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Growth hormone and prolactin immunoreactivity in the pituitary gland of postnatal little *(lit)* mice

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Summary. Homozygous little (*lit/lit*) mutant mice exhibit a growth lag which is manifested at approximately two weeks postnatally. Functional aspects of the development of pituitary growth hormone (GH) cells and prolactin (PRL) cells were thus analyzed by means of colloidal gold immunocytochemistry at the ultrastructural level in *lit/lit* mice and their normal counterparts ranging in age from 5 days postnatally to adulthood. In the adult normal and *lit/lit* pituitaries, secretory granules in GH cells and PRL cells showed a positive immunoreaction to their respective antisera, as did granules in both cell-types at 5 days postnatally. By 14 days some GH cells in *lit/lit* pituitaries appeared to be less densely populated with granules than GH cells in normal pituitaries, but a positive immunoreaction continued to occur even in sparsely granulated GH cells. PRL cells showed ultrastructural features in *lit/lit* pituitaries which were similar to those in normal mice, and immunoreactivity was present at all stages examined. The results indicate that since differences in granule reactivity were not evident between *lit/lit* and normal GH cells, despite ultrastructural morphologic differences which were present by 14 days postnatally, manifestations of the defect in *lit/lit* may be primarily quantitative in terms of numbers of granules and/or numbers of GH cells. With respect to PRL cells, neither morphologic nor functional aberrations could be observed; thus, a deficit in PRL hormone production might be the result of a more subtle defect than that in GH cells.

Key words: Pituitary gland - Postnatal mice - Immunoreactivity

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Introduction

In homozygous little (*lit/lit*) mutant mice, there is a pituitary deficiency of growth hormone (GH), as well as of prolactin (PRL) (Eicher and Beamer, 1976; Beamer and Eicher, 1976). Despite these deficiencies, GH cells and PRL cells with ultrastructural features similar to those in normal mice have been observed in the anterior lobe of the pituitary in the adult, as well as in the immature, little mouse (Christensen and Wilson, 1981; Cheng et al., 1983). However, ultrastructural criteria based on conventional electron microscopy, particularly of PRL cells, can be imprecise, and the degree to which GH cells as well as PRL cells are functionally active, especially during development, is not known. Thus, the current immunoelectron microscopic study was undertaken to assess the functional status of GH and PRL cells in the little mutant mouse in the adult pituitary as well as during progressive stages of postnatal development ranging from 5 days to 9 weeks of age.

Materials and methods

Little (*lit/lit*) mice (on a C57BL/6J background) and normal mice (either *lit/+* heterozygotes, +/+ homozygotes, or C57BL/6J mice), were maintained on a 14 hour light, 10 hour dark cycle. Homozygous (*lit/lit*) matings were established to obtain litters consisting entirely of *lit/lit* mice at postnatal ages of 5 and 8 days, i.e. prior to gross manifestation of a definitive growth lag. C57BL litters of comparable age were used as controls. For later stages, *lit/lit* and normal (*lit/+*; +/+) mice were obtained from heterozygous matings. Pregnant females were checked daily for litters, with the date of birth counted as day 0. In the case of *lit/lit* litters where the female was not producing milk, the litter was fostered to a lactating

female. Pituitaries were removed and fixed in 3.0% glutaraldehyde in 0.1M phosphate buffer, pH 7.3, for 6 hours at 4°C. Tissue was rinsed in phosphate buffer and postfixed in 1% OsO_4 for 1 hour at 4°C. Following routine dehydration, tissue was embedded in Epon-araldite 502, and thick sections stained with methylene-azure blue were used for orientation. Thin sections (silver to gray) were collected on bare nickel grids.

Grids were washed for 2 minutes in glass distilled water, etched in 10% H_2O_2 for 2 minutes, rinsed again and stabilized in 0.3% BSA (Bovine Serum albumin fraction V, J.T. Baker or Sigma) in TBS (0.05 M Tris, 0.15 M NaCl, pH 7.6) for 5 to 10 minutes (BSA-TBS). The grids were incubated at room temperature for 1-3 hours in one of the following primary antibodies: rabbit anti-human growth hormone (Dako, diluted 1:500 with BSA-TBS), rabbit anti-rat prolactin (NIADDK, diluted 1:100 or 1:300 with BSA-TBS) or a 1:50 dilution of non-immune rabbit serum (Miles), which served as a control. Radioimmunoassay by the supplier showed the following specificities. For GH, with GH reactivity at 100%, cross reactivity was 1% for prolactin and less than 1% for HPL, FSH, TSH and LH. For PRL, cross-reactivity was less than 0.1% for FSH, LH, GH and TSH at a 1:300,000 antiserum dilution. Following a BSA-TBS rinse, grids were incubated for 1 hour at room temperature in secondary antibody-gold complex (goat anti-rabbit IgG fraction, Cooper Biomedical, conjugated to 4 to 9 nm colloidal gold or Janssen goat anti-rabbit IgG fraction conjugated to 15 nm colloidal gold diluted from 1:10 to 1:45 with BSA-TBS). Following rinses in BSA-TBS, TBS and glass-distilled water, grids were routinely stained in uranyl acetate and lead citrate and observed with a Zeiss 9 electron microscope at direct magnification from 1,800 to 28,000 x.

Results

In the following account, "normal" refers to C57BL, +/+, or *lit*/+ individuals; "abnormal" refers to *lit/lit* individuals.

Adult

In normal adult mice, GH cells are ultrastructurally identifiable on the basis of their round to oval shape and the presence of large, round, homogeneously dense granules of uniform size and shape (Fig. 1). In contrast, PRL cells tend to be less rounded, with homogeneously dense granules varying in size and shape. The GH cell granules were strongly reactive with anti-GH serum, even at a relatively low concentration; the reaction of PRL cells to anti-PRL serum was likewise prominent (Figs. 2, 3). Whereas most of the GH granules were labeled, it was not unusual to see some unlabeled granules in PRL cells. In the control sections, a negative reaction for GH and for PRL occurred when the primary antibody was replaced by a 1:50 dilution of non-immune rabbit serum.

In abnormal mice, GH cells and PRL cells showed ultrastructural features similar to those in normal mice. However, the arrangement of secretory granules in the GH cells appeared to be more sparse than in their normal counterparts (Fig. 4). As in the normal pituitary, the GH cells in the abnormal mice showed a strong reaction to anti-GH serum, with most of the granules labeled (Fig. 5). With anti-PRL, the reaction likewise appeared similar to that in the PRL cells of the normal pituitary (Fig. 6).

Sections that reacted with the same concentrations of anti-GH, but higher concentrations of labeled secondary antibody, showed more dense labeling over the secretory granules of GH cells. However, differences in the degree of labeling between normal and abnormal pituitaries were not evident.

Immature stages

At 5 days after birth, the normal and abnormal pituitaries showed immature but recognizable GH cells with secretory granules, some of which were immunoreactive with anti-GH (Fig. 7). PRL cells were also present and immunoreactive with anti-PRL, though not as consistently or as prominently as were the GH cells with anti-GH. Similar results were obtained at 8 days.

By 14 days, differences in overall granulation, particularly of GH cells, were apparent between normal and abnormal pituitaries (Fig. 8). Morphologically, the GH cells in abnormal pituitaries did not appear to have progressed much beyond their earlier status. whereas GH cells in normal pituitaries were more readily located and appeared to be more densely populated with secretory granules than at earlier stages. Despite their relative immaturity, the GH cells in the abnormals continued to show a positive reaction to anti-GH which was similar to that in their normal counterparts. In contrast to the GH cells, PRL cells in the normal and abnormal pituitaries showed a similar degree of morphologic, as well as functional, maturity.

At 24 days to 9 weeks of age, the GH cells in the normal pituitaries were readily observed and densely filled with granules, whereas those in abnormal pituitaries were less prominent and appeared to be less densely populated with granules. The degree of GH cell immunoreactivity. however, was similar to that in normal pituitaries. As in previous stages, the PRL cells continued to show both morphologic, as well as functional, similarities to their normal counterparts.

Figs. 1-6 Electron micrographs of adult anterior pituitaries from normal (+/+; *lit*/+) or abnormal (*lit/lit*) mice reacted with growth hormone antiserum (anti-GH)or with prolactin antiserum (anti-PRL).

Fig. 1. Normal, low magnification. Note dense population of secretory granules (large arrows) in GH cells (GH) and irregular granules (small arrows) in a PRL cell (P). x 5,400

Fig. 2. Normal, anti-GH. Note positive reaction (black particles) over secretory granules of a GH cell, whereas granules (arrows) of an adjacent non-GH cell are unlabeled. x 24,000

Fig. 3. Normal, anti-PRL. A positive reaction occurs over secretory granules of a PRL cell. x 24,000

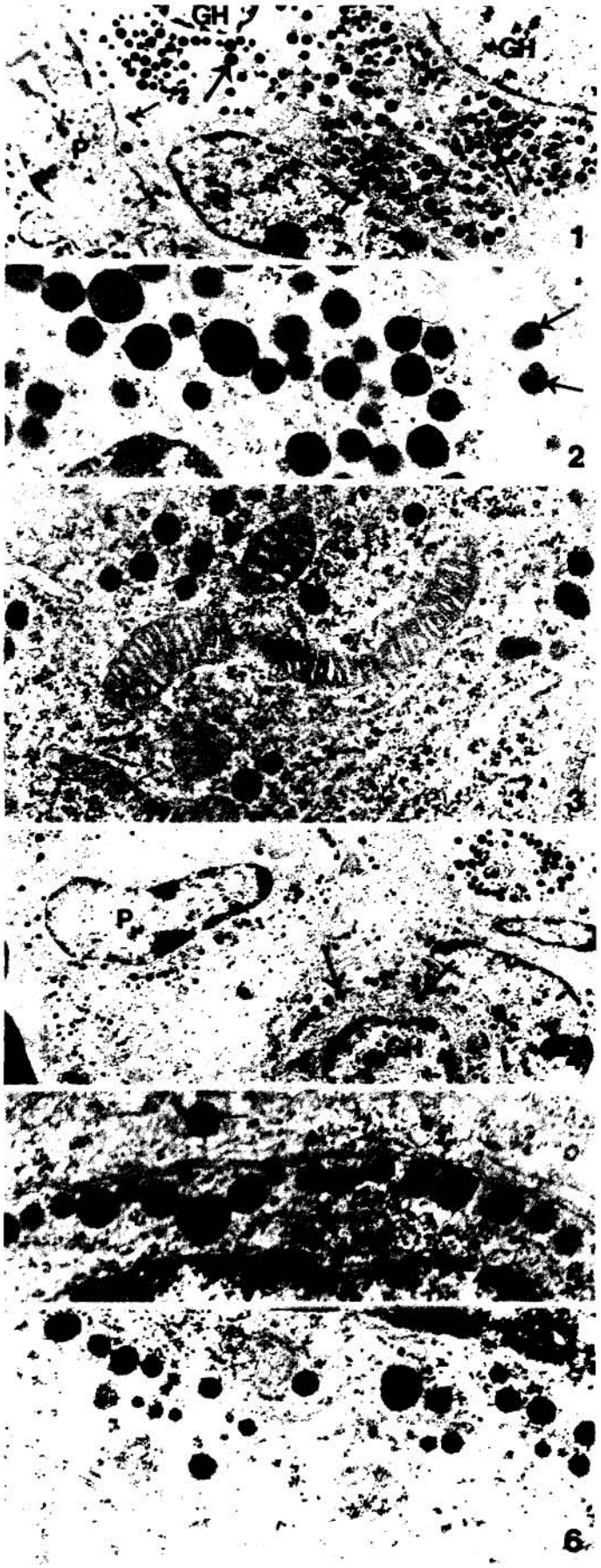
Fig. 4. Abnormal, low magnification. Note relatively sparse population of secretory granules (arrows) in a GH cell (GH) and normal appearance of a nearby PRL cell (P). x 5,400

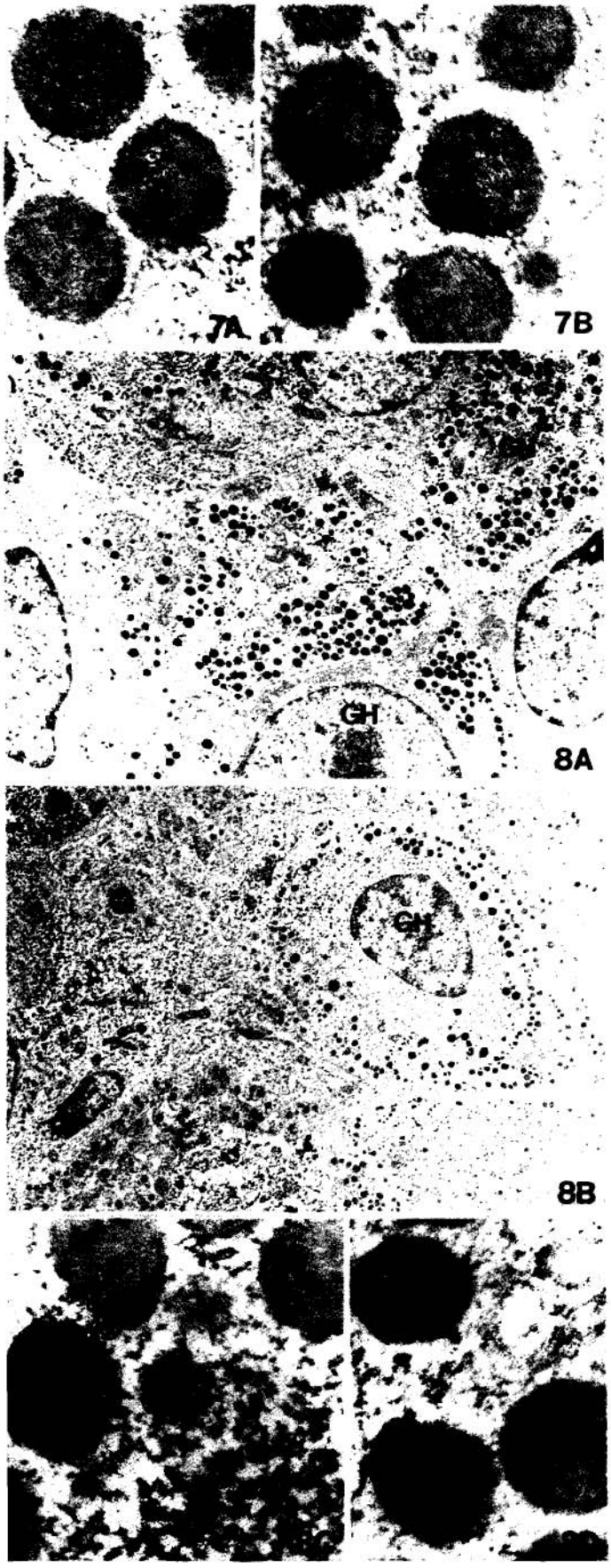
Fig. 5. Abnormal, anti-GH. Secretory granules show a positive reaction (black particles). Note lack of reaction over granule (arrow) of an adjacent non-GH cell. x 24,000

Fig. 6. Abnormal, anti-PRL. A positive reaction occurs over secretory granules of a PRL cell. x 24,000

Fig. 7. 5 days postnatal, anti-GH. High magnification shows a positive reaction over GH secretory granules in A, normal, and B, abnormal, x 84,000

Fig. 8. 14 days postnatal, anti-GH. A, low magnification of normal pituitary shows dense population of secretory granules in GH cells (GH) x 5,400. B, Same magnification of abnormal pituitary shows poorly granulated GH cell. C, High magnification of positive reaction to anti-GH in normal, x 84,000. D, A comparable positive reaction occurs in the abnormal, x 84,000





Discussion

Immunocytochemistry has proved to be of considerable importance in distinguishing various cell-types of the adenohypophysis during embryonic and postnatal development, when morphologic features are not definitive (Setalo and Nakane, **1976;** Tougard et al., **1977;** Li et al., **1977;** Watanabe and Daikoku, **1979;** Chatelain et al., **1979;** Wilson, **1986;** Wilson and Wyatt, **1986).** At the ultrastructural level, the technique is also of use in ascertaining whether cells which appear to be morphologically normal are capable of producing immunoreactive granules and whether all or most granules of a given cell show immunoreactivity. With respect to abnormal (*lit/lit*) adult pituitaries, although the GH cells appear to be less heavily populated with secretory granules than are their counterparts in normal pituitaries (Christensen and Wilson, **1981;** Cheng et al., 1983), the present study demonstrates that most, if not all, of the granules are immunoreactively labeled when exposed to anti-GH, even in very sparsely granuled GH cells. Since GH cells are not encountered in routine EM preparations of abnormal pituitaries as readily as in normal ones, it is possible that the pituitary hormonal deficit in little (Eicher and Beamer, **1976)** may result from a deficit in numbers of GH cells and/or the number of granules produced by individual cells. Although a specific target of the mutant gene lit still remains to be clarified, recent evidence suggests that the GH cells do not respond to growth hormone releasing factor: (GRF) and that this resistance may affect GRF-stimulated synthesis, as well as release of GH (Jansson et al., **1986).**

The situation with respect to PRL cells is more difficult to assess, since a deficit of pituitary PRL has been reported in *lit/lit* mice (Eicher and Beamer, **1976);** however, cells with ultrastructural features resembling normal PRL cells do occur (Christensen and Wilson, **1981).** In the current study, the presence of apparently normal PRL cells was confirmed by their immunoreactivity to anti-PRL. Since, even in normal pituitaries, the degree of granulation of PRL cells can be variable, morphologic differences between normal and abnormal pituitaries could not be readily detected.

Although *lit/lit* females do not lactate subsequent to their first pregnancy, their mammary glands are responsive to exogenous hormones (Keough and Wood, 1979), suggesting that the deficit in pituitary PRL (Eicher and Beamer, **1976)** may be of significance. A morphometric analysis of PRL cells and their granule content might possibly reveal subtle differences in the number, granulation and distribution of PRL cells.

During normal postnatal development of the anterior pituitary, there is a progressive accumulation of granules in GH and PRL cells that can be correlated with hormone content and with postnatal growth patterns (Birge et al., **1967;** Yamashita, **1969;** Wilson and Christensen, **1980).** The current immunocytochemical data indicate that, as early as 5 days postnatally, identifiable and functionally active GH cells are present in *lit/lit* mice, but that morphologic differences in terms of density of granulation may not be discernible until about **14** days. Thus, the fundamental

defect would appear to be one of arrested or decelerated maturation of GH cells, rather than of regression of mature cells. Moreover, the defect in *lit/lit* is clearly different from that in the mouse mutant Snell dwarf (*dw*), in which typical immunoreactive GH cells, as well as PRL cells, are lacking both prenatally and postnatally (Wilson and Christensen, 1981, 1982; Wilson, 1986; Wilson and Wyatt, 1986).

Although the precise time in development when the *lit* gene exerts its effect(s) is unknown, it is of interest that growth retardation in terms of significant weight differences is first detected postnatally at about 15 days (Eicher and Beamer, 1976; Wilson and Christensen, 1980). Subtle differences in GH cells may already be present at 5 days, or even earlier, since Eicher and Beamer (1976) have suggested that the mutants tend to weigh less, though not significantly so, at birth. Thus, a quantitative morphometric analysis of GH cells, particularly during the neonatal period, might provide valuable data concerning the etiology of the pituitary defect in this mutant.

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