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Pituitary Growth Hormone and Its Hypothalamic Releasing Factor in Normal and Genetically Diabetic Mice* (33474)

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Metabolic disturbances resembling the onset of diabetes mellitus at maturity in man occur in mutant diabetic mice of the C57BL/Ks strain (1). Mice homozygous for the diabetic gene (db/db) develop hyperglycemia between 4–5 weeks of age (2). Two weeks before hyperglycemia can be detected plasma insulin levels are unequivocally higher than normal indicating that the diabetic syndrome is not due to insulin insufficiency (2, 3). The failure of excess circulating insulin to maintain normal concentrations of blood sugar in diabetic mice suggests that the hypoglycemic action of insulin may be antagonized by excess growth hormone (4). These facts prompted us to compare the concentration of pituitary growth hormone (GH) and its hypothalamic releasing factor (GH-RF) in normal and diabetic mice during the transition from mild to severe hyperglycemia. The results show that mildly hyperglycemic mice had significantly less GH and GH-RF than normals and that severely hyperglycemic mice had significantly more GH and GH-RF than normals.

Materials and Methods. Normal mice (C57BL/6J) and outcrossed diabetic db/db mice of both sexes were obtained at 4–5 and 12–13 weeks of age (1). These age groups were chosen in order to study pituitary GH and hypothalamic GH-RF in diabetic mice shifting from mild to severe hyperglycemia. Previous studies revealed that young diabetic mice are mildly hyperglycemic (200 mg/100 ml vs 150 mg/100 ml for normal mice) and markedly hyperinsulinemic (350 μU/ml vs 40 μU/ml for normal mice) while older diabetic mice are severely hyperglycemic (500 mg/100 ml) with nearly normal concentrations of circulating insulin (65 μU/ml), (2,

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3). The mice were fed pelleted diet number three (5) *ad libitum* and housed in cages (15 × 15 × 30 cm) containing 4–6 mice. Animals were decapitated between 1 and 5 p.m. over a 9-month period to obtain pituitaries and hypothalami.

Pools of 80 pituitaries were collected for each genotype, sex, and age category. Pituitaries were homogenized (10 mg/ml) in 0.85% sodium chloride, centrifuged at 10,000*g* for 10 min at 5°, and the supernatant fluid from each pool was assayed for GH by injecting (i.p.) hypophysectomized rats (14 days after surgery) for 4 days. Twenty-four hr after the last injection the left tibia was removed and the width of the proximal epiphyseal cartilage was measured (6). Five rats were used for each dose level of unknown or standard. Pituitary GH concentration was determined with 1.0-/and 2.0 mg-equivalents of fresh tissue and this was compared with 30 and 60 μg of NIH-GH-S8. Three replicate assays were performed on each pituitary pool. Relative potencies were calculated (7) and the results of replicate assays were combined (8). Potency estimates were subjected to analysis of variance and orthogonal comparisons were used to evaluate treatment differences.

Pools of 25 hypothalami were collected for each genotype and age category (one half from each sex). Control tissues were removed from the cerebral cortex of the same mice and treated as hypothalami except that the tissues from 4–5 and 12–13-week-old animals were pooled within genotype. Hypothalami were placed in 0.1 *N* HCl at 5°, homogenized, adjusted to pH 7.0 with 0.1 *N* NaOH, and centrifuged at 10,000*g* for 10 min at 5°. The supernatant fluid from each pool was assayed for GH-RF by injecting 1.0 ml (equivalent to 5 hypothalami) into the carotid artery of 5 male rats (250–300 g of body wt.) under ether anesthesia (9). Thirty min after administering the extract, the rats were decapitated, pituitaries were collected, pooled, homogenized, and centrifuged as outlined. The GH remaining in each pool of five pituitaries was assayed by measuring the width of the proximal epiphyseal cartilage of five hypophysectomized rats after injecting 2.5 mg-equivalents of fresh pituitary tissue per rat over 4 days (9).

TABLE I. Mean Concentration of Pituitary Growth Hormone (GH) in Normal and Diabetic Mice.

Genotype	Sex	Age (weeks)	Pituitary GH (95% limits)*
Normal	♂	4–5	20.2 (15.7–25.1)
	♀	4–5	21.3 (16.1–26.7)
	♂	12–13	22.7 (16.8–28.9)
	♀	12–13	19.8 (12.2–27.8)
Diabetic	♂	4–5	11.3 (6.3–16.4)
	♀	4–5	12.7 (6.5–19.0)
	♂	12–13	36.9 (27.4–48.6)
	♀	12–13	38.2 (29.3–47.4)

* Each value represents the combined potency and 95% confidence limits of three replicate assays expressed in μg-equivalents of NIH-GH-S8 per mg of fresh pituitary. Combined index of precision was 0.21.

In a preliminary experiment, a linear response was obtained between the logarithm of the concentration of mouse hypothalamic extract injected and the amount of rat pituitary GH released indicating that this procedure could be used to measure the GH-RF content of mouse hypothalami.

Results. The mean concentrations of pituitary GH in normal and diabetic mice are summarized in Table I. Analysis of variance revealed that within the same genotype and age the differences in pituitary GH concentration due to sex were not significant ($p>0.25$). Consequently, the GH concentrations of males and females within the same genotype and age category *were* combined to evaluate differences in GH concentrations among ages and between genotypes. The mean pituitary GH concentration of normal mice was approximately equal ($p>0.25$) at 4–5 and 12–13 weeks of age, but the mean pituitary GH concentration of diabetic mice increased from 12.0 to 36.7 μg/mg between 4 and 12–13 weeks ($p<0.02$). Pituitaries from normal mice contained an average of 8.7 μg/mg more GH than diabetics ($p<0.05$) at 4–5 weeks of age but at 12–13 weeks the mean pituitary GH concentration of normals was 16.4 μg/mg less than diabetics ($p<0.05$) of the same age.

TABLE II. Growth Hormone Content of Rat Pituitaries Following Intracarotid Injections of Hypothalamic Extracts from Normal and Diabetic Mice.

Treatment[a]		Age (weeks)	Width of epiphyseal cartilage[b]
Normal CC ext.		4–13	251 ± 10.4
Normal Hyp ext.		4–5	164 ± 6.8
		12–13	169 ± 7.1
Diabetic CC ext.		4–13	247 ± 10.1
Diabetic Hyp ext.		4–5	209 ± 9.6
		12–13	125 ± 4.9
Growth hormone (μg)	0	—	108 ± 3.8
	30	—	151 ± 4.4
	60	—	223 ± 9.4

[a] CC ext. = cerebral cortex extract; Hyp ext. = hypothalamic extract.

[b] Pituitary growth hormone content expressed as the mean ± SE of the width (μ) of the proximal tibial epiphyseal cartilage of five hypophysectomized rats.

The mean pituitary GH content of rats receiving intracarotid injections of hypothalamic extracts from normal and diabetic mice is shown in Table II. Hypothalamic extracts from normal and diabetic mice reduced the pituitary GH content of recipient rats significantly more than extracts prepared from cerebral cortex ($p<0.05$) indicating that hypothalami contained GH-RF. Hypothalamic extracts from normal mice of both age groups depleted the pituitary GH content of rats similarly ($p>0.25$) suggesting that these hypothalami contained equivalent amounts of GH-RF. In contrast, hypothalami from 4–5-week-old diabetics did not deplete the pituitary GH stores of rats as effectively as hypothalami from 12–13-week-old diabetics ($p<0.05$). Hypothalamic extracts obtained from normals at 4–5 weeks reduced the pituitary GH content of rats significantly more than extracts prepared from 4–5-week-old diabetics ($p<0.05$), but hypothalami from 12–13-week-old normals evoked significantly less GH release from rats than hypothalami from 12–13-week-old diabetics ($p<0.05$). This suggests that the hypothalami of the young mildly hyperglycemics contain less GH-RF than normals and that the older severely hyperglycemics have more GH-RF than normals.

Discussion. The results presented here indicate that the concentration of pituitary GH and the neurohumor that controls its release are markedly altered in diabetic mice during the transition from mild to severe hyperglycemia. The concentrations of pituitary GH and hypothalamic GH-RF were lower than normal in mildly hyperglycemic mice, but during severe hyperglycemia the concentrations of GH and GH-RF were greater than normal. The changes in pituitary GH were positively related to changes in hypothalamic GH-RF in this study and hence it may be suggested that in mildly hyperglycemic mice GH release was greater than normal since minimal levels of pituitary GH and hypothalamic GH-RF were recorded. Conversely, the detection of abnormally high levels of pituitary GH and hypothalamic GH-RF may reflect the absence of GH release in severely hyperglycemic mice. This interpretation of the present results agrees with the observations of Müller *et al.* (10) that the concentration of plasma GH is raised above normal. when both the concentrations of pituitary GH and hypothalamic GH-RF are depressed. Other evidence also confirms the existence of a reciprocal relationship between the level of GH in the blood and the neurohumor that controls its release (11).

Since prolonged GH administration antagonizes the hypoglycemic action of insulin and increases pancreatic insulin secretion in a number of species (4), it is possible to argue that excess GH secretion during the early stages of diabetes may account for the mild hyperglycemia and the greater than normal concentrations of plasma insulin characteristic of young diabetic mice (2, 3). This conclusion is consistent with the present findings that GH release appeared to be in excess of normal in mildly hyperglycemic mice. In contrast to mild hyperglycemia, severe hyperglycemia resulting from glucose infusions suppresses GH release in primates (12) and supports the suggestion that the severe hyperglycemia characteristic of older diabetic mice may have inhibited GH release and thus ex-

plain the abnormally high concentrations of pituitary GH and hypothalamic GH-RF detected at 12–13 weeks of age. This interpretation also agrees with the observation that hyperglycemia produced by glucose administration leads to an increase in pituitary GH content (13).

Whether the transition from mild to severe hyperglycemia was directly associated with the observed changes in pituitary GH and hypothalamic GH-RF or caused by the coexistent secretion of other hormones remains to be shown. Nevertheless, insulin antagonism and hyperglycemia have been consistently related to GH (4).

Summary. Pituitary GH and hypothalamic GH-RF were determined in normal and in genetically diabetic (db/db) mice during mild and severe hyperglycemia. Mildly hyperglycemic mice had above normal concentrations of GH and GH-RF ($p<0.05$), whereas the concentrations of GH and GH-RF were below normal in severely hyperglycemic mice ($p<0.05$). The results suggest that mild hyperglycemia may be stimulated by excessive GH release during the early stages of diabetes and that GH release may be inhibited by severe hyperglycemia during the latter stages of diabetes.

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