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Identification of a Placental Lactogen in Pregnant Snell and Ames Dwarf Mice (41775)

MICHAEL J. SOARES,*[,1] ANDRZEJ BARTKE,† PETER COLOSI,* AND FRANK TALAMANTES*

*Department of Biology, Thimann Laboratories, University of California, Santa Cruz, California 95064, and †Department of Obstetrics and Gynecology, The University of Texas Health Science Center, San Antonio, Texas 78284

Abstract. Sera and placentas from pregnant dwarf mice contain a placental lactogen. This placental lactogen has immunological and electrophoretic properties similar to those of placental lactogen from normal mice.

Snell (dw/dw) and Ames (df/df) dwarf mice are characterized by a deficiency in pituitary prolactin (PRL), growth hormone (GH), and thyroid stimulating hormone (1). A polypeptide hormone, placental lactogen (PL), of the PRL–GH family, has recently been purified from mouse placentas (2) and a radioimmunoassay (RIA) developed for its measurement (3). Female dwarf mice treated hormonally can be brought to reproductive maturity, and if inseminated, their pregnancies can be maintained by treatment with pituitary PRL (4). The objective of this report was to determine whether the deficiency in PRL and GH in dwarf mice also extended to a deficiency in a related hormone, PL.

Materials and Methods. Since PL is a product of the placenta it was essential for the placentas from the dwarf females to be homozygous for the dwarf alleles. Placentas from dwarf females mated to normal males would be heterozygous and thus expected to contain PL. For this reason Snell and Ames dwarf female mice were mated to dwarf male mice homozygous for the same mutation. Snell and Ames dwarfs of both sexes were brought to reproductive maturity by a series of thyroxine injections and pituitary PRL treatment. The animals were treated with *l*-thyroxine, NA (Calbiochem, La Jolla, Calif.) three times weekly for 1 month at 2 μg/injection, for another month with a similar injection regime at 4 μg/injection, and a twice weekly injection regime for a third month at 2 μg/injection. Toward the end of the second month of treatment an anterior pituitary from a normal adult female was transplanted to the kidney capsule (4). In some Snell dwarf mice a second anterior pituitary graft was transplanted 1 month later. Treated females were placed with treated males during the third month of hormonal therapy and inspected daily for the presence of a vaginal plug (Day 0 of pregnancy). For each of the two strains utilized in this study genetically normal (Dw/? and Df/?, respectively) females were mated with genetically normal males and served as controls for the experiment. On Day 15 or 16 of pregnancy the females were decapitated, serum collected, and stored for later measurement of PL and PRL by specific RIAs and total PRL-like activity by radioreceptor assay (RRA). At the time of sacrifice placentas were removed from the uterus, dissected free of extraneous tissue, frozen in tubes submerged in a dry ice/ethanol mixture and stored frozen until used for electrophoretic analysis of PL.

PL, PRL RIAs, and PRL RRA. PL and PRL were measured with specific RIAs as previously described (3, 5). Sera were assayed in duplicate at three dilutions (1, 0.1, and 0.01 μl) for PL and at two dilutions (10 and 1 μl) for PRL. The sensitivities of the peptide hormone assays were: PL, 5–10 pg/tube and PRL, 10–20 pg/tube.

PRL-like activity was measured with the lactating rabbit mammary gland RRA as previously reported (6). Purified mouse PL (2) was used for radioiodination and as a reference standard for the RRA. The sensitivity of the PRL RRA was 0.1–0.25 ng/tube.

[1] Present address: Department of Cell Biology, Baylor College of Medicine, Texas Medical Center, Houston, Tex. 77030.

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Electrophoresis. Placentas were homogenized in 25 m*M* Tris–HCl, 20% sucrose, pH 6.8, in ground-glass homogenizing vessels immersed in ice. The homogenate was centrifuged at 4000*g* and an aliquot of the supernatant was fractionated by discontinuous polyacrylamide gel electrophoresis on 6% gels according to procedures described previously by our laboratory (6, 7). After electrophoresis the gels were cut into 4-mm segments along the entire length of the gel and eluted overnight in 0.3 ml of 10 m*M* Hepes • NaOH, 144 m*M* NaCl, pH 7.4. The presence of PL in the eluates was determined by RIA.

Results. Sera from pregnant dwarf mice were found to contain PL immunoactivity (Table I). Pituitary PRL was present in serum samples from the dwarf mice due to the presence of anterior pituitary grafts in their kidney capsules; however, the levels were too low to influence the PL RIA. Mouse PRL cross-reacts less than 0.002% in the PL RIA (3). Additionally, the PRL RRA detected activity far in excess of the PRL-like activity that could be accounted for by pituitary PRL present in the serum samples. Mouse pituitary PRL is at least 10 times less potent than mouse PL in this assay (2). Although serum PL concentrations appeared to be greater in dwarf females than in the normal controls (see Table I), the small sample sizes prohibit any quantitative analysis. Our sample sizes for these experiments are small due to the difficulties in getting the dwarf females pregnant by dwarf males and in maintaining their pregnancies.

Placental extracts from dwarf and normal females contained similar amounts of PL (df/df: 3.6 ± 0.6 μg/placenta, $N = 3$; Df/?: 3.9 ± 0.5 μg/placenta, $N = 2$; dw/dw: 5.1 μg/placenta, $N = 1$; Dw/?: 4.9 ± 0.5 μg/placenta, $N = 2$). Electrophoresis of placental extracts from both dwarf and normal animals demonstrated that the PL immunoactivity present in the placentas eluted from the gels at a position identical with purified mouse PL standards ($R_f = 0.2$–0.3). Mouse pituitary PRL migrates with the ion front in this system.

Discussion. Our results indicate that sera and placentas from pregnant dwarf mice contain a PL. This PL has immunological and electrophoretic properties similar to those of PL from normal mice. Although PLs are known to be biochemically and biologically related to PRLs and GHs (8), the deficiency in PRL and GH gene expression in dwarf mice is not accompanied by a deficiency in PL gene expression. Recent studies on the immunohistochemistry and molecular genetics of the dwarf mouse anterior pituitary have provided evidence that the lack of PRL and GH gene expression in these genetic dwarfs is associated with a lesion in the development of the mammotrophs and somatotrophs (9–11).

Daughaday *et al.* (12) have shown that hypophysectomy of pregnant rats results in a significant elevation of serum PL levels. Our findings, although not definitive, are consistent with this work, implicating the anterior pituitary in an inhibitory role in the control of PL secretion. If the difficulties in breeding dwarf mice could be alleviated, then these mice would seem to be good animal models for evaluating a relationship between the anterior pituitary and placenta. Another PRL-like hormone of conceptus origin has been identified from the midpregnant mouse conceptus (13), whether dwarf mice produce this hormone remains to be evaluated.

TABLE I. SERUM PLACENTAL LACTOGEN AND PROLACTIN ACTIVITY IN PREGNANT AMES (df/df) AND SNELL (dw/dw) DWARF MICE

Genetic composition	*N*	Number of fetuses	PL (ng/ml)	PRL (ng/ml)	PRL-like[a] (ng/ml)
df/df	3	8 ± 1[b]	1224 ± 203	196 ± 22	2151 ± 378
Df/?	2	13	339 ± 71	23 ± 1	291 ± 43
df/df	Nonpregnant pool	—	ND[c]	ND	ND
dw/dw	1	6	622	6	644
Dw/?	2	14 ± 1	536 ± 121	9 ± 6	562 ± 81

[a] Expressed as ng mouse PL equivalents/ml.
[b] Mean ± SEM.
[c] Not detectable.

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