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Inhibition of Gonadotropin Secretion During Lactation in the Rat: Relative Contribution of Suckling and Ovarian Steroids[1]

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ABSTRACT

The relative effects of the suckling stimulus and ovarian steroids on gonadotropin inhibition during lactation were studied in intact and ovariectomized lactating rats. On Day 2 postpartum, litter sizes were adjusted to 0 or 8 pups and ovariectomy was performed. Serum LH concentrations in the intact lactators were barely detectable and approximately five-fold lower than basal cycling levels reported previously by this laboratory. Serum and pituitary LH concentrations gradually increased in ovariectomized lactators but did not differ markedly from intact lactators until Days 15 to 20 postpartum. At all times studied, the postcastration rise in serum and pituitary LH was clearly inhibited in ovariectomized lactators when compared to nonlactating, ovariectomized females.

Serum FSH concentrations in the intact lactating animals remained at values similar to basal cycling levels, while pituitary FSH concentrations gradually increased. Serum and pituitary FSH concentrations in the ovariectomized lactators increased dramatically throughout lactation but did not equal those observed in nonlactating, ovariectomized females until Days 15 to 20.

Serum progesterone concentrations increased during lactation, reaching maximal values near Day 10 and declining to baseline values by Day 20. Estradiol concentrations increased from very low levels on Day 2 to reach values similar to basal cycling levels by Day 10 and diestrus-2 levels by Day 20.

These results demonstrate that in the absence of ovarian steroids, the suckling stimulus: 1) can effectively inhibit the postcastration rise in LH and FSH secretion for at least 15 days postpartum; 2) is a less potent inhibitor of FSH than LH secretion; 3) can not maintain serum LH and FSH below basal cycling values. The increase in estradiol concentration during lactation in the intact animals was unexpected since it occurred in the presence of unchanging, suppressed basal levels of LH.

INTRODUCTION

The anovulatory state that accompanies lactation and pseudopregnancy in the rat is characterized by an inhibition of gonadotropin secretion, an increase in prolactin secretion and maintenance of corpus luteum function. During pseudopregnancy, basal levels of gonadotropin are not diminished, only the ovulating surge of gonadotropin is inhibited. Furthermore, pituitary levels of LH and FSH increase during pseudopregnancy similarly to rats castrated on Day one of the estrous cycle, at least for the first 8 days (Schwartz and Rothchild, 1964; Van Rees and DeGroot, 1965; Rothchild, 1966). Therefore, during pseudopregnancy, gonadotropin inhibition probably results from progesterone secretion maintained via the action of prolactin on the corpus luteum (Rothchild, 1960).

In contrast, during lactation, serum and pituitary levels of LH and FSH are suppressed in both intact and castrated lactators (McCann et al., 1967; Ford and Melampy, 1973; Hammons et al., 1973). Lactation, then, is associated with decreased synthesis and release of gonadotropin from the pituitary. Since this effect is still manifest in the absence of the

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ovaries, it must be mediated independently of ovarian steroids and result from events associated with the suckling stimulus.

The purpose of the present investigation was to study the relative contribution of the suckling stimulus and ovarian steroids on both basal gonadotropin secretion and the postcastration rise in gonadotropin secretion. Serum and pituitary concentrations of LH and FSH along with serum concentrations of progesterone and estradiol were determined in intact lactating, ovariectomized lactating and ovariectomized nonlactating rats.

MATERIALS AND METHODS

Female, Sprague-Dawley rats (ARS/Sprague Dawley, Madison, Wisconsin) were housed in a room maintained at 24°C and illuminated daily from 0600–1800 h. Two days prior to the expected day of parturition, the pregnant females were isolated in maternity cages containing wood shavings for nest building. The day of parturition was designated as Day 0. On Day 2 postpartum, litter sizes were adjusted to 0 or 8 pups, and sham ovariectomy or ovariectomy was performed under halothane anesthesia by midventral abdominal laparotomy at 1700 h. The lactating animals were subsequently divided into three groups: lactation, intact; lactation, ovariectomy; and nonlactation, ovariectomy (litters removed at the time of ovariectomy).

Blood samples were collected by rapidly decapitating animals at 1700 h on Days 5, 10, 15 and 20 postpartum. Intact, lactating animals were also decapitated at 1700 h on Day 2 to serve as controls for the three experimental groups. Immediately following decapitation, the anterior pituitaries were removed, weighed, and homogenized in phosphate buffered saline, pH 7.0 (2 mg pituitary/ml). The supernatant was then appropriately diluted for LH and FSH analyses.

Pituitary and serum LH analyses were performed as previously described (Neill, 1970). The limit of sensitivity for the LH assay was 0.075 ng/ml of serum and the rat LH reference preparation (LER-1240) had a biological potency of 0.6 × NIH-LH-S1. FSH analyses were performed using the double antibody radioimmunoassay developed by Gay et al. (1970) and reported by our laboratory previously (Smith et al., 1975). We have validated the use of this assay by showing that rat LH does not interfere in the assay, that ovariectomized serum produces dose response curves that are parallel to the rat FSH reference preparation (NIAMD-RP-1, 2 IU/mg), and that a number of FSH preparations have comparable immunological and biological activities. The limit of sensitivity for the assay was 50 ng/ml. Progesterone concentrations were determined by the radioimmunoassay procedure described by Thorneycroft and Stone (1972) and validated in our laboratory (Smith et al., 1975). Using progesterone antibody #42 (provided by G. D. Niswender), the sensitivity of the assay was 5–10 pg so that 2–5 μl of serum were routinely assayed. Estradiol concentrations were measured by the radioimmunoassay procedure previously described (Smith et al., 1975). The sensitivity of the assay was 1–2 pg.

Statistical significance between means was determined by analysis of variance and the Duncan Multiple Range Test.

RESULTS

Serum and Pituitary LH Concentrations During Lactation

The effect of lactation on basal LH secretion and on the postcastration rise in serum and pituitary LH can be seen in Table 1. The concentrations of LH observed on Day 2, prior to either ovariectomy or removal of pups, serve as control values to which LH concentrations on subsequent days of lactation in the various groups are compared. In the intact females nursing 8 pups, serum LH was significantly lower (approximately five times) than basal cycling values reported previously by this laboratory (Smith et al., 1975; range of 40 means from 0700 h on estrus to 1300 h on proestrus, 0.4 to 0.8 ng LH/ml), or pseudopregnancy (unpublished observations). However, by Day 20 postpartum, LH concentrations approached cycling levels. Except for the increase in pituitary LH concentrations on Day 5, there was good parallelism between changes in serum and pituitary LH.

Serum and pituitary LH in the ovariectomized lactators gradually increased throughout lactation. By Day 10, the suckling stimulus alone was no longer sufficient to maintain gonadotropin secretion below basal cycling levels. However, this pattern of LH release following ovariectomy during lactation was in sharp contrast to the postcastration rise in serum and pituitary LH observed in the ovariectomized females with no litters. Serum levels had increased dramatically by Day 5 (3 days after ovariectomy and removal of litter) and had increased 100- to 150-fold by Day 15, a pattern similar to cycling or pseudopregnant rats castrated for comparable periods of time (Gay and Midgley, 1969; Swerdloff et al., 1972; unpublished observations). By Day 20 postpartum, pituitary levels of LH were similar in the ovariectomized lactators and nonlactators, while serum LH concentrations were still significantly different (3.73 versus 12.65 ng/ml, respectively).

TABLE 1. Serum and pituitary LH concentrations during lactation.

Days post partum	Lactation, Intact		Lactation, Ovariectomy		Nonlactation, ovariectomy	
	Serum LH*	Pituitary LH**	Serum LH	Pituitary LH	Serum LH	Pituitary LH
2	0.13 ± 0.02 (6)***a	0.16 ± 0.03a	0.13 ± 0.02a	0.16 ± 0.03a	0.13 ± 0.02a	0.16 ± 0.03a
5	0.12 ± 0.04 (7)ax	0.27 ± 0.01bx	0.12 ± 0.02 (10)ax	0.33 ± 0.03bx	4.00 ± 0.51 (6)by	0.50 ± 0.07by
10	0.11 ± 0.02 (5)ax	0.51 ± 0.10bx	0.31 ± 0.13 (9)ax	0.32 ± 0.04bx	7.90 ± 0.95 (6)cy	0.95 ± 0.11cy
15	0.08 ± 0.002 (6)ax	0.23 ± 0.03bx	0.50 ± 0.23 (9)by	0.38 ± 0.04bx	16.00 ± 1.73 (7)dz	1.80 ± 0.38dy
20	0.34 ± 0.07 (6)bx	0.47 ± 0.04bx	3.73 ± 1.19 (6)cy	1.23 ± 0.41cy	13.65 ± 1.08 (6)dz	1.62 ± 0.06dy

*ng LH/ml serum.

**μg LH/mg pituitary.

***Mean ± S.E. followed by number of animals in parentheses.

a,b,c,dRefer to comparisons of means within groups and between days. Means having different superscripts are significantly different ($P<0.05$).

x,yRefer to comparisons of means within days and between groups. Means having different superscripts are significantly different ($P<0.05$).

Serum and Pituitary FSH Concentrations During Lactation

The effect of lactation on FSH secretion can be seen in Table 2. Serum FSH in the intact females remained at values similar to basal levels of FSH during the cycle reported previously (Smith et al., 1975; range of 40 means from 0700 h on estrus to 1300 h on proestrus, 50 to 125 ng FSH/ml), or during pseudopregnancy (unpublished observations). However, pituitary FSH concentrations did not follow the same pattern. A significant increase in pituitary FSH was observed on Day 5 and a further elevation occurred on Day 10.

In contrast to the intact females, the ovariectomized lactators showed a dramatic rise in serum and pituitary FSH during lactation. By Day 20, serum FSH had increased 13-fold. However, ovariectomy and simultaneous removal of the litter resulted in a more rapid increase in serum FSH to reach maximum values by Day 5 postpartum, a pattern similar to cycling or pseudopregnant females castrated for a similar time (unpublished observations). The increase in pituitary FSH was more gradual, since maximum concentrations were not obtained until Day 10. By comparing the postcastration rise in FSH in the lactating and nonlactating animals, the results indicate that by Day 15, pituitary FSH concentrations were similar, and by Day 20, serum levels were also comparable.

Serum Progesterone and Estradiol Concentrations During Lactation

In order to establish if there was any correlation between ovarian steroids and levels of LH and FSH in the intact lactating females, serum progesterone and estradiol concentrations were determined in these animals. Also, progesterone and estradiol were measured in the ovariectomized, lactating females as an indication of adrenal steroid secretion. The results are shown in Table 3. Progesterone levels increased during lactation to reach maximum values on Day 10 then declined to approach basal levels by Day 20 postpartum. The concentration of progesterone in the ovariectomized lactators (1.5 ng/ml) was significantly below basal values.

Table 3 also shows the concentrations of estradiol during lactation. On Day 2, estradiol levels in the intact lactators (2.6 pg/ml) did not differ from those observed in ovariectomized,

TABLE 2. Serum and pituitary FSH concentrations during lactation.

Days post-partum	Lactation, Intact		Lactation, Ovariectomy		Nonlactation, Ovariectomy	
	Serum FSH*	Pituitary FSH**	Serum FSH	Pituitary FSH	Serum FSH	Pituitary FSH
2	68.3 ± 8.3 (6)***a	1.05 ± 0.12[a]	68.3 ± 8.3[a]	1.05 ± 0.12[a]	68.3 ± 8.3[a]	1.05 ± 0.12[a]
5	66.7 ± 12.5 (7)ax	1.92 ± 0.11[bx]	121.3 ± 14.7 (10)by	2.72 ± 0.28[bx]	1027.0 ± 65.7 (6)bz	4.04 ± 0.48[by]
10	79.3 ± 9.8 (5)ax	3.26 ± 0.48[cx]	162.4 ± 23.1 (9)by	3.57 ± 0.45[bx]	1182.0 ± 71.5 (6)bz	6.57 ± 0.75[cy]
15	58.1 ± 3.8 (6)ax	2.96 ± 0.21[cx]	336.5 ± 113.0 (9)cy	5.15 ± 0.70[cy]	1269.0 ± 93.2 (7)bz	6.51 ± 0.35[cy]
20	94.6 ± 12.8 (6)bx	3.81 ± 0.67[cx]	875.6 ± 105.3 (6)dy	8.46 ± 0.80[dy]	1200.0 ± 52.2 (6)by	6.28 ± 0.34[cy]

*ng FSH/ml serum.

**μg FSH/mg pituitary.

***Mean ± S.E. followed by number of animals in parentheses.

a,b,c,d Refer to comparisons of means within groups and between days. Means having different superscripts are significantly different ($P<0.05$).

x,y,z Refer to comparisons of means within days and between groups. Means having different superscripts are significantly different ($P<0.05$).

TABLE 3. Serum progesterone and estradiol concentration in intact, lactating rats.

Days post-partum	Progesterone[a] (ng/ml)	Estradiol[b] (pg/ml)
2	13.7 ± 1.9 (6)[c]	2.6 ± 1.0 (6)
5	56.3 ± 4.0 (7)	4.0 ± 0.9 (7)
10	69.0 ± 4.4 (5)	6.5 ± 1.4 (5)
15	38.3 ± 5.1 (6)	8.1 ± 3.4 (5)
20	22.6 ± 6.7 (6)	17.5 ± 3.4 (5)

[a]Mean serum progesterone concentration at Day 10 in ovariectomized, lactating females was 1.5 ± 0.3 ng/ml.

[b]Serum estradiol concentrations did not differ at any time in the ovariectomized lactators (average 3.25 ± 0.8 pg/ml) nor in the ovariectomized nonlactators (average 1.8 ± 0.4 pg/ml).

[c]Mean ± S.E. (number of animals).

lactating females, indicating an absence of any ovarian estradiol secretion. However, concentrations gradually increased between Days 5 and 10 of lactation to reach levels observed during estrus and the morning of diestrus-1 of the estrous cycle (5–10 pg/ml; Smith et al., 1975). By Day 20 of lactation, estradiol concentrations were equal to those observed on the evening of diestrus-2 (15–20 pg/ml; Smith et al., 1975).

DISCUSSION

The dramatic difference in serum LH and FSH concentrations between ovariectomized lactators and ovariectomized nonlactators clearly demonstrates that suckling inhibits and ovariectomy stimulates gonadotropin secretion, confirming the results of others (Ford and Melampy, 1973; Hammons et al., 1973). In addition, lactation depresses serum concentrations of LH in intact lactators below normal basal levels observed during the estrous cycle, while serum FSH concentrations are not affected. The suppression of basal gonadotropin secretion during lactation has been suggested previously using indirect measures. Rothchild and Dickey (1960) showed that ovarian compensatory hypertrophy does not occur in rats unilaterally ovariectomized on Day 1 postpartum. Furthermore, the occurrence of delayed implantation during lactation indicates that estrogen secretion, which is necessary for implantation to occur (Yoshinaga, 1961), is

suppressed, suggesting that gonadotropin levels are also suppressed. However, implantation is normally only delayed until Days 9–12 postpartum (Mantalenakis and Ketchel, 1966; Zeilmaker, 1964). While we did not observe any increase in serum gonadotropin levels until Day 20, we did demonstrate that by Day 10 of lactation, estradiol concentrations have increased to reach basal levels observed during the estrous cycle (Table 3). The increase in serum LH and FSH on Day 20, coupled with the decline in progesterone (Tables 1 and 3) would correlate with the pattern of 20α-hydroxyprogesterone secretion reported by others (Tomogane et al., 1969; Yoshinaga et al., 1971). Twenty α-hydroxyprogesterone had begun to increase by Day 18 to reach high levels by Day 21 postpartum.

The decreased basal levels of LH but not FSH demonstrates that lactation is a much more potent inhibitor of LH secretion. The factors responsible for this inhibition are unclear. However, gonadotropin secretion is not suppressed during pseudopregnancy, suggesting that the suckling stimulus causes an inhibitory influence in addition to the influence of progesterone secreted by the corpora lutea. The pattern of progesterone observed during lactation (Table 3) agrees with earlier studies although the absolute concentrations are somewhat lower (Grota and Eik-Nes, 1967; Tomogane et al., 1969; Yoshinaga et al., 1971). It is difficult to differentiate the effects of the suckling stimulus from progesterone secretion on basal gonadotropin secretion. Even though basal LH concentrations approach normal cycling values in females nursing 2 pups, progesterone concentrations are lower than in 8-pup lactators (Yoshinaga et al., 1971; unpublished observations). However, during the last half of pregnancy, when progesterone concentrations are similar to those observed during lactation (8 pups), basal LH secretion is decreased two- to three-fold compared to the estrous cycle (Brown-Grant et al., 1972; Morishige et al., 1973) and, therefore, inhibited to a similar degree as during lactation. Furthermore, in the ovariectomized lactators, the suckling stimulus can not maintain serum LH below normal basal levels. These results support the concept that progesterone might be responsible for the inhibition of basal gonadotropin secretion.

The effect of the suckling stimulus is most clearly evidenced in the ovariectomized lactators, demonstrating a dramatic inhibition of the postcastration rise in LH and FSH. Both pituitary and serum levels are decreased as compared to ovariectomized nonlactators, indicating an inhibition of both synthesis and release from the pituitary. Even though there was a tendency for pituitary FSH levels to increase prior to serum FSH, the role of the suckling stimulus in synthesis and release processes is unclear.

The results from the ovariectomized lactators also indicate that the relative contribution of the inhibitory influences of ovarian steroids and the suckling stimulus is not the same for LH and FSH, confirming the results of Ford and Melampy (1973). The suckling stimulus is a much more potent inhibitor of LH than FSH secretion. Indeed, by Day 20, the suckling stimulus has become completely ineffectual as an inhibitory influence on FSH secretion, while still exerting an inhibition on LH.

The mechanism by which the suckling stimulus provides as potent an inhibition of gonadotropin secretion as ovarian steroids is unclear. However, one difference in the mechanism of action of the suckling stimulus is the tendency for the system to become less sensitive to the suppressive effects of suckling as lactation continues. This phenomenon has been called "escape" by Hammons et al. (1973). The increase in basal LH and FSH secretion in the intact lactators and the postcastration rise in LH and FSH in the ovariectomized lactators supports the concept of this phenomenon.

The inhibition of gonadotropin secretion due to the suckling stimulus could result from the neural reflex initiated by suckling or from the increase in prolactin which follows suckling. There is considerable evidence that prolactin may act at the hypothalamus and pituitary to inhibit both prolactin and gonadotropin secretion. Prolactin implants in the hypothalamus not only inhibit lactation, but result in ovulation even though the pups continue to suckle (Clemens et al., 1969). In addition, treatment of ovariectomized rats with methallibure (a nonsteroidal gonadotropin suppressor) augments perphenazine-induced prolactin release (Ben-David et al., 1971). Moreover, if prolactin secretion is inhibited in postpartum women with 2-Br-α-ergocryptine, gonadotropin secretion increases and ovulation occurs (Rolland et al., 1975).

On the other hand, previous research has also demonstrated that prolactin, itself, has no inhibitory influences on gonadotropin secre-

tion. Desclin (1947) showed that prolactin did not prevent the formation of castration cells in pituitaries of castrated, lactating rats with no litters. The postcastration rise in LH in animals nursing a small litter is similar to castrated lactators deprived of their litters (Ford and Melampy, 1973; Hammons et al., 1973). These results suggest that the inhibition of gonadotropin secretion is related to the intensity of the suckling stimulus itself. However, suckling of a small litter also causes a smaller increase in prolactin (Ford and Melampy, 1973) and progesterone (Yoshinaga et al., 1971), making it difficult to separate the neural and hormonal effects.

One of the most surprising observations of these studies is the increase in estradiol that occurred during lactation in the intact females. Estradiol concentrations are below basal cycling values (Smith et al., 1975) until 5 to 10 days postpartum. However, these concentrations seem to be biologically effective since FSH secretion has increased in the ovariectomized lactators by Day 10. Furthermore, Ford and Melampy (1973) found that in females ovariectomized on Day 2, uterine weight was significantly decreased on Day 6 postpartum compared to intact controls. The stimulus for the increase in estradiol secretion is unclear at this time since LH and FSH levels remain at unchanging concentrations until Day 20 postpartum. The ability of the ovaries to respond to the low levels of gonadotropin on Days 10 and

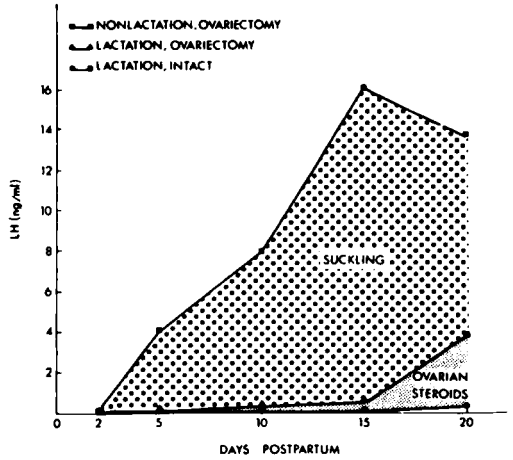

FIG. 1. The relative contributions of ovarian steroids and the suckling stimulus on serum and pituitary LH concentrations during lactation.

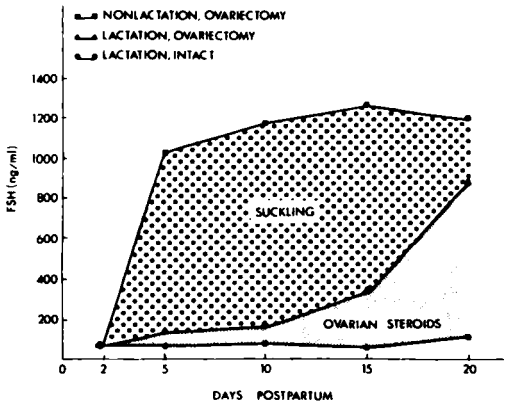

FIG. 2. The relative contributions of ovarian steroids and the suckling stimulus on serum and pituitary FSH concentrations during lactation.

15 but not on Day 2 suggests that the ovaries might be refractory to gonadotropin stimulation during the early stages of lactation. Rothchild (1960) has shown previously that ovaries from cycling, pseudopregnant or lactating rats responded equally to exogenous gonadotropin stimulation. However, his studies were performed after Day 10 of lactation. In humans, the ovary appears to be refractory during the early postpartum period since no ovarian response occurs after 5 days of HMG treatment (Zarate et al., 1972).

Figures 1 and 2 illustrate the relative contributions of the suckling stimulus and ovarian steroids on gonadotropin inhibition. While the suckling stimulus might play a role in decreasing basal secretion in the intact animals, the high levels of progesterone would also seem to be contributory. The fact that gonadotropin levels increase above basal levels in the ovariectomized lactators would support this concept. The ovariectomized lactators also demonstrate that the suckling stimulus is a very potent inhibitor of the postcastration rise in gonadotropin secretion, having a more pronounced effect on LH than FSH secretion. Since the inhibitory influence of the suckling stimulus becomes relatively ineffective between Days 15 to 20 postpartum, ovarian steroids become necessary to maintain basal levels of gonadotropins during this time.

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