Changes in Pituitary Prolactin and Mammary Nucleic Acid Content during Pseudopregnancy in the Rat* (33675)

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During pseudopregnancy in the rat, ovulation is suppressed and the corpora lutea are maintained, presumably under the influence of prolactin. Schwartz and Rothchild (1) observed a steady rise in the pituitary concentration of luteinizing hormone (LH) during pseudopregnancy, reflecting a reduction in LH release which prevented ovulation. The mammary gland, another target organ of prolactin, also grows during pseudopregnancy. The extent of growth is comparable to that during the first 11–12 days of pregnancy (2). Although a sharp decrease in pituitary prolactin concentration at the induction of pseudopregnancy has been reported (3), no data for an extended period of time are available. Thus, the present study was undertaken to determine the changes in pituitary prolactin content during the entire course of pseudopregnancy and its relationship to biochemical changes in the mammary gland.

Materials and Methods. Adult rats of CFE strain obtained from Carworth Inc., New York City, N. Y. were used. Rats were maintained in a room lighted between 6 a.m. and 6 p.m. and received Agway rat and mouse pellets and water *ad libitum*. Stage of the estrous cycle was determined by daily examination of vaginal smears. Pseudopregnancy was induced by vibrating the cervix for 20–25 sec on the day of estrus with a glass rod attached to a dental drill (4). Rats that began cycling after stimulation were discarded.

Rats in groups of eight were killed by decapitation immediately after stimulation, at 0.5, 2, 8, 16, and 24 hr, and at 2, 4, 6, 8, and 12 days after vibration of the cervix. One unstimulated group and one group showing proestrus smear after the termination of pseudopregnancy were also killed. The anterior pituitary glands were removed, weighed and stored at −20° for prolactin assay. The eight pituitaries from each group were pooled and prolactin was assayed by a modification of the method of Reece and Turner (5) using a four-point assay design. Low doses of pituitary (0.5 mg) and NIH-P-S_8 ovine prolactin[1] (1.0 μg) were injected into right and left sides of the crop sacs, respectively, of eight adult pigeons (Homer breed), and high doses of pituitary (1.5 mg) and standard prolactin (3.0 μg) were injected similarly into eight additional birds. Potency and the several criteria of the validity of the assays were calculated by the method of Bliss (6).

The six abdominoinguinal mammary glands were removed and analyzed for nucleic acids after separating the parenchyma from the surrounding connective tissue (7). The weights of the adrenal glands were recorded.

Results. Anterior pituitary weights (Table I) increased slightly (not significant) 30 min after cervical vibration, remained high until 16 hr, and then gradually declined to a low value on day 10 of pseudopregnancy ($p<0.01$). Adrenal weights declined 10% ($p<0.05$) by 2 hr after cervical stimulation and remained at low levels thereafter. The pituitary prolactin concentration did not change appreciably between 0 and 16 hr after stimulation but decreased 23% between 16 and 24 hr. It continued to decline until day 10 of pseudopregnancy when it reached the lowest level. This amounted to a 73% drop in prolactin concentration in comparison to the unstimulated control group. Prolactin concentration then increased between days 10 and 12 of pseudopregnancy and remained at the same level on the day of proestrus. Pituitary prolactin content (IU/pituitary) fol-

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TABLE I. Pituitary Prolactin Concentration of Rats during Pseudopregnancy.

Time after vibration	Ant. pit. wt. (mg/100 g of body wt.)	Adrenal wt. (mg/100 g of body wt.)	Prolactin conc. (IU/mg)
Unstim	4.8 ± 0.23[a]	30.6 ± 0.6[a]	0.045 ± 0.018[b]
1–2 min	5.1 ± 0.15	31.4 ± 1.1	0.043 ± 0.012
0.5 hr	5.3 ± 0.18	29.7 ± 1.1	0.042 ± 0.011
2 hr	5.0 ± 0.19	28.2 ± 0.9	0.053 ± 0.014
8 hr	5.1 ± 0.16	28.1 ± 1.3	0.045 ± 0.010
16 hr	5.1 ± 0.22	25.6 ± 0.7	0.047 ± 0.005
24 hr	4.7 ± 0.30	27.9 ± 1.0	0.036 ± 0.019
2 days	4.6 ± 0.10	26.6 ± 0.9	0.030 ± 0.008
4 days	4.5 ± 0.20	26.1 ± 1.0	0.035 ± 0.008
6 days	4.2 ± 0.12	26.3 ± 0.3	0.019 ± 0.051
8 days	4.1 ± 0.09	27.3 ± 1.2	0.020 ± 0.018
10 days	3.7 ± 0.11	25.0 ± 0.5	0.012 ± 0.041
12 days	4.4 ± 0.15	23.7 ± 1.4	0.029 ± 0.008
Proestrus[c]	4.0 ± 0.17	—	0.029 ± 0.012

[a] Standard error of the mean.

[b] Standard error of the assay; the index of precision (λ) for the assays averaged 0.33 ± 0.03.

[c] Time after vibration averaged 13 days.

lowed essentially a similar pattern throughout.

Mammary deoxyribonucleic acid (DNA) content (Table II) did not change between 0 and 2 days after cervical stimulation but increased 15% between days 2 and 4 of pseudopregnancy. It then increased linearly ($p<0.01$) until day 12 of pseudopregnancy totaling an increase of 109%. Between day 12 and the next proestrus (day 13), mammary DNA content decreased slightly (8%). Mammary ribonucleic acid (RNA) content fluctuated irregularly between 0 and 2 days after stimulation but increased 25% between days 2 and 4 after cervical stimulation. The increase continued linearly ($p<0.01$) until day 12, totaling an increase of 183%. Like DNA, mammary RNA then decreased 18% between day 12 and the day of proestrus. trimmed mammary gland weight paralleled the changes in mammary DNA content until day 12 of pseudopregnancy but did not decline between day 12 and the day of proestrus. Total increases in the trimmed and fresh mammary gland weights averaged 53 and 33%, respectively, during the course of pseudopregnancy.

The correlation coefficients between prolactin concentration and mammary DNA and RNA contents were −0.74 ($df = 12$; $p<0.01$) and −0.70 ($df = 12$; $p<0.01$), respectively.

Discussion. Pituitary prolactin concentration did not decrease until 24 hr after the induction of pseudopregnancy. This is contrary to the findings of Herlyn *et al.* (3) who observed a significant decrease in pituitary prolactin content within 30 min after glass rod stimulation of the cervix. The method of stimulation or the strain of rats may have made the difference; however, Taleisnik *et al.* (8) did not find any evidence of LH release in rats 1 hr after a glass rod stimulation of cervix although normal mating resulted in a significant discharge of the hormone. Mating in the rabbit also causes release of LH, adrenocorticotropic hormone (ACTH) and prolactin (9) but ordinarily they do not ovulate following mechanical stimulation of the vagina (10).

After 24 hr pituitary prolactin concentration dropped markedly, reaching the lowest level on day 10 of pseudopregnancy. This trend was just opposite of the changes in LH concentration observed by Schwartz and Rothchild (1). The drop in pituitary prolactin concentration was followed by an increase in mammary DNA and RNA contents. Thus, if prolactin participates in mammary growth during pseudopregnancy, the concen-

TABLE II. Nucleic Acid Content of Mammary Glands of Rats during Pseudopregnancy.

Time after vibration	Body wt. (g)	Fresh MG wt. (g/100 g of body wt.)	Trimmed MG wt. (mg/100 g of body wt.)	Mammary DNA (mg/100 g of body wt.)	Mammary RNA (mg/100 g of body wt.)
Unstim	256	1.80 ± 0.07[a]	69.3 ± 3.0[a]	1.76 ± 0.09[a]	1.51 ± 0.08[a]
1–2 min	262	2.05 ± 0.01	65.4 ± 3.3	1.70 ± 0.11	1.56 ± 0.11
0.5 hr	258	2.00 ± 0.05	67.8 ± 3.0	1.75 ± 0.08	1.70 ± 0.09
2 hr	261	1.89 ± 0.12	64.1 ± 3.2	1.60 ± 0.12	1.49 ± 0.11
8 hr	267	1.81 ± 0.11	70.7 ± 3.5	1.63 ± 0.07	1.53 ± 0.09
16 hr	277	1.93 ± 0.11	72.8 ± 3.1	1.68 ± 0.07	1.77 ± 0.14
24 hr	263	1.81 ± 0.06	70.1 ± 2.5	1.68 ± 0.03	1.63 ± 0.06
2 days	274	2.14 ± 0.17	71.8 ± 3.6	1.70 ± 0.09	1.79 ± 0.15
4 days	274	2.03 ± 0.12	77.3 ± 2.7	1.95 ± 0.08	2.23 ± 0.19
6 days	265	2.03 ± 0.06	86.5 ± 4.8	2.34 ± 0.23	2.52 ± 0.25
8 days	263	2.12 ± 0.13	94.7 ± 6.0	2.95 ± 0.18	3.01 ± 0.09
10 days	286	2.29 ± 0.08	102.2 ± 6.7	3.43 ± 0.22	3.46 ± 0.07
12 days	278	2.24 ± 0.08	104.5 ± 7.3	3.68 ± 0.34	4.27 ± 0.14
Proestrus	261	2.39 ± 0.09	106.0 ± 8.4	3.39 ± 0.34	3.51 ± 0.19

[a] Standard error of the mean.

tration of prolactin in the pituitary gland probably reflected enhanced release rate of the hormone and corroborated the hypothesis that prolactin stimulates luteal function in the rat. Between days 10 and 12, pituitary prolactin concentration increased 2.4-fold, a time when pituitary LH concentration drops sharply (1), suggesting that prolactin release rate is decreased at this time in conjunction with luteal regression and an increase in follicular activity. But the continuance of increase in mammary RNA and DNA between days 10 and 12 suggested that enough prolactin and ovarian steroids were still available to cause further mammary growth.

Prolactin and ovarian steroids, however, are not the only hormones involved in mammary development. Although a change in adrenal size does not necessarily reflect its functional status, a significant decrease in adrenal weight within 2 hr of vibration may be indicative of a stimulation of ACTH secretion and adrenal function. If that is so, then adrenal hormones may have aided increase in mammary nucleic acids. Whether they are also involved in the induction of pseudopregnancy and corpus luteum function is not known.

Mammary DNA and mammary RNA increased 109 and 183%, respectively, between virgin state and 12 days of pseudopregnancy in the present study whereas Anderson and Turner (11) noted only a 31 and 45% increase, respectively, during the same period. Mammary glands in the present study were trimmed by removing the lymph nodes, blood vessels and the extraparenchymal connective tissue, which is considerable in a nonlactating rat mammary gland. Anderson and Turner (11) measured DNA and RNA on the entire mammary tissue. As is evident from Table II, the weight of the total mammary gland changed considerably less than the trimmed weight. Therefore, the presence of the connective tissue may have masked some of the changes in the parenchymal tissue of their experiment.

Summary. Acute and long-term changes in pituitary prolactin and mammary nucleic acids of rats following induction of pseudopregnancy by cervical vibration were investigated. After maintaining a constant level (0.045–0.047 IU/mg) between 0 and 16 hr of stimulation, prolactin concentration declined progressively to the lowest level (0.012 IU/mg) on day 10 of pseudopregnancy. This decrease was followed by substantial increases in DNA and RNA contents of the mammary gland. Pituitary prolactin and mammary nucleic acid content were highly but negatively correlated. The data suggest that prolactin in rats may be involved in

mammogenesis and luteal function during pseudopregnancy.

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Effect of an Adrenal 17α-Hydroxylase Inhibitor on Gonad Function (33676)

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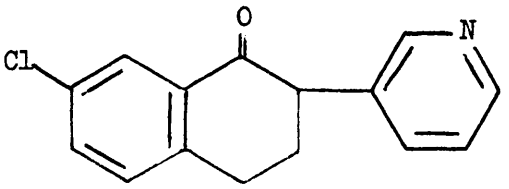
Since the original work with amphenone (1) and related compounds such as metyrapone, a large number of substances were shown to modify the pattern of steroidogenesis in the adrenal [Refs. (2, 3)]. One of these, designated Su-10603, synthesized by Bencze and Barsky (4) had considerable activity as an adrenal 17α-hydroxylase inhibitor both *in vivo* and *in vitro* (5–7).



7-chloro-3,4-dihydro-2-(3-pyridyl)-1(2*H*)-napthalenone (Su-10603)

In the dog it decreased the secretion of cortisol and 11-desoxycortisol, resulting in a compensatory increase in the secretion of corticosterone and 11-desoxycorticosterone.

This raised the question as to its possible effect on the secretion of gonadal hormones. It and related compounds have, indeed, been found to inhibit androgen synthesis *in vitro* in various systems and circumstances (8–15). The only report of gonadal effects in intact animals, however, is one in which an inhibition in male rats of the acute response to chorionic gonadotropin was found (6). The present report extends that work and includes the results of long-term treatment with Su-10603 in both male and female rats.

Methods. Two types of tests were done. In one, immature male and female rats (50–60 g initially) were treated daily for 30 days with varying doses of Su-10603 s.c. The drug was dissolved in polyethelene glycol at a concentration of 12.5–200 mg/ml. At the termination of the experiment, organ weights were taken and some tissues studied histologically.

In the second type of experiment rats like those used above were treated daily for 4 days simultaneously with Su-10603 and 2 units/rat of chorionic gonadotropin (APL-Ayerst). Autopsy was performed and organ weights obtained on the fifth day.

Corticosterone secretion rates were measured using whole blood from the left adrenal vein. Methods were based on those of Knigge (15) as described for the hamster.

Results. Non-effect on corticosterone secretion rate. If, as evidence cited above indicates, Su-10603 is a more or less specific inhibitor of 17α-hydroxylation, it might be expected not to alter the corticosterone secretion rate in rats. That was the case when 15