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Serum and Pituitary Concentrations of Growth Hormone and Prolactin in Pygmy Mice[1] (40651)

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Mutant pygmy (*pg/pg*) mice first described by King in 1950 (1) exhibit marked growth retardation, growing to only about half the size of normal littermates at 6 weeks of age (2). Although heterozygotes reproduce normally, the homozygotes of both sexes are highly infertile or sterile.

The *pg* mutation is not completely recessive since heterozygous *Pg/pg* mice tend to be somewhat smaller than homozygous *Pg/Pg* mice ((2), Wolff, unpublished data). Heterozygous *Pg/pg* females also tend to have somewhat smaller litters than *Pg/Pg* dams (Wolff, unpublished data).

The cause of pygmy's growth retardation is not known. Based on pituitary transplantation experiments, King (2) postulated that the small size of the mouse was not due to growth hormone (GH) deficiency, but rather to subresponsiveness of the body tissue to GH. Rimoin and Richmond (3) reached the same conclusion after unsuccessful attempts at stimulating body growth of these mice by injecting porcine GH, and suggested that the pygmy mouse could serve as a model of the human African pygmy. GH levels in the serum and pituitary gland of pygmy mice have not been measured.

In this study, we measured GH and prolactin (PRL) in pygmy mice of both sexes. PRL is another pituitary hormone known to stimulate body growth in dwarf mice (4). Serum PRL concentrations were determined under basal conditions as well as after treatment of pygmy mice with the PRL release inducer, perphenazine. In addition, we have investigated the effects of PRL administration on body weight gain of these mice.

Materials and methods. Pygmy (*pg/pg*) mice were produced by mating heterozygous VY/Wf-*Pg/pg* females with C3H/HeNIcrWf-*Pg/pg* males. Normal *Pg/Pg* (VY/Wf × C3H/HeNIcrWf) F-1 hybrids were used as controls. After weaning at 4 weeks of age, all mice were shipped by air from Jefferson to La Jolla. There they were kept in 36 × 30 × 18-cm plastic cages, 10–12 normal or 18–20 pygmy mice per cage, in a light (12-hr light:12-hr darkness)- and temperature (24 ± 1°)-controlled room. Wayne Lab Blox (6% fat) and tap water were available *ad libitum*. At approximately 90 days of age, control and pygmy mice of both sexes were decapitated within 30 sec after removal from the cage. Blood and pituitary glands were collected individually from groups of 17–19 animals. They were stored at 4° not longer than 2 hr before processing. Each serum was separated by centrifugation and each pituitary gland was weighed and extracted with 0.05 *M* Na_2CO_3–$NaHCO_3$ buffer, pH 10, as described earlier (5). GH and PRL were measured in duplicate samples by homologous radioimmunoassays specific for each hormone. The details of the assay procedure have been described previously (6, 7). The biological potency of the mouse prolactin standard used was 25 IU/mg and that of the mouse GH, 3.1 USP U/mg.

To determine their responses to stimulated release of PRL, pygmy and normal mice of both sexes were given single intraperitoneal injections of 1 μg perphenazine/g body wt. They were killed by decapitation at 0, ½, or 1 hr after treatment and serum prolactin concentrations were determined.

For growth stimulation study, a separate group of male and female pygmy mice were used. Beginning at 40 days of age and continuing for 8 weeks, half of the mice of each sex were injected subcutaneously twice daily (at

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8:30 AM and 4:30 PM) with 50 μg of ovine PRL (NIH-P-S_{12} Ovine; potency 35 IU/mg). The remaining mice were injected with equal volumes of saline solution (0.85% NaCl). The hormone was dissolved in saline to a concentration of 1 mg/ml. The addition of about 50 μl 1 *N* NaOH per 20 ml of PRL solution helped to dissolve the PRL. Body weights of the mice were recorded twice a week.

The results were analyzed statistically with a two-way analysis of variance.

Results. Pygmy mice weighed about 60% less than normal controls at 3 months of age (Table I). Pituitary glands from pygmy mice were significantly lighter than those from comparable normal controls ($P < 0.01$). The pituitary glands of male pygmies averaged only 29% as heavy as those of the normal males, while those of female pygmies were only about 21% as heavy as glands from normal females. The serum concentrations of GH and PRL in pygmy mice of both sexes were similar to those in controls ($P > 0.05$). However, GH and PRL concentrations in the pituitaries of pygmies were much lower than in the pituitaries of normal mice ($P < 0.01$). Among the males, pituitary GH concentration of the pygmy mice was only about 41% as great as that of the normal mice and PRL content was only 18% as high. Among pygmy females, the pituitary GH concentration averaged 49% and the pituitary PRL concentration was about 15% of the comparable values in normal female mice.

As expected, a single injection of perphenazine induced the release of large amounts of PRL by the pituitary glands of normal mice ($P < 0.01$) (Table II). In contrast, the pituitary glands of the pygmy mice did not respond to treatment with perphenazine ($P > 0.05$).

Daily administration of 100 μg of ovine PRL for 8 weeks did not enhance body weight gain of male pygmy mice appreciably ($P > 0.05$) (Fig. 1). However, in females, a slight increase was observed. The net gain in body weight of PRL-treated females was 20% higher than that of saline-treated controls ($P < 0.01$).

Discussion. While basal serum levels of GH and PRL in dwarf (*dw/dw*) mice have been reported to be clearly subnormal (8), the results of the present study demonstrate that, in the pygmy mouse, the basal serum concentrations of immunoreactive GH and PRL are in the normal range. In contrast, GH and PRL levels in the pituitary gland of pygmy mice and serum PRL levels after a provocative challenge were markedly subnormal. The lack of pharmacologic means of inducing GH release in the mouse has prevented us from performing a stimulatory test for GH release. However, since the pituitary contents of both PRL and GH were quite low, the release of GH may also be expected to be deficient. It is thus possible that the pulsatile secretion of GH and PRL (9), which is the normal mode of secretion for many pituitary hormones, may be lacking or deficient in the pygmy mouse. It should be noted that in the human African pygmy, GH levels were found to be normal not only under basal but also under stimulated conditions of release (10, 11).

The disproportionately undersized pituitary, the marked deficiency of the glandular GH and PRL contents, and the lack of PRL release in response to perphenazine all point to a defect of the pituitary–hypothalamic system in the pygmy mouse. Whether the growth retardation of the pygmy is due to a subresponsiveness of the body tissue to GH, as

TABLE I. PITUITARY AND SERUM CONCENTRATIONS (MEAN ± SEM) OF PROLACTIN AND GROWTH HORMONE IN 3-MONTH-OLD PYGMY (*pg/pg*) AND NORMAL (*Pg/Pg*) (VY/Wf × C3H/Wf) F-1 HYBRID MICE

Sex and phenotype	No. of mice[a]	Body wt (g)	Pituitary gland wt (mg)	Prolactin		Growth hormone	
				Serum (ng/ml)	Pituitary (μg/mg)	Serum (ng/ml)	Pituitary (μg/mg)
Male							
Normal	19	32.5 ± 0.6	2.01 ± 0.04	10.5 ± 0.7	1.68 ± 0.04	62.9 ± 11.9	121.7 ± 3.8
Pygmy	19	13.2 ± 0.2*	0.58 ± 0.01*	13.2 ± 1.2	0.30 ± 0.02*	45.6 ± 10.1	49.7 ± 1.6*
Female							
Normal	19	24.3 ± 0.2	2.17 ± 0.05	23.2 ± 3.7	7.24 ± 0.21	13.8 ± 2.4	68.2 ± 1.2
Pygmy	17	9.8 ± 0.2*	0.46 ± 0.01*	25.4 ± 2.2	1.08 ± 0.08*	19.2 ± 1.5	33.2 ± 2.3*

[a] Age: 90 days.

* $P < 0.01$, comparisons shown are between mice of the same sex.

proposed by King (2) and supported by Rimoin and Richmond (3), or whether it is due to a lack of other growth factors such as thyroid hormones, or a lack of pulsatile release of pituitary somatotrophic hormones, is not clear at this time. Other possibilities may also exist. For example, the immunoreactive GH and PRL circulating in the plasma of pygmy mice may have only weak biological activity. There is growing evidence to suggest that the preponderant isomers of GH and PRL found in the pituitary gland are prohormones that undergo chemical transformation during or after secretion in order to manifest their biological effects (12). We do not know if the enzymatic capabilities to catalyze such chemical transformations are deficient or aberrant in the pygmy mouse. The failure of pygmy mice to respond appreciably to the administration of GH (3) and PRL (Fig. 1) derived from the pituitary raises this type of possibility.

Dramatically subnormal body growth rate in the presence of normal basal GH and PRL concentrations in the blood and the lack of the normal response to stimulation of PRL release by the pituitary gland make the pygmy mouse an exceptionally interesting animal model for the study of mechanisms of hormonal regulation of growth.

Summary. Basal levels of GH and PRL in the serum and pituitary gland of normal (*Pg/Pg*) and pygmy (*pg/pg*) (VY × C3H) F-1 hybrid mice were measured by homologous radioimmunoassays. The serum concentrations of GH and PRL in pygmy mice at approximately 90 days of age were found to be no different from those in corresponding normal controls. The pituitary concentrations of the two hormones, however, were substantially subnormal in pygmy mice. Perphenazine injection failed to stimulate PRL release by pygmy (*pg/pg*) pituitaries, in contrast to the marked increase in serum PRL levels of the normal *Pg/Pg* mice. Daily administration of 100 μg of ovine PRL for 8 weeks produced none or very little increase in the body weight of pygmy mice. These results suggest that the *pg* mutation induces a multifaceted change affecting the synthesis, release, and function of growth hormone and prolactin.

TABLE II. SERUM PROLACTIN CONCENTRATIONS OF PYGMY MICE AFTER AN INJECTION OF PERPHENAZINE

Sex and phenotype	Hours after injection[a]		
	0	½	1
Male			
Normal	8 ± 1(8)	45 ± 2(8)	45 ± 2(7)
Pygmy	12 ± 1(6)	10 ± 2(6)	12 ± 1(5)
Female			
Normal	24 ± 7(7)	317 ± 32(6)	559 ± 39(8)
Pygmy	17 ± 3(7)	23 ± 2(6)	21 ± 2(6)

[a] Dose of perphenazine = 1 μg/g BW. Values given are mean ± SEM. Numbers in parentheses indicate the number of mice in each group.

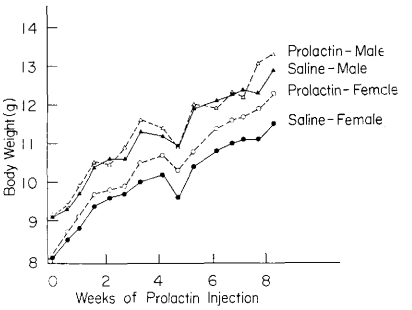

FIG. 1. Effect of daily administration of 100 μg ovine prolactin on body weight gain of pygmy (*pg/pg*) (VY/Wf × C3H/Wf) F-1 hybrid mice. 8–10 mice/group.

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