hormonal proteins and peptides

THYROID HORMONES

EDITED BY CHOH HAO LI

CHOH HAO LI 6

HORMONAL PROTEINS AND PEPTIDES

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The Hormone Research Laboratory
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VOLUME VI

Thyroid Hormones

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Preface

In young animals, removal of the thyroid gland results in an arrest of growth. In men, a similar effect is seen when the thyroid is atrophied at birth. It is now known that the thyroid hormone has profound effects on growth, differentiation, and development of tissues. The metabolism of carbohydrates, proteins, and lipids is influenced by the thyroid gland. The thyroid hormone has also been shown to influence the rate of red blood cell and hemoglobin production. In lower vertebrates, the action of the thyroid hormone is involved in a diversity of functions, including growth, metamorphosis, sexual maturation, migration, and nervous activity.

The presence of iodine in organic combination in the thyroid gland was first demonstrated by E. Baumann in 1896. Nearly twenty years later, E. C. Kendall isolated the active component, thyroxin, in crystalline form. This achievement paved the way for the elucidation of the structure of thyroxine as 3,5,3′,5′-tetraiodothyronine and its synthesis by C. R. Harington and G. Barger in 1927. The occurrence of an unknown iodine-containing compound in hydrolysates of the thyroid of the rat was discovered by J. Gross and C. P. Leblond in 1951. Subsequently, Gross and R. Pitt-Rivers showed that the unknown compound was 3,5,3′-triiodothyronine. Pitt-Rivers has been associated with Harington for many years. In the last chapter of this volume, she presents historical aspects of the thyroid hormones.

In addition to the chapter by Pitt-Rivers, this volume presents six chapters on various aspects of the thyroid hormones. The first of these, by Sairam and Li, deals with the chemistry of human thyrotropin. In Volume I of this series, Pierce *et al.* published a review of bovine thyrotropin. At that time, the amino acid sequence of the human hormone was not known. From comparison of their structures, it is now evident that the hormone specific subunit, β, is more resistant to changes during the course of evolution. In the most comprehensive review of this volume, Jorgensen surveys the chemistry of thyroid hormones and analogs, with special reference to synthesis and structure–activity relationships. Although several reviews related to this field have appeared in recent years,

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this contribution of Jorgensen in two chapters gives, I believe, the most critical and thorough study yet available of thyroid hormone chemistry.

For the last decade, various investigators have focused their studies to explain the physiological actions of thyroid hormones in terms of molecular mechanisms. In Chapter 4, Dratman presents an important concept of thyroxin action. She views thyroxin as an amino acid analog, and proposes possible direct participation of thyroxin in catecholamine pathways in the nervous system.

In the fifth article, Cohen *et al.* discuss in detail the thyroid hormone action during amphibian metamorphosis at the cellular and molecular levels. Cohen has studied biochemical and ultrastructural changes resulting from thyroid hormone treatment using the tadpole system for many years, and summarizes the current status of the problem in a systematic and coherent fashion. This chapter is followed by a short account by Gorbman on the evolution of thyroid function.

The Editor especially wishes to acknowledge the cooperation of the contributors. He is also indebted to the staff of Academic Press for their help and cooperation.

Choh Hao Li

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7. The Thyroid Hormones: Historical Aspects

Rosalind Pitt-Rivers

1

Chemistry of Human Pituitary Thyrotropin*

M. R. SAIRAM AND CHOH HAO LI

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I. Introduction*

Early experiments of Smith revealed the existence of a factor in the pituitary that influenced thyroid function in the rat (Siebert and Smith, 1930; Smith, 1930). It is now established that thyrotropin is one of the three distinct glycoprotein hormones secreted by the anterior pituitary gland. In comparison to the other pituitary hormones, the glycoprotein hormones have been difficult to isolate and characterize. These difficulties have been partly attributed to the low content in the gland, instability, tedious nature of bioassays, and lack of suitable standards. The development of techniques of protein fractionations enabled the isolation of the glycoprotein hormones in the early 1960's. During the last decade, notable progress has been made in unraveling the close structural interrelationships in the glycoprotein hormone family. This has been possible only because of simultaneous and independent contributions by several laboratories around the world.

Bovine TSH has clearly been the most widely investigated of all the mammalian thyrotropins. A detailed description of the chemistry of thyrotropins, with special reference to the bovine hormone, can be found in recent articles by Pierce (1974) and Pierce *et al.* (1973). They have admirably reviewed the field and set the stage for the present chapter. References to earlier literature can also be found in these reviews. In this article we shall restrict ourselves to the chemistry of human thyrotropin and to its interrelationships with other human hormones. A full-scale investigation of the detailed chemistry of human TSH had to await the complete structural analysis of the bovine hormone. The demonstration that bovine TSH, like ovine lutropin, was a subunit protein consisting of an α and β subunit, and the elucidation of the subunits' complete amino acid sequences, provided the basic information needed to launch similar studies on a limited supply of the precious human hormone. It is recognized that proteins from different species with similar biological profiles have similar molecular architectures. It was thus deemed reasonable to suppose a similar situation with respect to human and bovine TSH.

This chapter describes recent investigations conducted in both our own laboratories and those of others on the isolation, characterization, and structure of human pituitary thyrotropin; it also discusses this hormone's relationship to the structure of human lutropin and follitropin. These three pituitary glycoprotein hormones and human choriogonadotropin have now been shown to consist of a common α subunit and a β subunit that is

* Abbreviations: TSH, thyrotropin; CMC, carboxymethyl cellulose; HCG, human choriogonadotropin; HCT, human chorionic thyrotropin.

specific to each hormone. The human placenta is also known to secrete a substance with thyrotropic activity; this material has been referred to as human chorionic thyrotropin. For a variety of reasons, the information available on this hormone is restricted to certain aspects of its characterization; we have included here pertinent data on some of its properties for comparison with pituitary thyrotropin.

II. Isolation Procedures

The first isolation procedure developed for human thyrotropin was achieved by Condliffe in 1963. This procedure utilized the basic steps employed for the preparation of bovine thyrotropin. Using a side fraction obtained during the preparation of human somatotropin, purification was effected by a combination of CM-cellulose and DEAE-cellulose chromatography and gel filtration. Further improvements in the isolation method were reported by Bates *et al.* (1968). This method consisted in serial percolation of the pituitary material with a mixture of ethanol–NaCl–water in varying concentrations. Thyrotropin and lutropin were separated from follitropin by CM-cellulose chromatography at pH 6.0. The thyrotropin and lutropin present in the adsorbed fraction were separated by DEAE-cellulose chromatography at pH 9.5.

The wide interest in the clinical uses of human pituitary hormones has necessitated the development of procedures to provide highly purified preparations suitable for such clinical purposes as well as for detailed biochemical investigations. Efficient and simple methods are essential in order to accomplish this goal, given the relative scarcity of human pituitary glands. A procedure that is currently widely employed in a number of laboratories, including our own, is that developed by Stockell-Hartree in 1966. The principal advantages of this method (other than its simplicity) are its easy applicability to large amounts of glands and the achievement of a group separation of the major hormones at a very early stage of the extraction method. The method employed by us will be described below in some detail, as we have used these preparations in our characterization studies and structural analysis.

Using the "crude glycoprotein" fraction of the Hartree (1966) procedure, Shome *et al.* (1968a) obtained highly purified preparations of human thyrotropin by combined ion-exchange chromatography on CM-cellulose and DEAE-cellulose. This preparation was subjected to some physicochemical characterization (Shome *et al.*, 1968b). The same laboratory utilized essentially similar procedures in obtaining the hormone employed in later work on the isolation of subunits (see Section V).

In our laboratories, human thyrotropin has been isolated from a variety

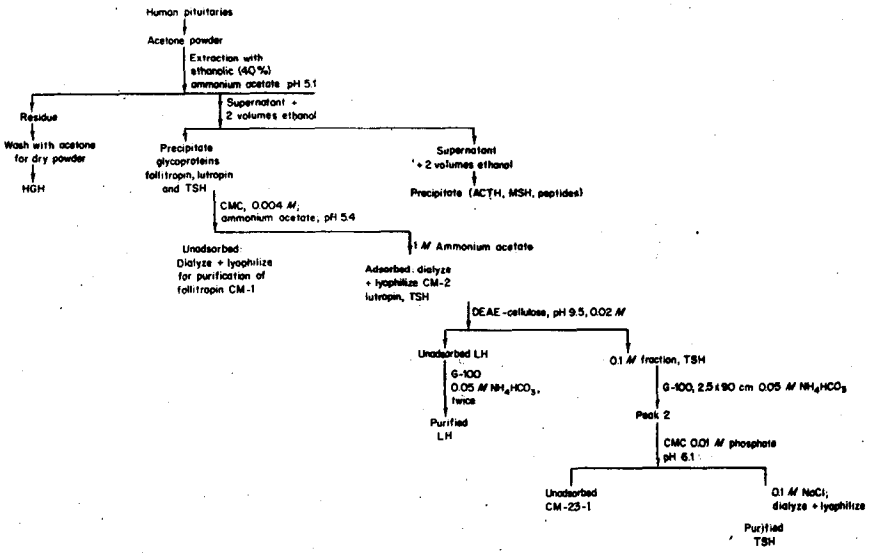

FIG. 1. Isolation scheme for human pituitary thyrotropin.

of starting materials, viz., fresh frozen glands, acetone dried glands, and crude glycoprotein fraction. The extraction procedure to be described below has employed fresh frozen glands. The procedure is that of Stockell-Hartree (1966) with some modifications. The complete isolation scheme is depicted in Fig. 1. In a typical experiment, an acetone powder of 500 glands was soaked in 600 ml of cold 6% ammonium acetate–40% ethanol mixture at pH 5.1 and mixed briefly in a Waring blender. The suspension was stirred overnight and the clear supernatant was recovered by centrifugation. The fibrous residue was reextracted in the blender with 400 ml of the same solvent. The residue was suspended in water, dialyzed, and lyophilized. This fraction is used for the preparation of clinical grade human somatotropin. The supernatants were combined and two volumes of cold ethanol (−20° F) were added slowly to effect precipitation of the glycoproteins. The mixture was left for 36–48 hours and the clear supernatant was decanted. The precipitate which was collected by centrifugation was suspended in cold distilled water, dialyzed, and lyophilized. This forms the glycoprotein extract, designated fraction A. The ethanol concentration in the supernatant of fraction A was increased to 85% and kept for 48–72 hours. The resulting precipitate was harvested, suspended in water, and directly lyophilized. This fraction was designated fraction B.

Fraction A, which is obtained in an average yield of about 1.5–2 g per 1000 glands, contains nearly all the gonadotropic and thyrotropic ac-

tivities. The same procedure has also been applied to acetone dried glands. Thus, more than 20,000 glands have been processed by this method.

A. Separation of Follitropin from Lutropin and Thyrotropin

CMC chromatography was used to separate the follitropin from lutropin and thyrotropin. About 2 g of fraction A was extracted overnight with 60 ml of 0.004 *M* ammonium acetate, pH 5.4. The supernatant was separated by centrifugation and residue reextracted. The small amount of insoluble residue that remained was discarded. The combined supernatant was loaded onto a 3 × 45 cm column of CM-cellulose (Schleicher and Schuell Inc., Keene, New Hampshire) equilibrated with the above buffer. The column was washed with the same solvent until the optical density at 280 nm was <0.1. The unadsorbed fraction, which contained nearly all of follitropin in the starting material, was dialyzed and lyophilized (designated CM-1). The adsorbed proteins, consisting of lutropin, thyrotropin, and others, were eluted by 1 *M* ammonium acetate. The solution was dialyzed and lyophilized. This fraction was designated CM-2. CM-1 has been used for purification of follitropin. CM-2 is obtained in an average yield of about 400 mg/g fraction A, and is found to contain low follitropic activity (0.3 × NIH-FSH-S8).

B. Chromatography and Gel Filtration

One of the persistent problems in the isolation of TSH has been the elimination of accompanying lutropic activity. The chromatographic behavior of the two hormones are so similar that separation is difficult to achieve in many systems; indeed, no satisfactory separation was accomplished until the introduction of DEAE-cellulose chromatography into the purification scheme. The first successful separation of the two hormones from bovine extracts was achieved by Condliffe and Bates (1957) by anion-exchange chromatography at pH 9.5. This step has been widely employed ever since for a number of other species, including human.

In a typical experiment, CM-2 was dissolved in 0.02 *M* Gly-NaOH, pH 9.5, and chromatographed on DEAE-cellulose (Bio-Rad, 0.67 meq/g) equilibrated in the same buffer. The protein fractions were eluted by a stepwise increase in buffer concentration (Fig. 2). Under these conditions, five major protein fractions were eluted and recovered by dialysis and lyophilization (designated fractions I–VI). A large part of lutropin present in CM-2 was unadsorbed under these conditions and could be easily re-

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