Growth Hormone Immunological and Biological Assays in Pituitary of Rat Under Different Experimental Conditions[1]

The studies on growth hormone (GH) regulation in the rat have been considerably hindered in the last years by a surprising discrepancy of results obtained by using radioimmunoassay (RIA) or bioassay (BA) methods. The important observation that insulin-induced hypoglycemia, α-2-deoxyglucose administration or exposure to stressful stimuli cause an increase in plasma GH in man, first made by ROTH et al.[2,3], has been confirmed using the bioassay method in rat, in which these stimuli have been shown to result in an acute decrease of pituitary GH concentration, indicative of a release of the hormone[4,5]. However, determinations of plasma GH levels by radioimmunoassay in the rat gave unexpected results, since the aforementioned stimuli evoked either no response, or a response opposite to that found in primates[6–8]. While in the rat many negative results have been reported on the determinations of GH in plasma by RIA, only few reports are available at present on GH levels in the pituitary and, to our knowledge, in only one work pituitary GH measurements have been performed using both types of assay on the same pituitary extracts[7]. In that instance surprisingly it was found that insulin hypoglycemia or cold exposure failed to alter significantly pituitary GH determined both biologically and immunologically. In the present research we have determined GH levels both by RIA and by BA in the pituitary of animals subjected to insulin hypoglycemia, cold exposure or starvation, all experimental conditions able to induce release of GH from the pituitary in primates[2,3,9].

Material and methods. Sprague-Dawley male rats weighing 150–180 g were used. All animals were fasted overnight before the experiments. The following day they were exposed to the experimental conditions described in the Table (for details on the experimental procedure see Table).

Animals were killed by decapitation at different time intervals. The cranium was then immediately opened and the posterior pituitary dissected free and discarded. The anterior pituitary was removed, weighed and homogenized in 0.9% saline with a 2000 rpm mechanical homogenizer with the vessel placed in ice.

For the determination of GH by RIA, samples of pituitary homogenates corresponding to 10 µl of the undiluted extract of each pituitary were used. Samples were diluted 1:10,000 in phosphate buffer 0.01 *M*, pH 7.6 and stored at −20 °C until assayed. The same pituitary extracts, remaining after having taken the aliquots necessary for RIA, were used for the biological determination of GH. These extracts were pooled by groups and kept frozen until the time of assay.

Radioimmunoassay. The methods described by SCHALCH and REICHLIN[6] with minor modifications was used. Rat growth hormone was used for immunoassay standards and preparation of antibody. Antibody to rat GH was induced in guinea-pigs and monkeys. Rat growth hormone was iodinated with I^{125} using the iodination method of GREENWOOD, HUNTER and GLOVER[10].

Rat GH^{125} before using was purified by gel filtration on Sephadex G 150 to remove the immunologically-inactive components[11]. Separation of bound from free hormone was obtained by precipitation of antibody bound hormone with rabbit anti guinea-pig gamma globulins.

Bioassay. GH activity of the samples was measured by the 'tibia test' method of GREENSPAN et al.[12]. Pituitary extracts equivalent to 0.5 mg of pituitary per assay rat were administered each day for 4 days to hypophysectomized assay animals. On the fifth day, the width of the epiphyseal cartilage was measured. Since GH standard was used in only one experiment, most of the results are expressed directly in terms of the width of the epiphyseal cartilage. When GH standard was used (experiment 3, see Table), the GH potencies of pituitary tissue were

Simultaneous immunological and biological assays of pituitary GH in rat under different experimental conditions

Experiment	Treatment[a]	Pituitary growth hormone RIA-GH[b] µgGH/mgAP	BA-GH[c] (expressed as epiphyseal width (µ) mean ± S.E.	Blood glucose (mg/100 ml)
1	Saline	46.32 ± 6.14	257 ± 2.8	69 ± 2.4
	Insulin (0.2 U/100 g body wt.)	64.59 ± 14.6	202 ± 9.5[d]	42 ± 9.9[c]
2	Saline	22.00 ± 4.2	267 ± 4.6	74 ± 5.6
	Insulin (0.2 U/100 g body wt.)	34.57 ± 5.2	213 ± 4.8[d]	40 ± 4.2[d]
	Cold exposure (4 °C/1 h)	31.91 ± 3.1	213 ± 5.2[d]	82 ± 6.6
	Fasting (60 h)	22.80 ± 1.4	224 ± 13.9[d]	52 ± 4.3[c]
3	Saline	25.81 ± 4.4	234 ± 6.0 (70.0)[f]	48 ± 1.4
	Insulin (0.3 U/100 g body wt.)	38.82 ± 5.2	194 ± 5.0[d] (14.2)[f]	25 ± 2.2[d]
	Cold exposure (4 °C/1 h)	48.52 ± 1.7[d]	180 ± 8.7[d] (4.1)[f]	51 ± 1.2
	Fasting (60 h)	37.24 ± 6.2	211 ± 4.6[c] (37.5)[f]	26 ± 2.3[d]

[a] Animals were injected i.p. with saline or insulin or exposed to a cold environment and killed 1 h later. Fasted animals were killed at the completion of the 60th hour. [b] Standard rat GH (Ellis, 1968) with potency of 2.7 U.S.P. units/mg. RIA = radioimmunoassayable. [c] $p < 0.01$ vs. saline. [d] $p < 0.001$ vs. saline. [e] BA = bioassayable. 6–8 hypophysectomized assay animals per group. [f] Estimates of GH content (µg GH/ mg AP) according to a bracketed 3-point assay. Ovine GH (NIH-GH-S8) was used.

[1] Supported by USPHS Research Grant No. HD01109-04.

[2] J. ROTH, S. M. GLICK, R. S. YALOW and S. A. BERSON, Diabetes *13*, 355 (1964).
[3] S. GLICK, J. ROTH, R. S. YALOW and S. A. BERSON, Recent Prog. Horm. Res. *21*, 241 (1965).
[4] L. KRULICK and S. M. MCCANN, Endocrinology *78*, 759 (1966).
[5] E. E. MÜLLER, T. SAITO, A. ARIMURA and A. V. SCHALLY, Endocrinology *80*, 109 (1967).
[6] D. S. SCHALCH and S. REICHLIN, Endocrinology *79*, 275 (1966).
[7] W. H. DAUGHADAY, G. T. PEAKE, C. A. BIRGE and I. K. MARIZ, Proc. First Int. Symp. Growth Hormone (Eds. A. PECILE, E. E. MÜLLER; Excerpta Medica Found. Int. Series No. 158, Milan 1967), p. 238.
[8] J. F. GARCIA and I. I. GESCHWIND, Proc. First Int. Symp. Growth Hormone (Eds. A. PECILE, E. E. MÜLLER; Excerpta Medica Found. Int. Series No. 158, Milan 1967), p. 267.
[9] C. C. GALE and M. JOBIN, Fedn. Proc. *26*, 255 (1967).
[10] F. C. GREENWOOD, W. M. HUNTER and J. S. GLOVER, Biochem. J. *89*, 114 (1963).
[11] G. GIUSTINA, L. CANTALAMESSA, E. RESCHINI, D. MIEDICO, D. GELLI and M. LAMPERTICO, Folia Endocrin. *21*, 632 (1968).
[12] F. S. GREENSPAN, C. H. LI, M. E. SIMPSON and H. M. EVANS, Endocrinology *45*, 455 (1949).

calculated by comparing tibia width responses to 1 dose of pituitary homogenates with the responses to 2 doses of GH standard (bracketed 3-point assay). Significance of differences in epiphyseal cartilage width was determined by Student's t test. A sample of venous blood was withdrawn from experimental rats at the time of sacrifice for blood glucose determination (Glucostat, Worthington, Biochemicals).

Results and discussion. From the results reported in the Table it appears that of the 3 experimental conditions investigated, i.e. insulin-induced hypoglycemia, exposure to a cold environment or fasting for 60 h, no one was able to induce significant changes in pituitary GH as measured by RIA, with the exception of cold exposure, which in experiment 2 induced a clearcut increase of pituitary GH levels. In contrast with these negative results is the dramatic decrease in rat pituitary GH concentration observed in the aforementioned situations when pituitary GH levels were determined by BA in the same pituitary extracts. It would appear, in agreement with our previous results[5], that the more effective stimulus in releasing pituitary GH, as judged by bioassay, is cold exposure (experiment 3 from 70.0 µg/mg to 4.1 µg/mg), but also insulin hypoglycemia and severe fasting were highly active as stimuli.

The almost all negative results obtained till now in the rat by measuring plasma and pituitary GH levels by RIA, reflect the inability to modify in whichever direction plasma or pituitary GH levels and raise again the problem of the adequacy of this assay in the non-primate species. Even if the BA certainly suffers from lack of specificity[14], nevertheless results obtained in the rat by BA are compatible with the findings obtainable in primates by RIA. In addition numerous indications reached by BA in rat often served to further progress in the clarification of some aspects of the CNS involvement in the control of GH secretion in primates[15,16].

The possibility that what is currently measured as GH by RIA in plasma or pituitary of the manipulated rat may not be identical to the molecule responsible for the metabolic and growth effect of the hormone[8], is not completely divorced from the present-day reality[17].

Efforts aimed to study further the specificity of the antisera currently used, as well as the possible presence in the pituitary extracts or in plasma of substance(s) aspecifically interferring with the antigen-antibody binding, are urgently needed. On this line of investigation work is under way also in our laboratories[18].

Zusammenfassung. Zur Bestimmung des Gehalts an Wachstumshormon in der Hypophyse wurden die radioimmunologische und die biologische Methode verwendet. Die Resultate der immunologischen Bestimmung von Wachstumshormon nach Insulinstress, Kälte oder Hunger waren negativ, während im biologischen Versuch eine deutliche Abnahme des Gehalts an Wachstumshormon in der Hypophyse festzustellen war.

E. E. Müller, D. Miedico[19], G. Giustina[19] and A. Pecile[20]

Department of Pharmacology School of Pharmacy, University of Pavia and Departments of Medicine and Pharmacology, School of Medicine, University of Milan, I-20129 Milano (Italy), 25 June 1969.

[13] G. Falconi and G. Rossi, Endocrinology *75*, 301 (1964).
[14] I. I. Geschwind and C. H. Li, in *The Hypophyseal Growth Hormone, Nature and Actions* (Eds. R. W. Smith Jr., O. H. Gaebler and C. N. H. Long; McGraw Hill, New York 1955), p. 28.
[15] E. E. Müller, S. Sawano, A. Arimura and A. V. Schally, Endocrinology *80*, 471 (1967).
[16] W. G. Blackard and S. A. Heidingsfelder, J. clin. Invest. *47*, 1407 (1968).
[17] J. C. Beck, Int. Symp. Integration of Endocrine and Non-Endocrine Mechanisms in the Hypothalamus, Stresa 1969 (Abstract).
[18] Acknowledgments. We would like to thank Dr. S. Ellis, Nasa, Moffet Field, Calif., USA, for the generous supply of rat GH and the Endocrinology Study Section of the NIH for ovine GH. The skilful technical assistance of Miss Vannisa Albrici is gratefully acknowledged.
[19] Department of Medicine, University of Milan (Italy).
[20] Department of Pharmacology, School of Medicine, University of Milan (Italy).

Iodine Accumulation by the Nephridia of *Sipunculus* (Sipuncula)

Previous studies related to the nephridia of Sipuncula have been concerned mostly with histological detail, especially those of Harms[1] and Kelley[2]; recent information on physiological and biochemical aspects of osmoregulation, nephridial contents or function of nephridia in osmoregulation can be found in the papers by Koller[3], Gross[4], Edmonds[5], Kamemoto and Larson[6] and Virkar[7]. Towle and Giese[8], while studying biochemical changes during reproduction and starvation, determined the water content and protein level of the nephridia of Phascolosoma.

The present report presents data on the iodine content in the nephridia of 2 species of Sipunculus, namely *S. multisulcatus* Fischer 1913[9] and *S. natans* Fischer 1954[10]. All specimens were collected at São Sebastião, on the littoral of the State of São Paulo, during the months of September and October 1968; no evidence was found of developing gonads, the animals therefore being considered immature.

Specimens of *S. multisulcatus* ranged in weight from 23–43 g, the size varying from 15–18 cm in length. In *S. natans* the weight varied from 80–120 g, the length being 20–25 cm; in both species the sac-like nephridia may attain 3–4 cm in length. The method used for the determination of iodine has already been reported[11].

[1] W. Harms, Arch. Entwmech. Org. *47*, 307 (1921).
[2] L. Kelley, *The Histology of the Nephridia of Golfingia gouldi*, Thesis (Library Fisk University, Nashville, Tenn., USA 1953).
[3] G. Koller, Verh. dt. zool. Ges. *41*, 440 (1939).
[4] W. J. Gross, J. exp. Biol. *31*, 402 (1954).
[5] S. J. Edmonds, Aus. J. mar. Freshwat. Res. *8*, 131 (1957).
[6] F. I. Kamemoto and E. J. Larson, Comp. Biochem. Physiol. *13*, 477 (1964).
[7] R. A. Virkar, Comp. Biochem. Physiol. *18*, 617 (1966).
[8] A. Towle and A. C. Giese, Comp. Biochem. Physiol. *19*, 667 (1966).
[9] W. Fischer, Mitt. naturh. Mus. Hamburg *30*, 93 (1913).
[10] W. K. Fischer, Ann. Mag. nat. Hist. *7*, 238 (1954).
[11] F. B. de Jorge and J. N. Kara, Nature *214*, 491 (1967).