COMPARATIVE BIOCHEMICAL ENDOCRINOLOGY OF PITUITARY GROWTH HORMONE

By

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It has been known for a long time that when the same protein hormone is isolated from tissues of various species, the products are not necessarily identical; however, comparative studies of the anterior hypophyseal hormones did not begin to attract wide interest until 1956, when it was demonstrated that the growth hormone (somatotrophin) isolated from primate pituitary glands is chemically distinct from the bovine hormone, and that the primate hormones are metabolically active in human subjects whereas the bovine hormone is not. In this communication, we wish to report some comparative investigations which have been carried out in the author's laboratory on the biochemical endocrinology of pituitary growth hormone. Recent reviews on some aspects of the subject have been published (Li 1957, 1958, 1959; Russell & Wilhelmi 1958; Knobil & Greep 1959).

PHYSICOCHEMICAL CHARACTERIZATION

There are now growth hormone preparations from pituitary glands of six different species, namely, ox (Li, Evans & Simpson 1945; Wilhelmi, Fishman & Russell 1948), sheep (Papkoff & Li 1958a), whale (Papkoff & Li 1958b), pig (Papkoff & Li 1959; Wilhelmi, private comm.), monkey and man (Li 1957, 1958, 1959; Li & Papkoff 1956), that can be obtained in a high degree of purity for physicochemical characterization. These preparations have been examined for homogeneity according to their behaviour in the ultracentrifuge and in electrophoresis and chromatography, as well as by N-terminal group analysis. In a few cases, immunological purity was also established. Some physical and chemical data for the six pituitary growth hormones are summarized in Table 1. It is of interest that the values for both molecular weight and iso-
### TABLE 1
Some Physicochemical Properties of Pituitary Growth Hormone from Various Species.

<table>
<thead>
<tr>
<th>Physicochemical characteristics*</th>
<th>Ox</th>
<th>Sheep</th>
<th>Pig</th>
<th>Whale (Hump-back)</th>
<th>Monkey (Macacus)</th>
<th>Man</th>
</tr>
</thead>
<tbody>
<tr>
<td>Sedimentation coefficient, (s_{20,w})</td>
<td>3.19</td>
<td>2.76</td>
<td>3.02</td>
<td>2.84</td>
<td>1.88</td>
<td>2.47</td>
</tr>
<tr>
<td>Diffusion coefficient, (D_{20,w})</td>
<td>7.23</td>
<td>5.25</td>
<td>6.54</td>
<td>6.56</td>
<td>7.20</td>
<td>8.88</td>
</tr>
<tr>
<td>Molecular weight, (M)</td>
<td>45000</td>
<td>48000</td>
<td>42000</td>
<td>39000</td>
<td>25000</td>
<td>27000</td>
</tr>
<tr>
<td>Isoelectric point, (p_I)</td>
<td>6.85</td>
<td>6.8</td>
<td>6.3</td>
<td>6.2</td>
<td>5.5</td>
<td>4.9</td>
</tr>
<tr>
<td>Specific optical rotation, ([\alpha]_{D}^{25})</td>
<td>-35.6°</td>
<td>-49.4°</td>
<td>-47.4°</td>
<td>-52.1°</td>
<td>-55.0°</td>
<td>-38.7°</td>
</tr>
<tr>
<td>Cystine</td>
<td>4</td>
<td>5</td>
<td>3</td>
<td>3</td>
<td>4</td>
<td>2</td>
</tr>
<tr>
<td>Tyrosine</td>
<td>12</td>
<td>13</td>
<td>13</td>
<td>12</td>
<td>10</td>
<td>9</td>
</tr>
<tr>
<td>Tryptophan</td>
<td>3</td>
<td>3</td>
<td>3</td>
<td>3</td>
<td>1</td>
<td>1</td>
</tr>
<tr>
<td>N-Terminal residue</td>
<td>Phe, Ala†</td>
<td>Phe, Ala</td>
<td>Phe</td>
<td>Phe</td>
<td>Phe</td>
<td>Phe</td>
</tr>
<tr>
<td>C-Terminal residue</td>
<td>Phe</td>
<td>Phe</td>
<td>Phe</td>
<td>Phe</td>
<td>Phe</td>
<td>Phe</td>
</tr>
</tbody>
</table>

* \(s_{20,w}\) (in S) determined in pH 9.9 borate buffer; \(D_{20,w}\) \((\times 10^7 \text{ cm}^2/\text{s})\), in pH 9.9 borate buffer; \([\alpha]_{D}^{25}\) in 0.1 M acetic acid; cystine, tyrosine and tryptophan, as residues per mole. † Phe: phenylalanine; Ala: alanine.

electric point of the primate hormones are the lowest of the various species whereas those of the bovine and ovine hormones are the highest: bovine = ovine > porcine = cetacean > simian = human. However, human and bovine hormones have an almost identical specific optical rotation, an index of the extent of the helical structure within the protein molecule; this value is much lower than that of the simian and cetacean hormones. These physical data have clearly demonstrated that the somatotrophins from pituitary glands of various species are distinctly different proteins.

Although amino acid analysis has shown differences in chemical composition, the six somatotrophins have an identical amino acid (phenylalanine) at both the C- and N-termini. The bovine and ovine hormones, however, have two N-terminal residues and the other four somatotrophins have only one. Further investigations (Parcells & Li 1958; Li, Parcells & Papkoff 1958) on the sequences adjacent to the C- and N-termini have revealed additional differences in these hormones as chemical entities. The known sequences at the
N-terminus are as follows: bovine somatotrophin, alanyl-phenylalanyl-alanyl ... and phenylalanyl-threonyl-alanyl..., and human, phenyl-alanyl-seryl-threonyl... At the C-terminus, the sequences are: bovine somatotrophin, ...leucyl-alanyl-phenylalanyl-phenylalanine; simian, ...alanyl-glycyl-phenylalanine, and human, ...leucyl-phenyl-alanine. From these physical and chemical data, it is evident that even the protein hormones isolated from species as closely related as man and monkey are not identical.

IMMUNOLOGICAL BEHAVIOUR

Immunological studies conducted with bovine (Hayashida & Li 1958a) and human (Hayashida & Li 1958b) growth hormones have demonstrated that the protein hormones form antibodies specific to the species, as evidenced by results of precipitin ring tests with rabbit antiserum and of anaphylactic shock experiments in guinea pigs. It has been further demonstrated in hypophysectomized rats that the rabbit antiserum possesses specific antihormone activity. In addition, the immunological homogeneity of the rabbit antiserum to human growth hormone has also been indicated by agar-gel diffusion tests performed by the Ouchterlony technique. Production of haemagglutinating antibodies to human growth hormone has also been reported by other investigators (Read & Stone 1958; Fishman, McGarry & Beck 1959).

Furthermore, it was shown by the Ouchterlony technique that antibodies to human somatotrophin cross-react almost completely with the simian hormone but manifest no reaction with the bovine, ovine, porcine or cetacean hormones (Hayashida & Li 1959; Li, Moudgal & Papkoff, unpubl.). Similar studies with rabbit antiserum to bovine somatotrophin indicated that bovine and ovine somatotrophin appear to be closely related antigenically, whereas bovine somatotrophin and simian hiraffiende ryrna hre primate omonnt in this respect. These observations on the species specificity of the immunological reactions were further supported by a demonstration that antibodies to bovine somatotrophin can completely neutralize the biological activity of bovine somatotrophin but not that of the human hormone.

It was also shown that rabbit antiserum to either the bovine or human somatotrophin could not only detect minute amounts of the homologous purified hormone in saline solution and neutralize its biological activity, but could also detect the hormone in a crude pituitary extract and significantly neutralize the growth-promoting effect of such an extract. These results have suggested that the antibodies to the purified somatotrophin may also be specific for the hormone as it exists in the crude state. This was further supported by the findings from the agar gel-diffusion studies, which revealed the presence of a substance in the crude extracts that was antigenically indistinguishable from the homologous purified somatotrophin. It is felt, therefore, that the similarities
and differences noted above in the somatotrophins from the various species are real, and not result of any artifact arising from the physicochemical manipulations required to purify the hormones (Hayashida & Li 1959; Li, Moudgal & Papkoff, unpubl.).

A purified gamma-globulin from rabbit antiserum to human somatotrophin has been prepared (Li, Moudgal & Papkoff, unpubl.), and with this gamma-globulin as the antibody, quantitative precipitin tests were performed on human and simian growth hormones. It was noted that the equivalence point for the human somatotrophin is located at 50 µg whereas that for the simian hormone is located at 70 µg. In a further experiment in which a solution of the gamma-globulin was treated with an equivalent concentration of monkey growth hormone and the supernatant fluid tested for any reactivity with human somatotrophin, no reaction occurred, indicating that all the precipitable antibodies to human growth hormone had been precipitated by the monkey hormone. Thus, it can be concluded that the cross-reaction between these two primate hormones is complete (Li, Moudgal & Papkoff, unpubl.).

The quantitative precipitin test with the purified gamma-globulin used as the antibody has been applied to achieve a quantitative estimation of the growth hormone content in single human pituitary glands and in sera of acromegalic subjects (Li, Moudgal & Papkoff, unpubl.). In five experiments an average estimate of the growth hormone content in a single human pituitary gland (wet weight of single glands varying from 463 to 701 mg) was 5.7 mg, with the full range extending from 3.8 mg to 8.8 mg. In addition, a preliminary estimate of the growth hormone content in lyophilized sera of several acromegalic patients, made on the basis of the quantitative immunochmical method, was approximately 0.2 µg of the hormone per ml of serum.

**GROWTH-PROMOTING ACTIVITY**

Since the isolation of bovine pituitary growth hormone in highly purified form (Li, Evans & Simpson 1945; Wilhelmi, Fishman & Russell 1945), the many attempts to demonstrate its activity as an anabolic agent in man have met with no success (Bennett, Weinberger, Escamilla, Margen, Li & Evans 1950; Shorr, Carter, Kennedy & Smith 1953). In addition, the bovine hormone has been shown to be incapable of promoting growth in the guinea pig (Michell, Guillemin & Selye 1954) and monkey (Knobil & Greep 1959). Although bovine growth hormone is active in both fish and rats, fish pituitary growth hormone preparations are active in fish but not in rats (Wilhelmi, 1955). As expected, primate growth hormones are very active in promoting body growth in primates. Studies related to the effect of human pituitary growth hormone in human subjects will be reported by Luft, Raben, Escamilla and others before this Congress.

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It is of interest that the growth-promoting activities of the growth hormones isolated from pituitary glands of ox, sheep, whale, pig, monkey and man as assayed by the four-day tibia test do not differ significantly from one another. A total dose of 60 µg produced an increment in the width of uncalcified tibial cartilage in hypophysectomized rats (Long-Evans strain; operated on at 28 days of age; daily injections begun 14 days after the operation) over the value of 155 µ for the controls, as follows: bovine, 91 µ; ovine, 76; cetacean, 95; porcine, 88; simian, 93; and human, 105. It was also noted that a straight-line relationship occurs when dose-response is plotted according to the following equation, in which the values quoted are for human growth hormone:

\[ W = 42.5 \log D + 184.4 \]

where \( W \) is the width of tibial epiphyseal cartilage in micra and \( D \) the total dose in micrograms.

Not only these six highly purified growth hormone preparations, but also crude extracts of whole pituitary glands obtained from the frog, rabbit, cat, horse, and dog (Solomon & Greep 1959; Li & Jordan unpubl.), have been demonstrated to possess significant somatotrophin activity as shown by the tibia test. Moreover, turtle and chicken (Solomon & Greep 1959; Li & Jordan unpubl.) pituitary extracts elicited a measurable growth stimulation, whereas shad pituitary material (Solomon & Greep 1959) failed to elicit any response. As already mentioned, bovine somatotrophin cannot promote growth in guinea pigs (Michell, Guillemin & Selye 1954), but the pituitary glands of the guinea pig were found to have growth-promoting activity in the hypophysectomized rat (Li & Jordan unpubl.).

When various growth hormone preparations were assayed on the basis of body-weight gain in hypophysectomized rats, it was found that the rats gained weight at a comparable rate for the first 10 days regardless of the origin of the hormone. After 10 days, however, animals initially injected with either human or monkey growth hormone ceased to gain weight, whereas those injected with the hormone from other species (whale, pig, sheep, or ox) continued to elicit a weight gain for several weeks (Li, Papkoff & Jordan 1959). It was further found that if the human or simian somatotrophin-injected rats whose growth had ceased were then injected with either the cetacean or bovine hormone, the body-weight gain resumed. The plateauing of effect observed with the primate hormones, and not seen in the non-primate hormones, for their part elicit continuous growth in the rat, probably reflects a peculiar chemical structure on the part of the hormone protein from man and the monkey. It might be that this structure endows the hormones of these species with a capacity to produce antibody in the rat, a rare property for a protein. At any rate, it is clear that the biological differences among proteins of various species reflect chemical differences in their structures.
DISCUSSION

It is of considerable interest that according to the aforementioned data growth is elicited in the rat by injections of pituitary somatotrophin from practically all the species studied so far, including ox, sheep, whale, pig, monkey and man, the only exception being the hormone from fish glands. In view of the fact that there are marked differences among these various growth hormones in chemical structure and physicochemical characteristics, it is remarkable that the rat is capable of such a wide response. On the other hand, although it has been demonstrated that man can respond to the growth-promoting activity in primate somatotrophins, practically all attempts to obtain a response in man with the bovine hormone have hitherto failed. The question has been raised whether a common biologically active core (Li 1957, 1958, 1959) or nucleus (Wilhelmi 1955) might possibly exist in the somatotrophin molecules derived from various species, and, further, whether the rat may possess the necessary enzyme system to utilize the whole hormone protein from non-primate sources whereas man does not (Li 1957, 1958, 1959). In order to explore this hypothesis, it is necessary to establish the primary assumption that the complete integrity of the protein molecule is not necessary for the hormonal action. This was earlier found to be the case for bovine somatotrophin (Li 1956), when the hormone was partially hydrolyzed with the enzymes chymotrypsin, trypsin and carboxypeptidase. Similar studies with ovine (Papkoff & Li 1958b), porcine (Papkoff & Li 1959), cetacean (Papkoff & Li 1958a), simian and human (Li 1957, 1958, 1959) somatotrophins have also shown that limited digestion of the hormone by chymotrypsin does not cause loss of growth-promoting potency. Indeed, a fraction (alpha core) prepared from a chymotryptic digest of bovine somatotrophin (Li, Papkoff & Hayashida 1959) was shown in preliminary studies to produce nitrogen retention in human subjects (Forsham, Li, DeRaimondo, Kolb, Mitchell & Newman 1958). It should pointed out that these observations are not sufficient to verify the assumption that the same active core occurs in various somatotrophins, but they do encourage further investigation along the lines of the “common core” hypothesis.

REFERENCES

Li C. H.: Laboratory Investigation 8 (1959) 574.