Increase in Plasma Growth Hormone (GH)-Like Activity After Administration of Porcine GH-Releasing Hormone[1] (35606)

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The existence of a neural control of growth hormone (GH) secretion has been repeatedly suggested in recent years (1, 2). This suggestion is based on the demonstration in the hypothalamus of many animal species of a specific activity, designated GH-releasing factor (GRF) or GH-releasing hormone (GH-RH) (1–4). Crude hypothalamic extracts (5), as well as highly purified preparations (2, 6), have been shown to stimulate the release of GH *in vitro* during "short-term" incubation or tissue cultures of rat pituitaries and to deplete pituitary GH content *in vivo* (2, 7–9). In all these *in vitro* and *in vivo* experiments, determinations of GH were made by bioassay, using "the tibia test" (10). Following recent introduction of a radioimmunoassay (RIA) for rat GH (11) capable of detecting minute amounts of this hormone in plasma, attempts were made to develop assays for GH-RH based on RIA for GH. However, the results of the pituitary-depletion test, when GH was measured by RIA appeared to be substantially different from those obtained when GH was measured by bioassays, since no significant changes in either plasma or pituitary RIA-GH concentration were observed in the rat following injection of hypothalamic extracts (12). Moreover, the validity of the pituitary-depletion assays as a measure of secretion of a pituitary hormone has been questioned by Rodger *et al.* (13) on the basis of experiments in which crude ovine hypothalamic preparations failed to deplete pituitary GH content in rats.

Nevertheless, purification procedures for GH-RH in the hypothalamic extracts of domestic animals were developed using the pituitary GH depletion method (2). These led to the isolation of GH-RH on a large scale from porcine hypothalami (14). In the work reported below, the ability of pure porcine GH-RH to deplete pituitary GH content and simultaneously to increase plasma GH-like activity was investigated in the rat using the "tibia test" method. This work offers new evidence that pituitary depletion usually detected by bioassay is specific and results from release of GH from the pituitary.

Materials and Methods. GH-RH preparation. Porcine GH-RH was isolated as described previously by Schally *et al.* (14, 15). The preparation was homogeneous chromatographically and electrophoretically and was active both *in vivo* and *in vitro* (14, 15).

Test for GH-releasing activity of GH-RH. Sprague-Dawley female rats (120–130 g of body wt) obtained from a local breeder, were used as recipient animals. While under ether anesthesia they were given 0.5 ml of acidified saline (0.9% sodium chloride solution in 0.0005 *M* acetic acid) or different doses of GH-RH in 0.5 ml of acidified saline by intracarotid injection (7).

Fifteen min after the injections the rats were decapitated and blood samples from each group were collected and pooled in heparinized centrifuge tubes. The plasma was separated by centrifugation. Anterior pituitaries were removed, weighed to the nearest 0.05 mg on a microtorsion balance and then pooled by groups and homogenized in 0.9% saline. The pituitary and plasma samples were kept frozen at −20° until assayed for GH.

GH assay. GH activity of the samples was measured by the "tibia test" method of

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Greenspan *et al.* (10). Female Wistar rats, hypophysectomized at 26–28 days of age by the transauricular approach of Falconi and Rossi (16) (obtained through the courtesy of Dr. G. Falconi) were used as the assay animals. Bovine GH (first standard, WHO) was used as the reference standard. GH potencies of the samples were calculated by a 4-point assay according to Finney (17). Significance of differences in epiphyseal cartilage width was tested by Student's t test or by factorial analyses.

Results. In the first experiment, plasma (1 ml) and pituitary homogenates from rats injected with saline or 350 ng of GH-RH were given ip to hypophysectomized rats daily for 4 days. The results are shown in Table I. Administration of GH-RH induced a significant fall in pituitary GH content with a simultaneous rise in plasma GH-like activity. The mean width of epiphyseal cartilage of hypophysectomized rats injected with 4 ml of plasma from saline-treated animals was 166 $\mu \pm 3.4$, a value not significantly different from that of animals not given GH (159 $\mu \pm 2.7$). Since the minimum effective dose of the WHO GH standard which caused a significant increase of tibial cartilage width was 5 μg, GH-like activity in plasma of saline-treated rats was less than 1.25 μg/ml. The mean cartilage width in response to 4 ml plasma from animals which received GH-RH was 212 μ, a value significantly higher ($p = 0.001$) than that found in response to plasma from saline-injected control rats. The cartilage width of 212 μ corresponded to 2.5 μg of GH-like activity/ml of plasma in terms of the WHO GH standard.

In a subsequent experiment GH-RH was given ic in doses of 80 and 400 ng, and again plasma GH-like activity and pituitary content of GH were evaluated and compared with those of saline-treated animals (Table II). Both doses of the neurohormone, elicited a significant depletion of pituitary GH content, but only with the higher dose was there concomitant rise in plasma GH-like activity. In this instance, the mean cartilage width in response to 4 ml of plasma from rats given 400 ng of GH-RH was 222 μ, a value which represents approximately 6 μg of GH-like activity/ml in terms of the standard used. There was no difference between cartilage widths of assay animals given saline or control plasma and those given plasma from rats treated ic with 80 ng of GH-RH.

Discussion. The present study demonstrates that intracarotid injection of 350 ng of pure porcine GH-RH depleted pituitary GH and this fall was accompanied by an unequivocal increase in plasma GH-like activity. These data are in agreement with those of Sawano *et al.* (18), who reported increased GH-like activity in plasma of animals injected with purified GH-RH and provide further evidence for the existence of a hypothalamic neurohormonal control over GH secretion.

The validity of the pituitary depletion assays as an index of secretion of a pituitary hormone has been criticized (13). However our work demonstrates that pituitary depletion of GH is followed by a rise of GH-like activity in the blood stream. This is most likely to be a specific effect of the injected substance on the pituitary (2, 4, 7–9, 18). Particularly, the results of the second experiment indicate that the depleting effect of pure GH-RH reflected true release of GH into plasma. When GH-RH was tested at two dose levels, 80 and 400 ng, it induced a dose-related fall in pituitary GH, which in the case of the higher dose was concomitant with a greater rise of plasma GH-like activity than in the first experiment when 350 ng were used. Eighty ng, which was active in depleting pituitary GH, did not elicit a rise in plasma GH detectable by the "tibia test."

Our efforts to evaluate levels of GH-like activity in 1 and 2 ml of plasma given daily and to compare these levels by a 4-point assay with the responses to 2 doses of the WHO GH-standard, were frustrated by the adverse effects observed in the assay animals after administering 2 ml of plasma. Bleeding at the injection sites, bloody feces, severe discomfort and death of many of the animals were observed. These phenomena, also noted by Sawano *et al.* (18), did not allow completion of this part of the study.

Without doubt, the sensitivity of the radioimmunoassay for rat GH in plasma exceeds

TABLE I. Pituitary and Plasma GH-Like Activities 15 min After Intracarotid Injection of 350 ng of Porcine GH-RH.

	Pituitary GH content				Plasma GH-like content			
Material injected	Dose (mg/rat)	Cartilage width[d] ($\mu \pm$ SE)	(μg/mg with 95% fiducial limits)	p	Dose (ml/rat)[c]	Cartilage width[d] ($\mu \pm$ SE)	(μg/ml)	p
Saline[a]	2.0	253 ± 1.9 (8)	44.4 (29.9–61.9)[e]	—	4.0	166 ± 3.4[b] (6)	<1.25	—
	0.5	211 ± 3.8 (6)						
GH-RH[a]	2.0	226 ± 8.4 (6)	16.9 (5.3–32.3)[e]	0.001	4.0	212 ± 8.0 (6)	2.5	0.001
	0.5	198 ± 5.7 (6)						
GH standard	0 μg	159 ± 2.7 (6)						
	10 μg	212 ± 2.1 (6)						
	40 μg	229 ± 9.1 (6)						
	160 μg	271 ± 4.7 (6)						

[a] Twenty recipient rats/group.

[b] This cartilage width was not significantly different from that of hypophysectomized rat not given GH (159 ± 2.7).

[c] Sixty recipient rats/group.

[d] Number of hypophysectomized assay rats in parentheses.

[e] Although the 95% fiducial limits for pituitary content of GH slightly overlapped when calculated against the GH standard, the variance ratio F (33.9) indicated a significant difference between the groups.

TABLE II. Pituitary and Plasma GH-Like Activities 15 min After Intracarotid Injection of Different Doses of Porcine GH-RH.

	Pituitary GH content					Plasma GH-like activity			
	Dose								
Material injected	GH-RH (μg)	(mg/rat)	Cartilage width[c] (μ ± SE)	(μg/mg with 95% fiducial limits)	p	Dose (ml/rat)	Cartilage width[c] (μ ± SE)	(μg/ml)	p
Saline[a]		3.0	260 ± 2.0 (6)	27.5 (16.0–58.9)[d]	—	4.0	178 ± 10.4[b] (6)	<1.25	—
		0.75	229 ± 1.4 (6)						
GH-RH[a]	0.08	3.0	245 ± 2.1 (6)	15.8 (13.8–22.3)[d]	0.001	4.0	174 ± 1.6[b] (6)	<1.25	NS
		0.75	221 ± 3.1 (6)						
GH-RH[a]	0.4	3.0	231 ± 4.2 (6)	8.5 (2.6–13.5)	0.001	4.0	222 ± 5.3 (6)	>6.0	0.01
		0.75	212 ± 3.9 (6)						
GH standard		0 μg	167 ± 2.8 (6)						
		10 μg	216 ± 3.5 (6)						
		40 μg	233 ± 1.5 (6)						
		160 μg	278 ± 1.6 (6)						

[a] Twenty recipient rats/group.

[b] This cartilage width was not significantly different from that of hypophysectomized rats not given GH (167 ± 2.8).

[c] Number of hypophysectomized assay rats in parentheses.

[d] Although the 95% fiducial limits for pituitary content of GH slightly overlapped when calculated against the GH standard, the variance ratio **F** (30.8) indicated a significant difference between the group treated with saline and that which received 0.08 μg GH-RH.

that of bioassay by a factor of at least 100. In spite of this, however, we were able to detect by bioassay a net increase in plasma GH-like activity, while negative results have been reported in the rat after administration of hypothalamic extracts when GH was measured by radioimmunoassay (12). No compelling hypothesis can be formulated at the present time to explain the discrepancy in these results. It must be recalled, however, that the adequacy of radioimmunoassay for GH in plasma has been questioned recently as a result of the studies of Bala *et al.* (19) in man. These authors showed that RIA of GH in plasma is different from RIA of GH extracted from the pituitary and that probably radioimmunoassayable GH molecules are present in plasma in aggregated forms. On the other hand, Knobil (20), Garcia and Geschwind (21), and Machlin *et al.* (22) observed that administration of crude hypothalamic extracts of domestic animals induced significant increases in plasma GH levels in monkeys or in sheep when measured by RIA.

It is unlikely that the large difference in the "tibia test" after administration of pituitary or plasma samples of animals injected with GH-RH can be accounted for by the action of pituitary hormones other than GH (23). The GH-RH used in these experiments is known to be free of other known releasing and inhibiting factors (2, 14, 15). Contamination with TRH is also excluded by the results obtained by Sawano *et al.* (18), so that participation of TSH in the response of the tibia cartilage to the injections of plasma is highly improbable.

Prolactin can affect the width of epiphyseal cartilage to a small degree (23). However, possible contamination of GH-RH with a prolactin-inhibiting factor would result in blockade of prolactin release from the pituitary (24), which is not in agreement with the narrower tibial width induced by pituitary homogenates following administration of GH-RH. In any case, the amounts of prolactin present in the rat pituitary can make only a very small contribution to the increase of tibial epiphyseal width (Arimura and Schally, unpublished) and cannot account for the result of this and other similar studies (2, 4, 7–9, 18).

In conclusion, the present results clearly prove that the pituitary depletion of GH observed after administration of what we considered to be pure GH-RH, is indicative of release of GH and offer further evidence for the biological significance of the material purified on the basis of the bioassay method.

Summary. The ability of pure growth hormone (GH)-releasing hormone (GH-RH) to deplete pituitary GH content and simultaneously to increase plasma GH-like activity was investigated in the rat using the "tibia test" method. GH-RH (350 ng) induced a significant fall in pituitary GH content which was accompanied by increased GH-like activity in plasma. GH-RH administered at two dose levels, 80 and 400 ng, induced a dose-related fall in pituitary GH. There was a concomitant rise in plasma GH-like activity of rats given the larger dose. Plasma GH-like activity in rats injected with saline was not detectable. These results indicate that the pituitary depletion of GH content in rats caused by GH-RH reflects true release of the hormone.

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