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Receptor-Associated Resistance to Growth Hormone–Releasing Factor in Dwarf "Little" Mice

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Anterior pituitaries from the dwarf mouse strain "little" did not release growth hormone or accumulate adenosine 3′,5′- monophosphate (cyclic AMP) in response to human and rat growth hormone–releasing factor (GRF). Dibutyryl cyclic AMP, as well as the adenylate cyclase stimulators forskolin and cholera toxin, markedly stimulated growth hormone (GH) release. The basis of the GH deficiency in the little mouse may therefore be a defect in an early stage of GRF-stimulated GH release related either to receptor binding or to the function of the hormone-receptor complex.

MOST CASES OF CONGENITAL HUMAN growth hormone (GH) deficiency are idiopathic; that is, no organic lesion or other etiological factor can be identified. The disorder may involve only GH (isolated GH deficiency) or may be associated with deficiencies of other pituitary hormones (*1*). Since histological and ultrastructural studies of pituitaries from patients with isolated GH deficiency have revealed somatotrophs capable of GH synthesis (*2*), it has been suggested that stimulation of the pituitary by hypothalamic GH-releasing factor (GRF) is defective in many of these patients (*1*). Such a defect could be attributed to impaired GRF synthesis, release, or transport or to insensitivity of the somatotrophs to GRF.

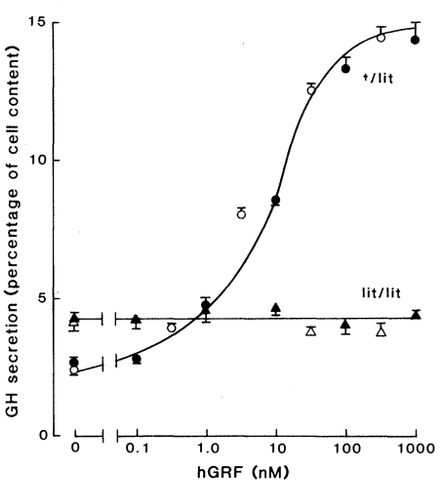

Fig. 1. Effect of hGRF on GH release from dwarf *lit/lit* and normal +/*lit* pituitary cells during a 4-hour incubation. Each point represents the mean ± SEM of four to eight observations. The dose-response curve of +/*lit* mice was analyzed by the ALLFIT program (*22*). Results are pooled data from two separate experiments. Filled and open symbols represent experiments 1 and 2, respectively.

The recessive autosomal little (*lit*) mutation in mice results in decreased growth and partial GH deficiency. It has been suggested that the little mouse represents a useful model for isolated human GH deficiency, especially the inherited isolated GH deficiency type I (*3*). We now report that pituitaries of little mice are completely insensitive to GRF, and we address the cellular basis for this phenomenon.

Homozygous 60- to 80-day-old female little mice (*lit/lit*) were compared with their heterozygous litter mates (+/*lit*), which are phenotypically identical to normal C57 BL/6J (+/+) mice. Anterior pituitaries were dissociated (*4*) and cultured for 3 days before being used. The yield from *lit/lit* and +/*lit* mice ranged from 0.45 to 0.5 × 10^6 and 1.2 to 1.4 × 10^6 cells per pituitary, respectively. Mouse GH was measured by a rat GH radioimmunoassay (RIA) (*5*).

The little mouse (*lit/lit*) pituitary contained only 9 percent of the normal concentration of GH in heterozygotes [902 ± 25 ng (mean ± SEM) compared with 9748 ± 298 ng per 10^5 cells], confirming previous observations (*3*). Incubation of +/*lit* pituitary cells with human GRF (1-40)-OH (hGRF) at 300 to 1000 n*M* for 4 hours stimulated GH secretion by a factor of six (Fig. 1). The half-maximal stimulatory concentration (EC_{50}) of hGRF, was 6 n*M*. In contrast, pituitary cells from *lit/lit* mice did not respond to hGFR, even at a concentration (1000 n*M*) that was more than 100 times that of the EC_{50} for +/*lit* pituitaries. These results are supported by the observation that anesthetized little mice do not release GH in response to the intravenous injection of a GRF fragment (*6*).

Exposure of +/*lit* pituitary cells to hGRF for 24 hours also caused a dose-dependent stimulation of GH release (Table 1), whereas GH secretion from *lit/lit* somatotrophs remained unaffected. Human GRF increased total GH (medium + cells) in cultures of +/*lit* but not *lit/lit* somatotrophs. These results suggest that hGRF-stimulated transcription of the GH gene and subsequent GH synthesis (*7*) does not occur in *lit/lit* pituitaries.

We next examined the cellular mechanisms responsible for the absence of GRF responsiveness in *lit/lit* pituitaries. GRF induces a rapid increase in intracellular adenosine 3′,5′-monophosphate (cyclic AMP) concentrations, which seems to mediate the hormone's effects on GH synthesis and release (*8, 9*). Human GRF had a pronounced dose-related effect on cyclic AMP accumulation in pituitary cells from +/*lit* but not *lit/lit* mice (Fig. 2). The absence of increased cyclic AMP concentrations in *lit/lit* somatotrophs may therefore constitute an etiological factor in the lack of GH response to GRF. To test this possibility, we investigated the effects of a cyclic AMP analog and stimulators of the adenylate cyclase (AC)–cyclic AMP system other than GRF. Dibutyryl cyclic AMP induced a dose-related increase in GH secretion from +/*lit* as well as *lit/lit* pituitaries (Fig. 3). Forskolin, a plant diterpene capable of stimulating the catalytic subunit (C) of the AC system in the absence of the regulatory subunit (G_S) (*10*), stimulated GH secretion in *lit/lit* and +/*lit* cultures. Cholera toxin, an agent that in-

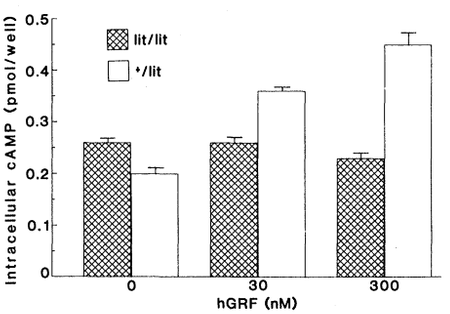

Fig. 2. Effect of hGRF on intracellular cAMP levels as measured by RIA (*23*) in pituitary cells from +/*lit* and *lit/lit* mice. Incubations were of 4 hours' duration. Results are the mean ± SEM of four observations.

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creases the fraction of G_S that is activated (*11*), also induced a marked increase in GH secretion in both genotypes. In contrast, rat GRF enhanced GH secretion from +/*lit* but not from *lit/lit* somatotrophs, further supporting the assumption that *lit/lit* somatotrophs are unresponsive to endogenous mouse GRF.

These results indicate that the G_S and C units of the AC complex, as well as the GH release pathway distal to this complex, are functional in *lit/lit* somatotrophs. Moreover, the absence of a response to GRF is not due to depletion of a readily releasable pool of GH despite the marked reduction of total GH content in the *lit/lit* genotype. Rather, impaired AC activation by GRF seems to be the basis for its lack of effect on GH secretion. The mechanism by which GRF stimulates AC is unknown. However, the stimulatory effect of GRF on AC has been reported to be dependent on guanosine triphosphate (GTP) and more affected by cholera toxin than by pertussis toxin (*12*). These data are compatible with a stimulatory influence of GRF on the G_S subunit after the peptide binds to its membrane receptor (*13*), as has been shown for several other AC activators (*14*). Since the receptor is the only component besides the G_S and C units that is essential for cyclic AMP generation (*15*), and since the defect seems to be limited to the somatotrophs (*3*), the abnormality in *lit/lit* mice is probably due to the absence of GRF receptors or an alteration in their function.

A primary change in somatotroph function is not the only possible explanation for the absence of GRF responsiveness in the *lit/lit* mutant. Extrapituitary factors controlling the *lit/lit* somatotrophs must also be considered. Glucocorticoid and thyroid hormones enhance the responsiveness to GRF (*16*), and glucocorticoid deficiency reduces the number of GRF binding sites (*13*). However, circulating thyroxine and corticosterone concentrations were not decreased in *lit/lit* mice. It is unlikely that somatomedins eliminate GRF responsiveness in *lit/lit* mice, since serum somatomedin activity, which is GH-dependent, is markedly decreased in this strain (*17*). Several observations also argue against the possibility that somatostatin is responsible for the GRF resistance in the *lit/lit* mouse. Most evidence suggests that the inhibitory effect of somatostatin on GRF-induced GH release occurs in part at a step distal to the elevation of cyclic AMP (*8*). It is therefore unlikely that somatostatin could totally inhibit GRF-induced GH release in *lit/lit* mice without affecting the response to dibutyryl cyclic AMP or to AC activators. Furthermore, enhanced somatostatin activity could not explain the decreased GH gene transcription and GH content in the pituitary (*3*), since several studies indicate that somatostatin inhibits GH release but not GH synthesis (*9, 18*).

Our results do not exclude the possibility that the primary defect caused by the *lit/lit* mutation is the absence of hypothalamic synthesis and release of GRF. Prolonged absence of stimulation by endogenous GRF in GH-deficient patients may decrease the GRF responsiveness of the somatotrophs, possibly as a consequence of reduced receptor function (*19*). Long-term treatment of humans and rodents with GRF can also enhance the response to subsequent GRF administration (*20*). However, 15 to 60 percent of patients with GH deficiency (*19, 21*) as well as little mice (*6*) do not release GH in response to GRF even after priming. Our results raise the possibility that GRF-resistant human isolated GH deficiency, like dwarfism in the little mouse, is caused by a GRF receptor–associated abnormality.

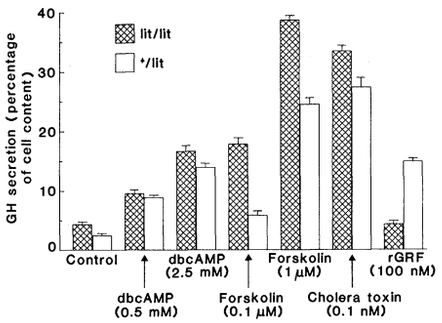

Fig. 3. The effect of dibutyryl cyclic AMP (dbcAMP), forskolin, cholera toxin, and rat GRF on GH release from pituitary cells of +/*lit* and *lit/lit* mice. Incubations were of 4 hours duration, and each value represents the mean ± SEM of four to eight determinations. Results are pooled data from two separate experiments.

Table 1. Effect of hGRF on GH secretion and cell content in pituitary cell cultures from +/*lit* and *lit/lit* mice. Each value represents the mean ± SEM of four observations.

Treatment	GH (nanograms per well per 10^5 cells)		
	Secretion*	Cell content	Total
		+/lit	
Control	1,256 ± 48	6,568 ± 220	7,823 ± 254
hGRF (10 n*M*)	2,932 ± 162†	7,360 ± 296	10,292 ± 243†
hGRF (100 n*M*)	3,870 ± 139†	6,169 ± 268	10,038 ± 399†
		lit/lit	
Control	453 ± 21	903 ± 34	1,356 ± 17
hGRF (10 n*M*)	409 ± 20	950 ± 30	1,358 ± 36
hGRF (100 n*M*)	438 ± 7	1,017 ± 64	1,455 ± 70

*Secreted during 24-hour incubation. †$P < 0.01$ compared with the corresponding control group (single-factor analysis of variance followed by Duncan's new multiple-range test).

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