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Inhibition of Prolactin Secretion of Rat Placental Extract[1] (41521)

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Abstract. Intact and gonadectomized male rats were injected for 4 days with aqueous extracts of midgestation rat placentae to study the effects of this treatment on pituitary secretory function. Experimental rats received daily doses of extract equivalent to one, two, or four placentae; controls were injected with liver extract. At autopsy, 24 hr after the last injection, serum and anterior pituitary glands were collected for radioimmunoassay of FSH, LH, and PRL. Placental extracts caused a dose-related reduction of serum PRL concentration in both intact and gonadectomized rats and depressed circulating LH levels of intact animals. Serum FSH concentration was not affected by this treatment. Pituitary levels of the three hormones were not significantly different between animals injected with placental extract and their liver-injected controls. We conclude that rat placental extract contains a substance, likely to be chorionic mammotropin (rCM), capable of modifying pituitary secretory function during pregnancy.

Rat chorionic mammoluteotropin (rCM), also known as rat placental lactogen (rPL), is detectable by bioassay at midgestation in placental extract (1) and maternal serum (2). As judged by a radioreceptor assay developed for human placental lactogen, rCM appears in maternal serum as early as Day 8 of pregnancy and reaches high levels on Days 11 and 17 (3), reckoning the day of finding sperm in the vaginal smear as Day 0 of gestation. The time course of pituitary prolactin (PRL) in the serum of pregnant rats is well known (4–6). Surges of PRL have been reported to occur twice daily soon after mating and to disappear near midgestation; i.e., when the rat placenta assumes its full functional role. There is abundant evidence (e.g. (7)) that the hormone is capable of acting on its hypothalamic control centers via a short-loop feedback system. The question may be asked, therefore, whether rCM influences PRL secretion in an analogous manner and is responsible for the cessation of PRL surges at midgestation.

We decided to investigate the effects of placental extract on pituitary and serum PRL concentrations, but chose male rather than female rats as test animals. Male rats have measurable levels of PRL not subject to endogenous fluctuation (8), making detection and interpretation of response to injected extract less problematical. To obtain additional information as to how rCM may affect pituitary gonadotropic function, we also measured pituitary and serum concentrations of follicle stimulating hormone (FSH) and luteinizing hormone (LH) following administration of placental extract.

Materials and Methods. Long–Evans rats, born and raised in our animal colony, were used in this study. The animals were kept in air-conditioned quarters (23°) on a 12- to 12-hr light–dark cycle, fed commercial rat diet,[3] and given tap water *ad libitum*.

Female rats used as donors of placental material were bred on the afternoon of proestrus and killed on Day 11 of gestation (day of finding sperm = 0). The uterus was excised, and each gestation sac was opened and the fetus removed. All subsequent procedures were performed at 4°. The fetal portions of the placentae and adjoining decidua, separated by blunt dissection from uterus

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[3] Special White Diet, Feedstuffs Processing Co., San Francisco, Calif.

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and fetal membranes, were collected, weighed, and homogenized in 6 vol of phosphate-buffered saline. The resulting homogenate was centrifuged at 5000*g* for 30 min. After this preliminary centrifugation, the supernatant was aspirated and centrifuged at 41,000 rpm for 60 min with an SW-56 rotor in a Beckman L5-65 ultracentrifuge.[4] This supernatant was then used for the day's injection at dosages equivalent to one, two, or four placentae.

Pieces of liver, obtained from the same female donors, were prepared according to the procedures just described, and the extract was injected into control animals at a dose equivalent by weight to four placentae/day.

Male rats, averaging 45 days of age, were used as recipients. Equal numbers of intact animals and castrates, 1 week postoperative, were injected daily, sc, for 4 days, with either liver extract or placental extract. All were killed on the 5th day, by exsanguination under light ether anesthesia; the serum was separated and stored frozen until assayed for FSH, LH, and PRL. Testes and/or ventral prostate gland were removed and weighed, and the presence of motile sperm was verified in all intact animals. The anterior pituitary gland was removed, immediately weighed, frozen on dry ice, and stored at −20° until assayed for FSH, LH, and PRL.

[4] This procedure results in a supernatant that is free of particles 100 S or larger.

Radioimmunoassays (RIA) were performed on the stored serum and on pituitary homogenates, using kits and instructions kindly supplied by Dr. A. F. Parlow and the National Pituitary Agency, NIAMDD–NIH. The hormones were iodinated with ^{125}I using the chloramine-T method (9). The reference preparations in each case were those supplied by NIAMDD: namely, rat-FSH-RP1 (2.1 × NIH-FSH-S1 by hCG-augmentation assay), rat-LH-RP1 (0.03 × NIH-LH-S1 by ovarian ascorbic acid depletion assay), and rat-PRL-RP1 (11 IU/mg by mouse deciduoma assay). The unknown values were calculated using a computer program developed in this laboratory. All samples were assayed in a single RIA. The within-assay variations were 9.7, 9.9, and 9.8% for FSH, LH, and PRL assays, respectively.

All data were analyzed statistically by one-way analysis of variance (ANOVA) drawing on computer programs in the Statistical Package for the Social Sciences (SPSS) (10). Data that were significantly different in the analysis of variance were analyzed by Dunnett's test (11) for the difference between the means of experimental groups and the controls. In each case, unless stated otherwise, $\alpha = 0.05$.

Results. Table I lists mean organ weights at autopsy. As expected, ventral prostate glands were heavier in intact animals ($P < 0.01$). Anterior pituitary weights did not differ significantly when corresponding groups

TABLE I. EFFECTS OF RAT PLACENTAL EXTRACT ON ORGAN WEIGHTS AND ANTERIOR PITUITARY HORMONE CONCENTRATIONS IN MALE RATS[a]

Treatment group	Daily dose[b] (equivalents of placenta)	Testes (g)	Ventral prostate (mg)	Anterior pituitary (mg)	Pituitary concentrations (μg/mg gland)		
					FSH	LH	PRL
Intact	0	2.59 ± 0.12[c]	136 ± 14	6.2 ± 0.2	27.2 ± 2.3	133 ± 16	7.1 ± 0.5
	1	2.48 ± 0.10	125 ± 10	6.1 ± 0.4	32.4 ± 5.4	139 ± 13	6.7 ± 0.9
	2	2.72 ± 0.06	138 ± 13	5.9 ± 0.3	29.3 ± 2.7	113 ± 12	5.5 ± 0.7
	4	2.55 ± 0.11	145 ± 12	6.1 ± 0.3	30.4 ± 3.5	120 ± 10	4.7 ± 0.5
Castrate	0	—	18 ± 1	6.8 ± 0.3	17.3 ± 2.2	114 ± 13	5.2 ± 0.7
	1	—	19 ± 1	6.3 ± 0.3	22.2 ± 1.8	133 ± 11	5.4 ± 0.7
	2	—	21 ± 3	6.9 ± 0.4	19.3 ± 1.1	122 ± 6	4.8 ± 0.6
	4	—	16 ± 1	6.7 ± 0.4	17.7 ± 2.2	136 ± 4	3.6 ± 0.3

[a] Intact and castrates (1-week postoperative), approximately 45 days of age and 153 g in weight at onset of injections.
[b] 0 dose = liver extract-injected controls.
[c] All values are mean ± SEM ($n = 6$).

of intact and castrate rats were compared; however, when all the gonadectomized animals were compared with all intact rats, the castrate animals collectively had significantly heavier anterior pituitary glands ($P < 0.01$). The only remarkable observation relative to pituitary hormone concentration (Table I) was the finding that FSH concentration of the combined groups of intact rats significantly exceeded that of all castrate groups combined ($P < 0.01$).

Compared to like-treated intact animals, gonadectomized rats always had lower serum PRL values (Fig. 1). In both intact and castrate animals, however, injection of placental extract produced a statistically significant, dose-dependent reduction in serum PRL concentrations. The same treatment had no effect on serum LH concentrations of gonadectomized rats (Fig. 2), but decreased serum levels of the hormone significantly in intact animals. These effects, therefore, were probably mediated through the testes. We observed no significant differences in the levels of circulating FSH within groups of intact or castrate rats; and, like serum LH concentrations, circulating FSH levels were always higher in castrates than in intact animals ($P < 0.01$).

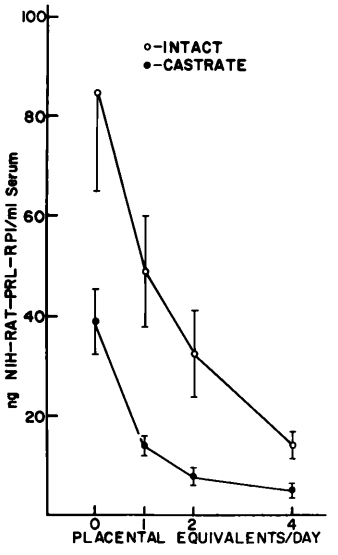

FIG. 1. Serum PRL concentrations in male rats injected with different doses of rat placental extract. Each point represents the mean value for a group of six (6) animals, and bars are the SEM. 0 dose = liver extract-injected controls.

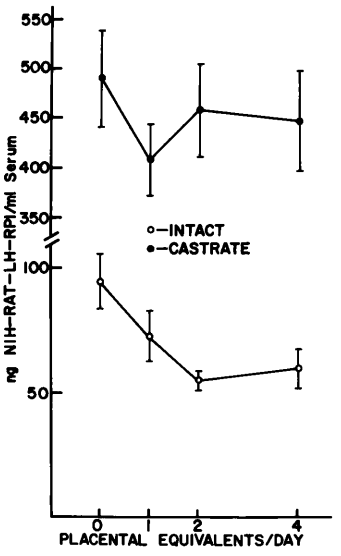

FIG. 2. Serum LH concentrations in male rats injected with different doses of rat placental extract. Each point represents the mean value for a group of six (6) animals, and bars are the SEM. 0 dose = liver extract-injected controls.

Discussion. We have shown that extracts of rat placenta, obtained at midgestation, contain a substance capable of producing a dose-related reduction in serum PRL concentrations. It is probable that this attenuation of circulating hormone resulted from inhibition of pituitary PRL secretion; and, as the extract was equally effective in intact and gonadectomized animals, we may assume that the inhibition was imposed directly upon the hypothalamus and/or anterior pituitary gland.

Others, before us, have concluded that PRL secretion is inhibited by a substance of placental origin, because (i) cessation of PRL surges in the rat (12) and mouse (13) correlates well with onset of placental function, (ii) hysterectomy of pregnant rats before Day 11 of gestation (14), or controlled, numerical reduction of implantation sites on Day 8 (15), prolongs the cycle of PRL surges, and (iii) in nursing rats, rebred during postpartum estrus, serum PRL concentrations rise in response to suckling after placentae are experimentally removed (16). However, all

previous attempts to demonstrate an inhibitory effect of placental material on pituitary PRL secretion *in vivo* (6, 14, 16) or *in vitro* (16) have been unsuccessful. The failure of such extracts to terminate PRL surges in pseudopregnant or hysterectomized rats, and their inability to block elevation of serum PRL in response to suckling, have been blamed on the acute nature of the treatment or loss of biologic activity of the extract during preparation or after its administration. The results of this study, therefore, constitute, to our knowledge, the first direct evidence that a placental factor is coupled in direct feedback regulation with pituitary PRL. That factor most likely is rCM, for it is present in large amounts in aqueous extracts of midgestation rat placenta (1–3). If these inferences are applied to the female rat in the context of normal pregnancy, they appear to be consistent with the hypothesis that the twice-daily surges of PRL are terminated at midgestation as the result of high circulating levels of rCM. Our observations (Laherty and Srebnik, unpublished), and those of others (4, 5), that serum PRL concentration increases at the end of gestation while serum levels of rCM are declining (2, 3) also suggest the existence of such a control mechanism.

Our data corroborate the reports of others, reviewed by Meites *et al.* (17), that castration results in lower serum PRL levels, presumably due to the loss of gonadal steroids.

Injection of placental extract into male rats of this study produced a decrease in serum LH concentration of intact males but had no effect on serum levels of the hormone in gonadectomized rats. Animals of the former group, despite lower circulating LH values, had normal testis weights and function (Table I) and motile spermatozoa in the epididymis. We have previously observed a decrease in serum LH at midgestation in the rat (Laherty and Srebnik, unpublished). These data are consistent with the concept of an LH-like placental hormone, the presence of which has been postulated but not proven (18–20), capable of inhibiting pituitary LH secretion by stimulating testosterone production. An alternative explanation would be that rCM has LH-like as well as PRL-like activity.

Neither castration nor injection of placental extract caused significant changes in the pituitary potencies of LH or PRL as measured by RIA. Whereas injection of placental extract produced no changes in the pituitary concentration of FSH, castration resulted in an overall statistically significant decrease. These data are in agreement with those of other investigators who have employed RIA to measure pituitary gonadotropin levels after gonadectomy (21, 22). They are in conflict, however, with the large body of evidence obtained by bioassay showing significant postcastration rises of pituitary FSH and LH concentrations (e.g. (23)). The question of which method yields more valid quantitative estimates of hormones present in anterior pituitary glands remains unanswered. RIA and bioassay procedures measure different properties of the hormone, i.e., immunoreactivity and biologic activity, respectively; the results obtained with one may not necessarily duplicate, therefore, those derived by the other.

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