Stimulation of Release and Synthesis of Growth Hormone (GH) in Tissue Cultures of Anterior Pituitaries in Response to GH-Releasing Hormone (GH-RH)[1] (34588)

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Demonstration of growth hormone-releasing hormone (GH-RH) activity in hypothalamic extracts prompted extensive research on this neurohumor. GH-RH has now been demonstrated in several species including man on the basis of *in vivo* and *in vitro* work (1, 2) and has now been obtained in pure form (3).

Highly purified GH-RH has been reported to stimulate synthesis of GH *in vitro* by this laboratory (1, 4). In this paper, the problem of stimulation of synthesis, as well as release, was restudied using a 5-day organ culture system and pure or highly purified GH-RH.

Materials and Methods. Tissue cultures were carried out by the method of Nicoll and Meites (5) with slight modifications. Male rats of the Sprague-Dawley Strain (Cheek-Jones Co., Houston, Texas) were used as donors for cultured tissue, except for Expt. 4, Table I in which females were used. The animals, weighing around 125–150 g, were decapitated and the pituitary glands were removed under aseptic conditions. Matched control and experimental explants (one pair from each pituitary) were prepared by slicing off the most lateral quarters of each anterior pituitary.

The cultures were performed in 3.5 × 1-cm sterile disposable plastic petri dishes (Falcon Plastics, Inc.) each containing 3 ml of Trowell's T8 medium (Microbiological Associates, Bethesda, Maryland). In each dish, 6 to 12 lateral fragments were placed upon a strip of washed lens paper which was supported on a stainless steel mesh platform. For each experiment, pituitary quarters from opposite halves of the same pituitaries were used to provide matched populations of cells. Standard organ culture techniques were followed throughout (6).

An atmosphere of 95% O_2–5% CO_2 and a temperature of 36° were maintained for 5 days. The GH-RH preparations used were of various degrees of purity: carboxymethylcellulose (CMC) purified (1, 2), triethylaminoethyl (TEAE) cellulose purified (3), as well as two preparations representing essentially pure GH-RH (3). They were added dissolved in 0.1 *M* acetic acid. Usually, 1 to 3 μl of this solution were added each morning and evening to 3.0 ml of the strongly bicarbonate-buffered Trowell's medium.

At the end of the 5-day culture period, media and tissues were frozen for assay. At the end of culture, the control fragments weighed an average of 0.96 mg; the experimentals weighed 0.92 mg. In Expts. 2 and 3, Table I, some of the explants were fixed for electron microscopy. "Compensatory additions" of GH-RH were made to control medium samples for bioassay at the end of culture (7).

GH was assayed by the tibia test method of Greenspan *et al.* (8) using 5 to 8 hypophysectomized rats/point and NIH-GH-S7 as a reference material. Mean potency and 95% fiducial limits were calculated by 4-point factorial analysis (9) or by other methods (10) when the assays failed to provide data on two dose levels within the linear portions of the log–dose response curves.

In the experiments reported in Table II, a generally labeled tritiated L amino mixture (New England Nuclear NET-250) contain-

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TABLE I. Effects of GH-RH on Medium and Tissue GH Contents.

Exp. no.	Treatment	Total of 10 doses of GH-RH/mg of tissue	GH (as μg of NIH-GH-S7/mg of pituitary tissue) Medium[a]	Tissue[a]
1	Control	—	41 (31–51)	43 (27–59)
	Stimulated[b]	0.81 μg	100 (80–126)[c]	80 (54–116)[d]
2	Control	—	84 (60–118)	27 (21–36.8)
	Stimulated[b]	2.90 μg	228 (163–309)[c]	50 (36.9–70)[c]
3	Control	—	141 (93–213)	38 (32–48)
	Stimulated[b]	21.7 pg	312 (297–401)[c]	48 (40–60)[d]
4	Control	—	34 (20–39)	24 (16–36)
	Stimulated[b]	225 pg	64 (50–107)[c]	28 (19–41)

[a] Data presented as means and 95% fiducial limits.

[b] GH-RH code nos.: AVS 35–73 no. 111–123 (Exp. 1 and 2), GH-RH purified by CMC and AVS 11–83 no. 185–210, GH-RH at final purity stage (in Exp. 3 and 4).

[c] $p < .01$ vs. controls (when 95% fiducial limits of both experimentals and controls failed to overlap).

[d] $p < .05$ vs. controls (when 95% fiducial limit of the relative potency estimate of the experimental sample failed to overlap the potency for the control and vice versa).

ing 15 amino acids with a mean activity of 6.67 μCi/mμmole amino acid was used. The amino acid mixture, 1 μCi of tritium/μl 0.1 *M* HCl, was added to the medium before culture. In Expts. 1 and 2, the concentration was 4 μCi/ml. In Expts. 3 to 8, 6 μCi/ml were used. At termination of culture, these explants were weighed, frozen, and later homogenized in upper gel buffer for disc gel electrophoresis. Medium samples were dialyzed, lyophilized, and later redissolved in upper gel buffer. A saturated solution of sucrose was used to increase the density of sample gels that failed to polymerize so that electrophoresis could be run.

Duplicate to quadruplicate aliquots of the homogenates and media were separately subjected to polyacrylamide gel electrophoresis essentially as described by Davis (11).

The separating gel was 7.5 % acrylamide gel buffered at pH 9.5. Separation of proteins was achieved at 2 mA/gel. Tissue homogenates representing 1 to 3 mg of tissue or dialyzed medium samples representing 0.5 to 2 mg of explant were run on each gel.

The proteins in the gel were stained with amido black. Segments of the polyacrylamide gels containing the GH zones (one band in tissue; three closely spaced bands in medium) and prolactin zones were dissected out and the radioactivity was determined by liquid scintillation techniques essentially as described by Tishler and Epstein (12).

Identification of the GH and prolactin bands was in agreement with Lewis *et al.* (13), assuming absence of interfering substances, and with Kragt and Meites (14), MacLeod *et al.* (15), Yanai and Nagasawa (16), and Catt and Moffat (17).

Results are presented as counts per minute (cpm) per milligram of tissue after a correction for quenching through addition of an internal standard. No correction was made for exchange of tritium or for nonspecific binding of labeled amino acids. Significance of differences between groups of radioactivity measurements (one measurement per gel) was determined by Student's *t* test.

Results. 1. Release and synthesis as measured by tibia assay. The results of 4 experiments are presented in Table I showing medium and pituitary contents per milligram of pituitary tissue (final weight). These results demonstrate that rat pituitary tissue can release appreciable amounts of GH activity in this organ culture system during 5 days and that GH-RH can produce significant increases in both medium and tissue, relative to the control. The experimental means averaged 231 % of the controls for the media and

TABLE II. Effect of GH-RH on Incorporation of Labeled Amino Acids into Medium and Tissue GH Zones.[a]

Exp. no.	Sample	Total of 10 doses of GH-RH/mg of tissue (ng)	(cpm/mg of tissue) Medium	(cpm/mg of tissue) Tissue	Total increase over control medium and tissue
1	Control	—	1050 ± 110	3190 ± 40	
	Stimulated[b]	2.5	2540 ± 130[c]	7640 ± 760[c]	+5940
2	Control	—	620 ± 70	5920 ± 380	
	Stimulated[b]	1.4	1670 ± 80[c]	6590 ± 1940	+1720
3	Control	—	—	10,200 ± 810	
	Stimulated[b]	673	—	13,100 ± 2300	—
4	Control	—	18,000 ± 1040	7500 ± 200	
	Stimulated[b]	545	26,800 ± 1230[c]	17,100 ± 2040[c]	+18,400
5	Control	—	18,400 ± 30	18,200 ± 40	
	Stimulated[b]	579	32,500 ± 1100[c]	16,800 ± 6030	+12,700
6	Control	—	33,100 ± 5400	—	
	Stimulated[b]	5.6	66,200 ± 6450	—	—
7	Control	—	16,800 ± 1210	34,000 ± 2490	
	Stimulated[b]	4.6	51,700 ± 160[c]	70,400 ± 1840[c]	+71,300
8	Control	—	19,900 ± 1720	5900 ± 130	
	Stimulated[b]	6.1	35,600 ± 4310[c]	17,700 ± 5220	+27,500

[a] Data are presented as mean ± standard error without correction for exchange or nonspecific binding.

[b] GH-RH code nos.: AVS 11-83 no. 185–210 (Exp. 1 and 2), AVS 11-103 no. 152–195 (Exp. 3–5) both representing GH-RH at final purity stages and AVS 37-19 no. 101–111 TEAE purified GH-RH (Exp. 6–8).

[c] $p < .05$ vs. matched control.

151 % for the tissues. In Expt. 3, the weight of GH activity produced, expressed as NIH-GH-S7, was 8,400,000 times the dose of GH-RH added.

2. Release and synthesis as measured by amino acid incorporation. The results of 8 experiments are presented in Table II showing radioactivity associated with the GH bands. The data are presented (cpm/mg of tissue) after correction for quenching.

The amount of radioactive amino acid incorporation was increased in both medium and tissue GH bands upon stimulation with GH-RH. Calculations from the data on Table II show an average apparent experimental medium GH incorporation as 218 % of the controls and stimulated tissue GH as 186 % of the controls.

Discussion. The present results clearly indicate that pure or highly purified porcine GH-RH can stimulate the release and apparent synthesis of GH and are consistent with earlier findings from this laboratory using GH-RH acting acutely on pituitaries from cold-stressed rats (4). The mean control release in the work reported here, 0.62 μg/mg/hr, is comparable to that obtained in short-term incubations (4). The high "multiplier ratio" of weights of GH/GH-RH observed is also in agreement with previous reports (3). The increase in tissue GH with chronic exposure contrasts with acute depletion *in vivo* (3) and may be considered evidence that a separate synthesis-stimulating hormone is not necessary for sustained secretion. Deuben and Meites (18) and Symchowicz *et al.* (19) previously reported net increases in GH activity *in vitro* as measured by bioassay. These findings are also comparable to recent work from this laboratory (20) in which thyrotropin-releasing hormone (TRH) caused increased bioassayable TSH in both medium and tissue during culture.

The increased radioactivity associated with the experimental GH bands is further evidenced for stimulation of synthesis. Previously, Catt and Moffat (17), Rao *et al.* (21), and MacLeod and Abad (15) obtained labeled GH from pituitary tissue incubated *in vitro*. MacLeod and Abad (22), M. C. Robertson (personal communication), and K. Yamamoto (personal communication) obtained vigorous incorporation of radioactive amino acids into GH during short-term (3–21 hr) incubation experiments; but hypothalamic preparations surprisingly had no detectable effect on the rate of incorporation. Nicoll (23) and Daughaday (24) have suggested that part of the GH pool may be activated and unstable as a possible explanation for stimulation of release and synthesis as measured by bioassay in contrast with certain observations based on radioimmunoassay, stain uptake by proteins on gels, or radioactive amino acid incorporation in short-term experiments.

After this work was completed, Krulich and McCann (25) reported stimulation of synthesis of bioassayable GH activity *in vitro* using partially purified ovine GH-RH in a 5-hr incubation procedure.

Summary. Addition of small amounts of pure or highly purified porcine GH-RH twice daily to rat anterior pituitary tissue, maintained for 5 days in Trowell's T8 medium, significantly increased the amounts of GH in the medium and tissues relative to the controls as measured by bioassay. Apparent incorporation of radioactive amino acids into the GH bands on polyacrylamide gels after electrophoresis of medium and tissue was increased also. These results support other findings from this laboratory on the identity and properties of this neurohormone.

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