hormonal proteins and peptides

GROWTH HORMONE AND RELATED PROTEINS

EDITED BY CHOH HAO LI

volume 4

HORMONAL PROTEINS AND PEPTIDES

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VOLUME IV

Growth Hormone and Related Proteins

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Preface

This volume presents four contributions dealing with various aspects of somatotropin (growth hormone) and its related proteins. The first of these considers the bioassay methods for somatotropin. There is no need to emphasize the importance of a sensitive and specific bioassay method for the isolation and characterization of a physiologically active molecule. For the last decade some investigators have employed exclusively radioimmunoassay techniques and have failed to recognize the fact that the biological activity of a molecule is not always correlated with its immunoreactivity.

It has long been known that somatotropin is intimately involved with fat metabolism. In experimental animals, chronic treatment with bovine somatotropin induces a reduction in the fat content of the tissues. In the second article, Rao and Ramachandran review the *in vitro* lipolytic actions of the hormone with the aim of clarifying the molecular mechanisms involved.

Human choriomammotropin (human chorionic somatomammotropin, HCS, human placental lactogen), a protein isolated from human placenta, possesses all the biological properties of human somatotropin (HGH). It consists of 191 amino acids, in a sequence almost identical to pituitary hormone. In the third review, Bewley gives a detailed discussion of the chemistry of HCS and compares it with that of HGH.

Prolactin is largely regarded as the mammotropin hormone. It has recently become clear that prolactin is also a growth hormone, especially in amphibians, reptiles, and birds. Clements and Meites discuss, in the fourth chapter, the control of prolactin secretion. An article on the control of somatotropin secretion will appear in a later volume.

The final chapter by Leathem presents an account of the early development of endocrinology based on the contributions of P. E. Smith. Investigators in pituitary research owe a great debt to Smith for the development of the hypophysectomy technique in the rat and for his original observations on the effect of hypophysectomy on various bodily functions in experimental animals.

Choh Hao Li

Contents of Previous Volumes

Volume I

Volume II

Volume III

Contents

3. The Chemistry of Human Chorionic Somatomammotropin

Thomas A. Bewley

4. Control of Prolactin Secretion

James A. Clemens and Joseph Meites

5. Hypophysectomy and Philip E. Smith

James H. Leathem

1

Bioassay of Pituitary Growth Hormone*

CHOH HAO LI

* Abbreviations: GH, growth hormone, somatotropin; HGH, human pituitary somatotropin; BGH, bovine pituitary somatotropin; ACTH, adrenocorticotropic hormone; TSH, thyrotropin; ODC, ornithine decarboxylase.

I. Introduction

The bioassay of a hormone frequently gives nothing more than a quantitative statement or definition of its biological activity. Such a definition is necessarily complex because, unlike other adenohypophyseal hormones, growth hormone does not have a single, well-defined target organ, and its biological and metabolic effects are quite numerous. Hence, there have been a diverse number of methods developed to measure the activity of the hormone. It should be kept in mind, however, that even though we can observe the different biological activities produced by this hormone, we still do not have a precise description of its action mechanism. As such, it is misleading to think about this hormone simply in terms of its growth-promoting effects. Although the term growth hormone does not adequately define the scope of the biological action of the hormone (Li, 1956), it has a long history of usage and will undoubtedly continue to be used, even though the alternative, somatotropin,* has the advantage of eliminating some of the connotations associated with the term growth. In this discussion, the two terms will be used interchangeably.

Since it is now apparent that species specificity is an important factor in connection with the biological activity of GH, it seems advisable to reexamine the existing bioassay procedures in terms of the responses elicited by growth hormones of various species. In general, somatotropin will elicit a body weight increase in either adult rats or hypophysectomized immature rats and in normal and dwarf mice. It can also elicit an increase in the weight of such organs as the liver, kidneys, stomach, and viscera in rats. An increase in the tail length, as well as skeletal changes such as an increase in the width of the tibial epiphysis, can be observed in the hyposectomized rat when it is treated with the hormone. Growth hormone has also been found to influence those activities associated with protein, carbohydrate, and fat metabolism. Indeed, it has been suggested that perhaps this hormone might best be named the metabolic hormone (Li, 1956). For example, growth hormone produces nitrogen retention, elicits decreases in the level of plasma amino nitrogen and blood urea, and causes an increase in plasma phosphate, to mention only a few effects associated with protein metabolism. One of the effects of growth hormone with respect to carbohydrate metabolism is the maintenance of muscle or cardiac glycogen in the fasting hypophysectomized rat. The hormone exercises a diabetogenic action in dogs, cats, and the intact rat. Fat metabolism is affected by this hormone, evidenced by the fact that when rats are chronically treated with growth hormone, their fat deposits are soon depleted. Growth hormone has also been observed to be capable of

* This term has now been accepted by the Commission on Biochemical Nomenclature of the IUPAC-IUB (1975).

causing a rapid increase in plasma nonesterified fatty acid concentration in a number of species.

A great diversity of methods for the bioassay of growth hormone have been proposed, but the number used routinely are few. Table I presents a summary of several of these methods compiled by Russell (1955). We will limit our efforts in this discussion to the few which are actively employed at present and to those which appear to hold promise for the future.

II. Weight Increase of Normal Plateaued Rats

The acceleration of growth in normal rats was one of the first noted and most easily demonstrable effects of growth hormone (Evans and Long,

Table I—Methods for the Detection or Assay of Growth Hormone[a]

Test	Duration of treatment	Precision (λ)[*b]
A. Body size		
Body weight increase		
Mature intact female rat	15–20 days	0.2–0.3
Hypophysectomized rat	10–14 days	0.3–0.4
Dwarf mouse	14 days	0.2–0.7
Tail length, increase in hypophysectomized rat	7–14 days	0.2–0.5
Tibial epiphysis, increase in width in hypophysectomized rat	4 days	0.3
Organ weight (e.g., liver thymus) increase	4–14 days	*
B. Metabolism of nitrogen, phosphorus, or sulfur		
Nitrogen balance, intact dog or rat	1–5 days	*
Plasma amino nitrogen, decrease	2–6 hours	0.4
Blood urea, decrease	2–6 hours	—
Urea formation after protein hydrolysate	1–3 hours	0.2–0.6
Tissue constituents (e.g., amino nitrogen, amide nitrogen, glutathione)	Varied	—
Tissue enzymes (e.g., transaminases) increase or decrease	7–14 days	—
^{15}N Retention	2 days	—
Plasma phosphate increase (hypox. rat)	15 days	*
Plasma or tibial phosphatase increase (hypox. rat)	15 days	*
Uptake of ^{35}S-methionine into muscle protein	3 days	—
C. Carbohydrate metabolism		
Muscle or cardiac glycogen, maintenance in fasting hypox. rat	24 hours	0.6
Cardiac glycogen, increase in intact rat	6–12 hours	0.8
R.Q. depression in fed intact rat	2– 6 hours	*
Diabelogenic action, intact rat	4 days	*

[a] Taken from Russell (1955).

[b] The significant relationship between response and dose is demonstrated. Index of precision (λ = S.D. in terms of log dose) is given if estimate is available.

1921; Evans *et al.*, 1948). After about 100 days, the normal female rat reaches a growth stasis or "plateau" where weight and length remain relatively stable. One of the first satisfactory tests for the bioassay of growth hormone used this observation to see if indeed the animal would grow if given injections of growth hormone (Evans and Simpson, 1931). A "growth hormone unit," i.e., the minimum amount of hormone necessary to produce a significant increment in body weight, was defined as that amount of growth hormone which causes an average body weight gain of 2 g per day. Adult female rats of the Long-Evans strain, 5–6 months old weighing 220–280 g, are satisfactory for the experimental procedure if growth stasis has been established by the rat's failure to gain more than 10 g in 20 days when diet and animal room conditions are kept constant. The hormone injections are given intraperitoneally daily for a specified period of time and the animals are weighed at 5-day intervals. Originally, a 20-day period was used, but a 15-day period has since been found adequate (Marx *et al.*, 1942). Both Light *et al.* (1940) and Chou *et al.* (1938) have suggested shorter injection periods of 5–10 days, but in this laboratory these injection periods proved too short for quantitative determinations. Fønss-Bech (1947) has used an injection period of 21 days.

According to standard methods of evaluating biological assays, the accuracy of a method can be analyzed by the ratio (λ) of the variance of the slope of the log-dose response line, $\lambda = s/b$, where s is the standard deviation about the curve, and b is the slope of the curve (Bliss and Cattrell, 1943). This analysis has been applied to the data obtained by several authors with the plateaued rat weight test, and the calculations are pre-

Table II—The Accuracy of Several Methods for the Bioassay of Growth Hormone

Method	Duration of injection period (days)	S.D. (s)	Slope (b)	λ	Reference[a]
Plateaued rat weight test	15	5.0	25.2	0.198	(a)
	21	4.08	18.07	0.226	(b)
Hypophysectomized rat	10	6.65	25.12	0.265	(a)
	7	4.95	14.0	0.354	(b)
	14	4.18	10.40	0.402	(b)
Dwarf mice weight test	14	0.78	3.34	0.234	(b)
	14	0.166	2.48	0.670	(b)
Tail length test	7	0.158	2.97	0.532	(b)
	14	1.19	5.30	0.225	(c)
Tibia test	4	24.6	79.4	0.310	(d)

[a] (a) Marx *et al.* (1942); (b) Fønss-Bech, 1947; (c) Dingemanse *et al.* (1948); (d) Greenspan *et al.* (1949).

sented in Table II. The data obtained by Marx *et al.* (1942) with groups of 30 animals, $\lambda = 0.198$ at each dilution of hormone, represent a high degree of precision. Using a Wistar strain of rats and an injection period of 21 days with groups of 10 animals, Fønss-Bech (1947) found the index of precision to be $\lambda = 0.226$, which is satisfactory. It is apparent that the method has a fair degree of accuracy even among different laboratories.

The minimum amount of hormone necessary to produce a significant response under stated conditions is a good index of the sensitivity of a particular bioassay. It is impossible to compare the sensitivity of one method to another unless both have been standardized with the same preparation. Fortunately, there have been some studies using the same preparation to standardize several bioassay methods. For example, a single partially purified bovine growth hormone preparation (designated as a globulin fraction) was used in our laboratory to standardize the plateaued rat weight test, the hypophysectomized rat weight test, and the tibia test *(vide infra)*. In the plateaued rat weight test, 15 mg of this preparation represented a minimal effective dose, whereas in the hypophysectomized rat weight test and the tibia test, 0.5 mg and 0.005 mg, respectively, were necessary for minimal effect (Table III). The plateaued rat weight test requires a considerable quantity of hormone in order to obtain a satisfactory response. As calculated from the data cited in Table III

Table III—Comparison of the Sensitivity of Various Methods of Growth Hormone Bioassay Standardized with the Same Hormone Preparation

Growth hormone preparation	Bioassay method	Threshold response		Reference[a]
		Definition	Total dose growth hormone (mg)	
Globulin fraction	Plateaued rat weight test	Weight gain 2 g/ day for 15 days	15,000	(a)
	Hypophysectomized rat weight test	Weight gain 1 g/ day for 15 days	0.520	(b)
	Tibia test	Increased width epiphyseal cartilage 40 μm	0.048	(b)
Bovine growth hormone	Hypophysectomized rat weight test	Weight gain 1 g/ day for 10 days	0.090	(c)
	Tibia test	Increased width epiphyseal cartilage 40 μm	0.005	(c)

[a] (a) Marx *et al.* (1942); (b) Evans *et al.* (1943a); (c) Li *et al.* (1945).

(globulin fraction), the hypophysectomized rat test is about 30 times as sensitive, and the tibia test about 300 times as sensitive as the plateaued rat weight test.

The specificity of the plateaued rat weight test must be considered in light of factors controlling the animal's growth. Diet, environmental conditions, and the interaction of a number of hormones are all influencing factors. On the other hand, if these conditions are stabilized, somatotropin is the only substance which will cause a rapid and continuous growth of the animal, although testosterone, prolactin, and thyroxine can cause small weight increases in the normal animal. The problem of synergism between GH and other hormones must also be considered. For example, thyroxine plus GH will produce a larger increment in growth than GH alone (Smith, 1933; Evans *et al.*, 1939); whereas ACTH will antagonize some of the growth effects of GH (Evans *et al.*, 1943b; Li and Evans, 1947). Therefore, it is important to determine the degree of contamination by other hormones in order to assess the possibility of synergism or antagonism in the reaction. It should be pointed out, however, that GH preparations of different potencies tested by this method produced very nearly parallel dose–response curves (Marx *et al.*, 1942), which indicates the response is reproducible. This is well illustrated in the assay data obtained with a partially purified bovine growth hormone preparation (globulin fraction) and a highly purified BGH preparation, both presented graphically in Fig. 1. The assays were run on the same Long–Evans strain of rats under similar conditions, but seven years apart, and the slopes are very nearly

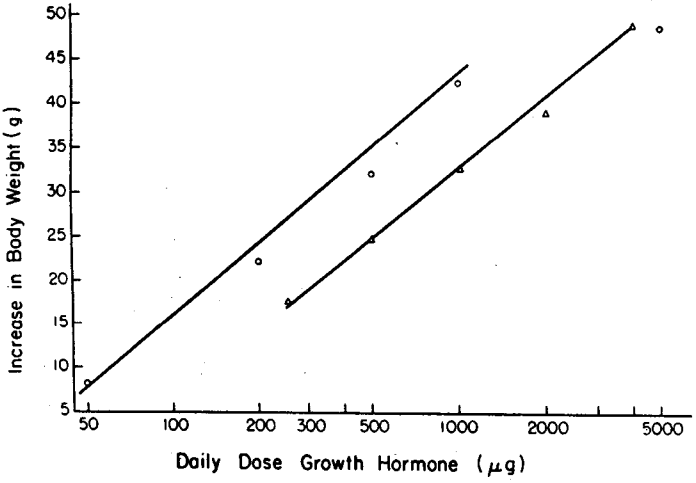

FIG. 1. Response of normal plateaued rats to partially purified BGH (△——△) and to pure BGH (○——○); 15 day test.

parallel. There is also some variation in the responsiveness of the animals at different times of the year. Hence, it is essential to control bioassays with a standard hormone preparation, whenever possible with the pure hormone.

The limitations of the plateaued rat weight method are that it is relatively insensitive and large amounts of hormones are necessary to produce a satisfactory response. In addition, the animals must be rigorously standardized, the assay must be controlled with a standard reference substance, and the possibilities of synergistic and antagonistic reactions of other hormones must be considered when impure preparations are assayed. Nevertheless, there are certain advantages of this method: it has a high degree of accuracy, the animals require no operative procedure, and they are easily maintained.

III. Weight Increase of Hypophysectomized Rats

Smith (1926, 1930) was the first to demonstrate the cessation of growth in the hypophysectomized rat and its resumption by implantation of whole pituitary glands. Van Dyke and Wallen-Lawrence (1930) first used these phenomena in the assay of growth-promoting extracts of the anterior hypophysis, but Evans *et al.* (1938) first standardized the procedure using female rats 2 to 3 months old, 20 to 30 days postoperative with a treatment period of 10 days. Under these conditions a GH unit was defined as that amount of hormone necessary to produce a weight increase of 10 g in 10 days in hypophysectomized rats. Later, immature female rats were used and the postoperative period shortened to 6 to 10 days (Fraenkel-Conrat *et al.*, 1940). The procedure for the hypophysectomized rat weight test can be summarized as follows: immature female rats are hypophysectomized at 26 to 28 days of age and are used in the assay 10 to 12 days later if the operation is complete. The criteria for judging a complete hypophysectomy are the limitation of body weight gain to 7 g in the preinjection period, impairment of body tone, maintenance of infantile hair, and finally the examination of the sella turcica at autopsy. Originally, the hormone was injected intraperitoneally daily for 15 days, but recently 10 days has been found to be satisfactory.

The hypophysectomized rat weight test is slightly less precise than the plateaued rat weight test, as indicated by the calculations of λ in Table II. This is substantiated by the fact that a larger multiple dose of the same hormone preparation is necessary to produce a significant increment of response with the hypophysectomized rat weight test than with the latter method. On the other hand, the hypophysectomized rat is considerably more sensitive than the normal plateaued rat in its response to GH. It has

already been noted that this test is about 30 times as sensitive as the normal plateaued rat test (Table III), and an even greater accuracy can be obtained in the hypophysectomized rat weight test if a longer injection period of 15 to 20 days is used.

As with the bioassays performed with normal rats, the specificity of the hypophysectomized rat weight test must take into consideration the synergistic and antagonistic effects of GH when combined with other hormones. It is interesting, however, that a partially purified GH preparation and pure hormone tested in the hypophysectomized animal several years apart yield standard curves of nearly parallel slope (Fig. 2).

The hypophysectomized animal is considerably more delicate than the normal animal and will not tolerate toxic extracts. Like the previous method, it is important to standardize the animals with regard to age, weight, and sex. Several investigators have pointed out the difficulties of using the same animals for more than one assay, especially since the response to the second assay series will be less than to the first (Marx *et al.*, 1942; Chou *et al.*, 1938).

The main advantage of the hypophysectomized rat weight test is the increased sensitivity of the assay method; the accuracy and specificity are about the same as that of the normal plateaued rat. The limitations of the method include the necessity of a careful operative procedure, the delicacy of the test animal, and the extreme importance of a rigorous standardization of the animals. As with the previous method, a standard reference substance should be used to control each assay and the possibilities of synergism and antagonism of other substances in impure preparations must be considered.

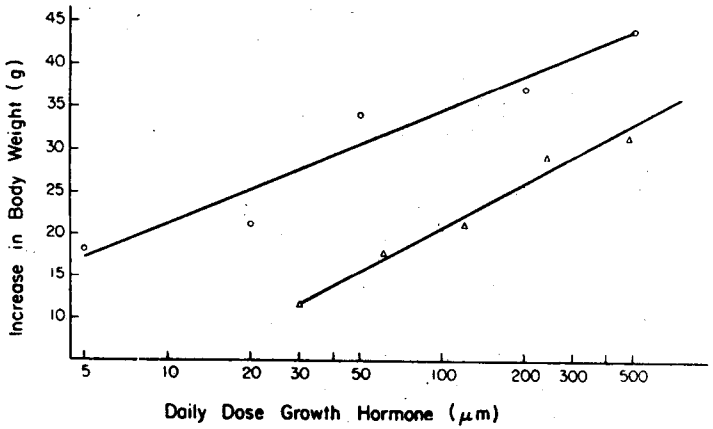

FIG. 2. Response of hypophysectomized rats to partially purified BGH (△——△) and to pure BGH (○——○); 15 day test.