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The Molecular Weight Forms of Rat Growth Hormone Secreted in Response to Growth Hormone-Releasing Factor (42317)

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Abstract. The different molecular weight forms of immunoreactive growth hormone (irGH) secreted by the anterior pituitary of rats were evaluated during basal and stimulated secretion *in vitro* and *in vivo*. Anterior pituitary cells maintained in a monolayer culture system secreted only a 22,000-Da form of GH based on Sephacryl S-200 column chromatography. This was also true for the irGH secreted in response to growth hormone-releasing factor and prostaglandin E_2 stimulation. In contrast, the molecular weight forms of irGH found in plasma under basal conditions included an approximately 90,000-Da form as well as the expected 22,000-Da form. The concentrations of both forms increased following growth hormone-releasing factor stimulation although there was a shift in the ratio of the forms secreted. These results suggest that the larger molecular weight forms of rat GH observed in plasma may be the result of some postsecretory process which occurs in blood and suggests a possible regulatory function for the larger molecular weight forms as it pertains to the bioavailability of GH *in vivo*. © 1986 Society for Experimental Biology and Medicine.

Circulating forms of immunoreactive growth hormone (irGH) in both humans and rodents are known to represent a heterogenous group of proteins (1–9). When irGH is fractionated by gel filtration chromatography, a major component is a monomeric form of GH, often called little GH, which has a molecular weight of 22,000 Da. It is now known that in the human little GH represents a number of variants of the 22,000-Da molecule (1–3). In addition, there are several larger circulating forms of irGH. Their elution profiles during gel filtration chromatography have led to the description of a big GH (molecular weight of 40,000 to 50,000 Da) and a big-big GH (molecular weight of greater than 60,000 Da) for both humans and rodents (4–9). It is presently unknown which molecular weight forms of GH are secreted by the rat pituitary *in vitro* and *in vivo* in response to growth hormone-releasing factor (GRF), the hypothalamic factor responsible for GH release under normal physiological conditions (10). Our results indicate that the monomeric form or little GH is the major species secreted *in vitro* and *in vivo* by the rat pituitary.

Material and Methods. *Animals.* Male Sprague–Dawley rats weighing 125–150 g were used in these experiments. They were acquired, maintained, and used in accordance with the guidelines established by the National Institutes of Health.[1] They were housed in a temperature- and humidity-controlled environment and exposed to a 14-hr light, 10-hr dark schedule. Food and water were freely available.

In vitro methods. Rat anterior pituitaries were collected and prepared for monolayer cell culture as described (11). On the third day cells were washed twice and then incubated in serum-free media supplemented with 0.1% bovine serum albumin. On the following day, the media were collected to determine basal GH secretion. The cells were then washed once and incubated in the presence of 10^{-10} *M* human GRF 1-44-NH_2 (12–14) and 10^{-7} *M* prostaglandin E_2 (PGE_2). The media were collected at 5 min, 30 min, 1 hr, and 3 hr of incubation. It was frozen at −20°C and subsequently fractionated by gel filtration chromatography within 48 hr.

In vivo methods. Eight rats were anesthetized with sodium pentobarbital (50 mg/kg, ip). Five to ten minutes later, the animals were fitted with a polyethylene catheter (0.28 mm

[1] Guide for the Care and Use of Laboratory Animals, Department of Health, Education and Welfare. Publication No. (NIH) 80-23, revised 1978, Office of Science and Health Reports, DRR/NIH, Bethesda, Md. 20205.

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i.d. × 0.61 mm o.d.; Clay Adams, Parsippany, N.J.) placed in an external jugular vein. Fifteen minutes after the injection of sodium pentobarbital, 5 μg/kg human GRF was administered iv to four of the animals while four received a control injection (0.5 ml saline). A single blood sample was drawn 3 min later. Samples were centrifuged and the plasma was collected and stored at −20°C until fractionated by gel filtration chromatography.

Gel filtration chromatography. Tissue culture media and plasma (1–2 ml) were fractionated on 1.7 × 88-cm Sephacryl S-200 columns using 0.067 *M* phosphate buffer, pH 7.4, as the eluting buffer. Fractions of 3.8–4.8 ml were collected and diluted as necessary for radioimmunoassay of irGH. The columns were calibrated with dextran blue, transferrin, ovalbumin (all obtained from Sigma Chemical Co., St. Louis, Mo.) and radioiodinated GH (NIADDK-rGH-I-5, National Institutes of Health, Bethesda, Md.).

Radioimmunoassay. The irGH was determined by a double antibody radioimmunoassay using reagents supplied by the National Institutes of Health except for the GH antiserum which was kindly provided by Dr. Y. Sinha (15). The GH concentrations are expressed in units of GH-RP-1, assay sensitivity

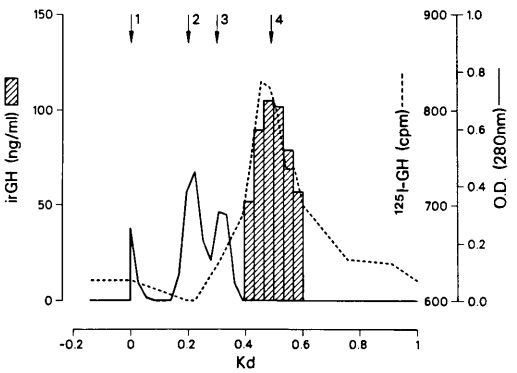

FIG. 1. Immunoreactive GH (irGH) elution profile of tissue culture medium obtained from anterior pituitary cells which were maintained in a monolayer culture system for 24 hr (basal secretion). Fractionation was done on Sephacryl S-200 column (1.7 × 88 cm) eluted with 0.67 *M* phosphate buffer pH 7.4. Arrows with numbers designate the elution profile for substances of known molecular weight: 1 = blue dextran (2,000,000 Da), 2 = transferrin (90,000 Da), 3 = ovalbumin (45,000 Da), 4 = I^{125}-GH (22,000 Da).

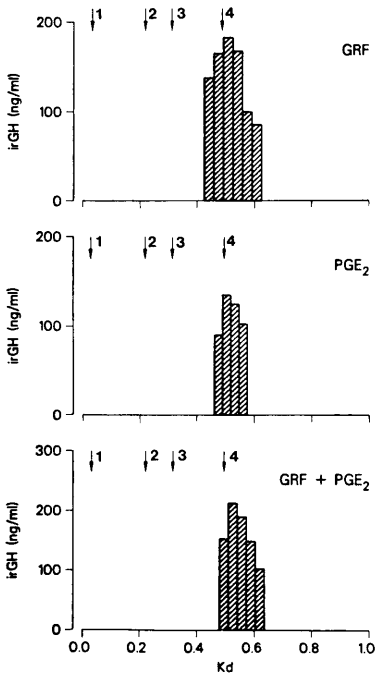

FIG. 2. Immunoreactive GH (irGH) elution profile of tissue culture medium obtained from rat anterior pituitary cells which were stimulated for 5 min in the presence of 10^{-10} *M* human growth hormone-releasing factor (GRF) and 10^{-7} *M* prostaglandin E_2 (PGE_2). Fractionation was performed as described in Fig. 1.

at 90% bound was less than 0.1 ng. The within and between assay variation averaged 8 and 14%, respectively.

Results. The results presented in Fig. 1 illustrate the calibration of a typical Sephacryl S-200 column and the elution profile of irGH released by the pituitary over a 24-hr period in serum-free media (basal secretion). As is evident the irGH eluted in the same position as the radiolabeled monomeric form of GH. Within the sensitivity limits of the radioimmunoassay there was no evidence of any larger molecular weight forms of irGH.

The molecular weight form of irGH secreted by the pituitary *in vitro* in response to a 5-min exposure to GRF was the 22,000-Da species, i.e., little GH. This was also true for irGH secreted in response to PGE_2 (Fig. 2, middle) and in response to the combined treatment of GRF plus PGE_2 (Fig. 2, bottom). The amount of irGH secreted in response to the combined

treatment of both secretagogues was greater than that of either of the individual treatments.

The elution profile of irGH secreted *in vitro* from cells stimulated for 30 min and 1 hr was virtually identical to those observed following 5-min stimulation (data not presented). As illustrated in Fig. 3, the sole molecular weight form of irGH secreted following 3 hr of stimulation with GRF was little GH. This was also true for PGE_2 stimulation. The treatment of pituitary cells for 3 hr with both GRF and PGE_2 resulted in only the monomeric form of GH being secreted. The combined treatment resulted in a greater release of irGH as compared to each treatment alone. Thus there was no evidence that any of the irGH secreted *in vitro* had a molecular weight different from the 22,000-Da form of GH.

Gel filtration of plasma obtained from control treated rats is illustrated in Fig. 4, upper panel. Two irGH peaks at K_d's of 0.195 and 0.473 are indicative of the presence of big-big

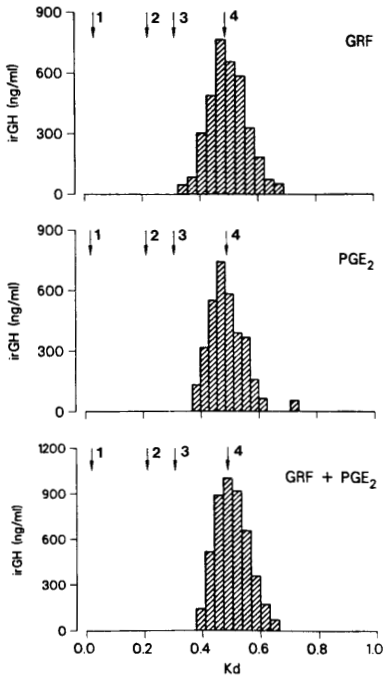

FIG. 3. Immunoreactive GH (irGH) elution profile of tissue culture medium obtained from rat anterior pituitary cells which were stimulated for 3 hr in the presence of 10^{-10} *M* human growth hormone-releasing factor (GRF) and 10^{-7} *M* prostaglandin E_2 (PGE_2). Fractionation was performed as described in Fig. 1.

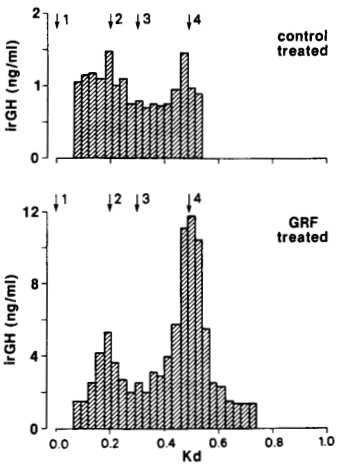

FIG. 4. A representative example of the immunoreactive GH (irGH) elution profile of plasma obtained from a rat treated with saline (control treated, $n = 4$) is illustrated in the upper panel and of plasma obtained from a rat treated with growth hormone-releasing factor (5 μg/kg iv, GRF treated, $n = 4$) is illustrated in the lower panel. The elution profile of each animal within treatment groups was identical. However, due to the heterogeneity of absolute (GH) concentrations between animals, the data were not pooled. Fractionation was performed as described in Fig. 1.

and little GH in the circulation. The elution profile of irGH secreted by the pituitary *in vivo* in response to GRF is illustrated in Fig. 4, lower panel. The presence of both big-big and little irGH in the plasma was again clearly observed. However, there was a greater increase in the amount of little GH secreted in response to GRF as compared to big-big GH. It is possible that big irGH was present in the plateau region between big-big and little irGH but no discernible peak was detected.

Discussion. The isolation and characterization of GRF (13, 14) allowed us to evaluate the molecular weight forms of GH secreted by the rat pituitary in response to its physiological releasing factor. The only detected form of GH secreted *in vitro* and the primary form secreted *in vivo* was the 22,000-Da monomeric species. There were oligomeric species of GH present in plasma which had molecular weights in the range of 90,000 Da (big-big GH). However, there was little evidence for the 45,000-Da form (big GH), although it has been previously detected in serum of humans and mice (4–9). The *in vitro* and *in vivo* results suggest that the secreted form of GH in the rat is little GH and

that postsecretory events lead to the creation of larger irGH molecules.

The molecular weight form of irGH secreted by rat pituitaries *in vitro,* that is little GH, is consistent with previous observations. Guyda (16) reported that of the total amount of human GH secreted *in vitro* only 5% was big GH and 1% was big-big GH. Similarly, Skyler *et al.* (17) reported that only 4% of the human GH secreted *in vitro* had a 40,000 to 50,000 molecular weight. An undetermined amount of irGH with a larger molecular weight (big-big GH) was also observed. Baumann and MacCart (18) reported that the human pituitary primarily secretes the 22,000 molecular weight form of GH *in vitro.* The fact that we did not detect any large molecular weight forms of rat GH *in vitro* may be a result of our RIA sensitivity. However, based on our ability to detect larger forms of irGH in rat plasma and their relative concentrations, it seems unlikely that this is the case. Regardless, there is little question that *in vitro* the primary secreted form of irGH in the rat is approximately 22,000 Da.

In contrast to our inability to detect any large molecular weight forms of rat GH *in vitro* we did observe big-big GH in plasma samples obtained from rats. This *in vivo* data suggests that dimerization, aggregation, or protein binding of little GH occurs as a postsecretory event rather than a large molecular weight form of GH being secreted. Evidence to support this hypothesis is found in the work of Antoniades (19) and Beitins *et al.* (20). They reported that radiolabeled little GH was quickly converted to larger molecular moieties following its iv administration to humans and rats. Interestingly, little GH does not appear to be converted to higher molecular weight forms when incubated *in vitro* with rat serum (19) but is converted in the presence of human serum (4, 20). There is evidence to argue against a postsecretory aggregation of little GH since big-big and big GH have been detected in pituitary extracts of both humans (4–8) and murine species (9, 21). Evidence suggests that the majority of these large molecules are not covalently linked, with a smaller fraction consisting of monomers linked by disulfide bridges (7, 8, 22).

The observation that GRF increases the plasma concentrations of both little GH and, to a lesser extent, larger molecular weight forms of GH in rats is consistent with observations made in humans (23, 24). As discussed by Stolar *et al.* (23), the molecular forms of irGH secreted in humans in response to GRF, the physiological regulator of GH secretion, are similar to the forms released after other pharmacological stimuli. Sassolas *et al.* (24) reported that in the human the circulating molecular forms of GH observed 30 and 60 min after GRF stimulation were similar to those observed before GRF. They also documented the biological activity of these molecular forms of GH. Yet the biological functions of the large molecular weight forms of GH and their implications in the bioavailability of the hormone and its metabolism remain to be established.

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