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Pituitary and Plasma Growth Hormone-Like Activity after Administration of Highly Purified Pig Growth Hormone-Releasing Factor* (32857)

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Evidence for the existence of a growth hormone-releasing factor (GRF) in the hypothalami of animals of various species and humans has been reported using *in vitro* and *in vivo* methods by many laboratories (1-10). The *in vivo* studies were based on the depletion of pituitary growth hormone (GH) content in recipient rats following the administration of extracts from hypothalamic tissue. Since 30-60% depletion of the pituitary stores in GH in rats was induced by crude extracts (5) or purified GRF preparations (7) from pig or beef hypothalamic tissue, it was hypothesized that it would be possible to detect increased plasma levels of GH-like activity in the rat, even with the relatively insensitive tibial cartilage bioassay, if the "depletion" truly reflects "release." However, crude hypothalamic extracts are reported to contain substances other than GRF, such as vasopressin or adrenaline, which stimulate the release of GH from the pituitary in monkeys as measured by radioimmunoassays (11,12). Thus it is desirable to utilize more purified GRF preparations free from these contaminants. Recently, purification procedures for GRF in the hypothalami of domestic animals have been developed in our laboratory and a highly

purified pig GRF preparation, which is free from vasopressin and other known releasing factors, has become available (13).

The purpose of the present study is to establish that the "depletion" of pituitary GH content in rats caused by injection of the highly purified GRF actually indicates the "release" of GH, by making simultaneous determination of pituitary and plasma GH-like activity with the "tibia test" method (14). The TSH activities of the pituitary and plasma were also determined, since it was reported that TSH can influence the "tibia test" (15) and also to verify the specificity of action of GRF.

Materials and Methods. Highly purified preparations of GRF were obtained from lyophilized, defatted pig hypothalami by extraction with 2 N acetic acid followed by heating, lyophilization, reextraction with glacial acetic acid, gel filtration on Sephadex G-25, free flow electrophoresis, and chromatography on CMC. The details of the procedures have been described elsewhere (13).

Test for GH-releasing activity of the GRF. Sprague-Dawley female rats (130-140 gm body wt.), obtained from Cheek-Jones Co., Houston, Texas, were used as the recipient animals of the test samples. The rats were given 0.5 ml of saline or 4 µg of GRF in 0.5

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ml saline by intracarotid injection under ether anesthesia (3). Fifteen min after the injection, the rats were decapitated and blood samples from each group were pooled in heparinized centrifuge tubes. The plasma was separated by centrifugation at 3000 rpm for 10 min at 4°C. Anterior pituitaries from each group were also pooled and homogenized in 0.9% saline. The pituitary and plasma samples were kept frozen until assayed for GH. The GH activity of the samples was measured by the "tibia test" method of Greenspan *et al.* (14). Sprague-Dawley female rats, hypophysectomized 26 days after birth, obtained from Hormone Assay Labs., Chicago, Ill., were used as the assay animals for GH. NIH-GH-S7 was used as the reference standard. The TSH activity of the pituitary and plasma samples was measured by the method of McKenzie (16) using the USP reference standard. The GH potencies of the samples were estimated from a bracketed 3-point assay by comparing the tibia width response to 1 dose of pituitary or plasma sample with the responses to 2 doses of the NIH-GH standard in each experiment, or 4-point assay according to Finney (17). Significance of the differences between the groups was determined by Student's *t* test or factorial analysis (17). The TSH potencies of the pituitary and plasma samples were calculated by a 4-point assay (18).

Results. To evaluate the plasma GH-like activity after the administration of GRF, 1 ml of plasma was injected intraperitoneally into a hypophysectomized assay rat daily for 4 days. The results are shown in Table I. Administration of 4 μ g of GRF resulted in a significant decrease in pituitary GH content with a concomitant increase in plasma GH-like activity in both experiments. In Exp. 1, the mean tibia width of assay rats injected with 4 ml of control plasma was 152 μ . This value was not significantly different as compared with that from animals not given GH, indicating no appreciable changes in plasma GH-like activity in the donor animals after saline injections. Since the minimum effective dose of the NIH-GH standard which stimulated a significant growth of tibia width was 5 μ g, GH-like activity in control plasma from saline injected rats was less than 1.25 μ g/ml. How-

TABLE I. Pituitary and Plasma GH-like Activities 15 Min after Intracarotid Injection of 4 μ g of Highly Purified Pig GRF.

Material injected	Pituitary GH content				Plasma GH-like activity			
	Dose mg/rat	Cartilage width ^d $\mu \pm$ SE	μ g/mg with 95% fiducial limits	<i>p</i>	Dose ml/rat	Cartilage width ^d $\mu \pm$ SE	μ g/ml	<i>p</i>
Expt. 1 ^a								
Saline	1.2	240 \pm 5.6 (8)	50.0	—	4.0	152 \pm 3.2 ^b (7)	<1.25	—
GRF	1.2	216 \pm 7.9 (8)	30.7	0.05	4.0	176 \pm 2.5 (7)	4.1	0.001
Expt. 2 ^c								
Saline	1.2	226 \pm 6.8 (6)	71.4 (57.7–90.3)	—	4.0	155 \pm 1.6 ^b (7)	<1.25	—
	0.3	192 \pm 3.3 (7)						
GRF	1.2	197 \pm 3.2 (8)	41.5 (32.9–49.6)	0.001	4.0	176 \pm 3.0 (6)	2.5	0.001
	0.3	170 \pm 3.3 (7)						

^a Twenty-seven recipient rats per group. The cartilage width of hypophysectomized rats not given GH in Expt. 1 was 144 \pm 3.3.

^b This cartilage width was not significantly different from that not given GH.

^c Eighty-one recipient rats per group. The cartilage width of hypophysectomized rats not given GH in Expt. 2 was 145 \pm 1.6 in pituitary GH group and 149 \pm 2.0, respectively, in plasma GH group.

^d No. of hypophysectomized assay rats in parenthesis.

TABLE II. Pituitary and Plasma TSH Concentration 15 Min after Intracarotid Injection of 4 μg of Purified Pig GRF.

Material injected ^a	Pituitary TSH content		Plasma TSH level (mU/ml)
	(mU/mg) ^b	95% fiducial limits	
Saline (81)	34.3 (10)	24.8–48.3	<0.05
GRF (81)	37.8 (10)	22.4–63.7	<0.05

^a Number of recipient rats in parenthesis.

^b Number of assay mice in parenthesis.

ever, the mean tibia width in response to 4 ml plasma from rats given 4 μg of GRF was 176 μ , which was significantly wider than that in response to plasma from donor rats given saline. The tibia width of 176 μ represented approximately 4.1 $\mu\text{g}/\text{ml}$ of GH-like activity in plasma in terms of the NIH-GH-S7. Similarly, in the second experiment, the GH-like activity in plasma increased significantly after injection of 4 μg of GRF. There was no difference between tibia widths of assay animals given control plasma and those given saline only. An average concentration of GH-like activity in plasma after injection of GRF was 2.5 $\mu\text{g}/\text{ml}$ in terms of the NIH-GH-S7.

Since TSH was reported to affect the tibia test (15), TSH potencies in the pituitary and plasma samples used in Expt. 2 were also measured. As shown in Table II, pituitary TSH content was essentially the same and plasma TSH was less than 0.05 mU/ml with the method used for both groups. These results suggest that TSH did not affect the results of the tibia test and that GRF releases only GH and not TSH.

Discussion. The present study clearly shows that an intracarotid injection of 4 μg of highly purified pig GRF increased plasma GH-like activity to a detectable level in rats, with a concomitant depletion of pituitary GH. This seems to provide some evidence for the existence of hypothalamic control over GH secretion from the pituitary gland. Many studies on this project reported previously were performed on the basis of the depletion of pituitary GH content in recipient rats following administration of crude hypothalamic extracts or purified GRF preparations (3–8, 10), but

an attempt to evaluate the simultaneous changes in plasma GH-like activity has not appeared previously in the literature. A question has arisen as to whether the acute decrease in pituitary GH is due to actual release of GH or to inhibition of GH synthesis. Depletion resulting from release of pituitary GH must be accompanied by increased plasma GH level. It was demonstrated in the present study that changes in plasma GH-like activities in rats induced by administration of highly purified GRF can be detected using the tibia test method. These results may indicate that the “depletion” of pituitary GH content in recipient rats accounts for the “release” of GH from the pituitary into the blood stream.

An evaluation of plasma levels of GH-like activity in experiment 2 (Table I) was, at first, designed to be analyzed by a 4-point assay comparing tibia width responses to 2 doses of plasma sample with the responses to 2 doses of the NIH-GH standard. However, almost all hypophysectomized assay rats, which had been scheduled to be given 2 ml of plasma samples daily over 4 days as high dose, suffered from severe bleeding at the sites of the injections or out of the external auditory meatus on the second or third day of the injection and died. This seemed to be due to a high heparin concentration in the plasma samples when the 2 ml was given daily. Therefore, this part was not completed. Ideally the tibia test should give a difference of over 40 μ in tibia width, but the levels of plasma GH-like activity were not sufficiently high to produce such a high response.

It has been reported that TSH (15) stimulates the width of epiphyseal cartilage to a small degree. The GRF used in these experiments is known to be free of other known releasing and inhibiting factors (13). In present studies, TSH activity in the pituitary of recipient rats treated with GRF was essentially the same as that in rats treated with saline, and plasma TSH activities were not detected in either group. These results confirmed that our GRF preparation, known to be free of thyrotropin-releasing factor, had no effect on TSH levels. Moreover, these results also indicate that TSH did not participate in the changes in tibia width responses to pitui-

tary or plasma samples. It is reported that prolactin can also affect the width of epiphyseal cartilage to a small degree (1,19). However, the administration of hypothalamic extracts of domestic animals is reported to inhibit rather than to stimulate the release of prolactin from the pituitary gland (20). It is, therefore, unlikely that production of a narrower tibia width by pituitary homogenates following administration of GRF was caused even in part by a decrease in the prolactin content in the pituitary samples. Estimates of GH-like activities in plasma using the tibia test method have been reported in rats and in humans. Contopoulos *et al.* (21) reported that GH activity in plasma from normal rats measured in terms of purified bovine GH was 1–2.5 $\mu\text{g/ml}$ and increased during pregnancy to 3.5–7.5 $\mu\text{g/ml}$. Segaloff *et al.* (22) observed that values for normal humans in terms of the Armour Standard 22KR2 varied from 0 to 40 $\mu\text{g/ml}$ plasma. In contrast with Segaloff's results, Greenspan (23) failed to demonstrate a significant tibial response by injecting plasma from growing children and adults. In the present study, no detectable GH-like activity was found in plasma from saline injected control rats.

Recently, Knobil (11), Garcia and Geschwind (24), and Machlin *et al.* (25) observed that the administration of crude stalk-median eminence extracts of domestic animals induced significant increases in plasma GH levels, which ranged between approximately 20–120 $\mu\text{g/ml}$, by means of radioimmunoassay in monkeys or sheep. The GH-like activity in plasma of rats obtained in our experiments by the tibia test method after stimulation by GRF are considerably higher. Although this discrepancy might be explained by the different assay methods, different assay animals, and different GRF preparations, other explanations may become apparent when experiments by means of radioimmunoassay for rat GH can be critically evaluated.

Summary. The effect of administration of highly purified pig GH-releasing factor (GRF) on the depletion of pituitary growth hormone (GH) content in recipient rats and levels of plasma GH-like activity were investigated using the tibia test method. It was demon-

strated that GRF induced a significant increase in plasma GH-like activity which was accompanied by a simultaneous decrease in pituitary GH content. The levels of plasma GH-like activity in recipient rats treated with saline were not detectable (less than 1.25 $\mu\text{g/ml}$). These results seem to confirm that the "depletion" of pituitary GH content in rats caused by GRF indicates the "release" of GH.

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The Protein and Protein-Bound Carbohydrates in Four Species of Nonhuman Primates' Sera (32858)

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Nonhuman primates constitute the group of animals phylogenetically, anatomically, physiologically, biochemically, and immunologically closest to man. Although it is widely believed and predicated that biochemical experimentation on nonhuman primates will throw light on human metabolism, our knowledge concerning the chemical composition of their various tissues and fluids is still very primitive.

Serum is the most important body fluid which is readily accessible. Among the major constituents of serum are the proteins of which the glycoproteins are an important subgroup. This paper reports comparative studies of total serum protein and glycoprotein levels of *Macaca mulatta* (rhesus), *Theropithecus gelada* (baboon), *Hylobates lar* (gibbon), and *Pan troglodytes* (chimpanzee).

Materials and Methods. Serum samples were obtained from various nonhuman primate colonies available at the Delta Regional Primate Research Center of Tulane University. Some serum samples of *Macaca mulatta* were kindly made available by Dr. D. R. Anderson of the USAF School of Aerospace Medicine, Brooks Air Force Base, Texas. All of the serum samples were kept in a deep freezer (-25°C) until analyzed.

Total serum protein was determined by the biuret method according to Weichselbaum (1)

using Lab-trol (Dade Reagents, Inc., Miami, Florida) as a standard. Hexose associated with protein was determined by the tryptophane- H_2SO_4 method described by Shetlar *et al.* (2). The Winzler (3) modifications of the cystein-sulfuric acid method and of the Elson-Morgan reaction were used for the determination of protein-bound fucose and hexosamine, respectively. Sialic acid associated with protein was determined by the thiobarbituric acid method of Warren (4) after hydrolysis of the protein sample with 0.1 *N* H_2SO_4 at 80°C for 1 hour. Detailed procedures for the hydrolysis of serum samples for sialic acid determination were recently described by Shetlar (5). Microelectrophoresis of the serum protein on a cellulose acetate membrane was performed according to the procedures described by Grunbaum *et al.* (6,7).

Results. The analytical data on serum protein and protein-bound carbohydrates of the four species of nonhuman primates are summarized in Table I. For comparison, the reported values for humans are also included.

The total serum protein concentrations of *Pan troglodytes*, *Theropithecus gelada*, and *Macaca mulatta* are very close to human total serum protein levels, while that of *Hylobates lar* is significantly lower. Protein-bound hexose concentration was found to be higher than