2 and 4 rats injected at 06.00 day 3. Statistical evaluation was done by ANOVA and by the Newman-Keuls multiple range test (* $P < 0.05$, ** $P < 0.01$).

cyclic rat, the neutralization of the biological activity of diestrous LH produces a prolongation in the peripheral serum progesterone during diestrous. The results also show, as has been previously demonstrated (1, 2, 18, 19), that the treatment with bromocriptine (which inhibits pituitary PRL secretion [17]) does not affect the concentration of progesterone during diestrous. We will comment on these data in terms of CL progesterone secretion because changes in peripheral serum progesterone levels in cyclic rats are related to changes in the rate of progesterone secretion (15) and also because the CL in the rat ovarian cycle are the only source of increased progesterone concentration in peripheral blood during the diestrous period (9).

The increase in progesterone secretion by the CL of the rat estrous cycle is independent of the pituitary (20, 21). On the contrary, the rapid fall in progesterone secretion observed on diestrus (day 3) (fig. 1) suggests that luteolysis is actively imposed (17, 20). The fact that neutralization of the biological activity of LH produces a prolongation in the CL progesterone secretion (18, figure 1) and that even in the absence of such luteolytic action of LH the CL of the cycle undergoes luteolysis (figure 1), strongly sug-

Table I. *Length of the diestrous period in 4-day cyclic rats injected with 0.5 ml of an anti-LH serum (LHAS) at different times of the estrous cycle.*

Treatment		Length of diestrous (days)
NHS*		2.0 ± 0.2 (38)
LHAS	12.00 estrus	4.7 ± 0.2 (5)
	18.00 estrus	5.0 ± 0.1 (4)
	24.00 estrus	5.5 ± 0.1 (5)
	06.00 metestrus	6.1 ± 0.1 (5)
	12.00 metestrus	6.6 ± 0.8 (4)
	18.00 metestrus	6.7 ± 1.0 (4)
	24.00 metestrus	7.8 ± 0.5 (5)
	06.00 diestrus	8.5 ± 0.7 (4)

* Includes all normal horse serum (NHS) injected rats regardless of the time of the treatment.

Table II. *Peripheral serum progesterone concentration (ng/ml) at 08.00 on diestrus (day 3) in 4-day cyclic rats injected with 0.5 ml of an anti-LH serum at 06.00 on metestrus (day 2) and with two injections of bromocriptine (0.4 mg/0.25 ml 70 % ethanol) at 06.00 and 18.00 on metestrus (day 2).*

Treatments	Serum progesterone
NHS + ETH	18.43 ± 2.50 (9)
LHAS + ETH	31.19 ± 3.13 (11)[a]
NHS + CB-154	16.50 ± 3.40 (9)
LHAS + CB-154	28.05 ± 1.67 (11)[a]

[a] Statistical evaluation by ANOVA and Newman-Keuls multiple range test. P 0.01. NHS, 0.5 ml of Normal Horse Serum. LHAS, 0.5 ml of anti-LH serum. ETH, 0.25 ml of 70 % ethanol. CB-154, 0.4 mg of bromocriptine.

gests that basal levels of LH stimulate some intrinsic luteolytic mechanism (17).

The nature of this luteolytic action of LH during the cycle is unclear. From the present results, both an association between the CL progesterone secretion activity and the ability of LH to accelerate the intrinsic luteolytic process are apparent. In 4-day estrous cyclic rat, the CL progesterone secretion life-span consists of rising, plateau and regression phases (17). The rising phase begins shortly around midnight of estrus (3, 11, 14). Peripheral serum progesterone during diestrous reaches a maximun by late afternoon of metestrus (day 2), and after an extremely short plateau, the regression phase begins and the peripheral serum progesterone levels reach basal values by late afternoon of diestrus (day 3) (2, 3, 14, figure 1).

The blockade of LH biological activity either shortly after ovulation (figure 1.A) or during the CL progesterone secretion rising phase (figure 1.B) prolongs the duration of CL progesterone production. Once the CL has reached maximum secretory capacity (late afternoon of metestrus), the luteolytic action of LH weakens (figure 1.C). Later on, the blockade of LH biological activity does not affect the intrinsic luteolytic mechanism (figure 1.D) and the CL progesterone secretion results in the typical pattern normally found in 4-day estrous cycle (14). Since LH does not act luteolytically shortly after noon of metestrus (day 2) in 4-day cyclic rats (figure 1), it seems that the intrinsic luteolytic mechanism (which is associated with the maximum capacity of the CL to make progesterone [17]), is at this time on its way, and it will produce the functional demise of the CL regardless of the action of LH. In fact, 5-day cyclic rats, whose CL secretes progesterone at the maximun rate (plateau phase) up to diestrus (day 3) afternoon (14, 19), LH is effective in shortening progesterone secretion up to 800 on diestrus (day 3) (10).

These facts point to a dual control of the rat reproductive cycle. First, through stimulating estrogen production by maturing follicles (4) and second by its stimulating action of the CL intrinsic luteolytic mechanism (figure 1). Whether the intrinsic luteolytic mechanism depends on CL ability to make luteolytic prostaglandins (17) is not yet certain, although the inhibition of ovarian prostaglandin synthesis with intraovarian bur-

sae indomethacin treatment tends to prolong CL progesterone secretion on both intact and hypophysectomized 4-day cyclic rats (SÁNCHEZ-CRIADO and ROTHCHILD, unpublished data). Further experiments will clarify this possibility.

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Resumen

La neutralización de la actividad biológica de la LH secretada durante la fase de diestro en ratas con ciclos estrales de 4 días de duración por administración subcutánea de 0,5 ml de un suero anti-LH (SALH) prolonga la secreción de progesterona por el cuerpo lúteo (CL) por aproximadamente 24 horas. Este efecto sólo se presenta cuando la inyección del SALH se realiza entre las 12,00 horas del día de estro y las 12,00 horas del día de metestro y no cuando el SALH es inyectado posteriormente. Se concluye que la secreción postovulatoria de LH hasta el momento de máxima capacidad secretora del CL de progesterona (16,00 horas del día de metestro) acelera el proceso luteolítico intrínseco, y que una vez que éste se ha puesto en marcha (mediodía del metestro) conducirá a la luteolisis funcional con independencia de la presencia de la LH.

Palabras clave: Ciclos estrales, Cuerpo lúteo, Progesterona, LH, Luteolisis.

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