HORMONAL PROTEINS AND PEPTIDES

Edited by CHOH HAO LI

VOLUME XI

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The Hormone Research Laboratory
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VOLUME XI
Gonadotropic Hormones

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Preface

In 1930, P. E. Smith wrote

> Without exception, all of my hypophysectomized rats have shown a pronounced retrogression and atrophy of all the reproductive organs. That this retrogression in these animals is due to the removal of the anterior lobe, and not to a brain injury, is strongly indicated by the restoration which is affected by a replacement therapy [*Am. J. Anat.* **45**, 205–273 (1930)].

This definitive evidence for the existence of gonadotropic factor(s) in anterior pituitary extracts was followed, a year later, by the observations of H. L. Fevold, F. L. Hisaw, and S. L. Leonard, who wrote

> In this paper we wish to present definitive evidence for the presence of two distinct anterior lobe hormones which promote follicular and lutein development in the ovary. One of these is the gonad stimulating hormone which causes precocious sexual maturity when injected into immature rats. Its primary function seems to be the stimulation of follicular activity in the ovary. The second is the luteinizing hormone which alone cannot affect ovaries of an immature animal. It does, however, cause luteinization of the follicles which are produced by the gonad stimulator [*Am. J. Physiol.* **97**, 291–301 (1931)].

It took 30 to 40 years for these two gonadotropins to be isolated in highly purified form: lutropin (LH) was isolated in 1959 [*J. Biol. Chem.* **234**, 520–525 (1959)] and follitropin (FSH) in 1967 [*Arch. Biochem. Biophys.* **120**, 434–439 (1967)]. Availability of pure hormones led to the determination of the primary structure, development of highly sensitive and specific radioimmunoassay, and elucidation of LH/FSH action in the reproductive processes.

In the opening chapter of this volume Sairam reviews the relationship of chemical structure to biological activity for gonadotropins, including chorionic hormones. This is followed by a short article by Ramachandran on *in vitro* bioassay methods. In the third chapter McIllroy and Ryan give a comprehensive discussion on some aspects of the molecular mechanism of gonadotropin action. The final contribution, by Sheela Rani and . loudgal, deals with immunobiology of gonadotropins, an area in which research has been very active since it began 21 years ago [*Arch. Biochem. Biophys.* **95**, 93–98 (1961)].

I wish to express my thanks to the staff of Academic Press for their cooperation in the task of preparing this volume.

Choh Hao Li

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Gonadotropic Hormones: Relationship between Structure and Function with Emphasis on Antagonists

M. R. SAIRAM

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I. Introduction

The gonadotropins belong to the family of hormones generally termed *glycoprotein hormones*. They are found in secretions from the anterior pituitary and the placenta. In humans and other primates, gonadotropins are also excreted into the urine, and in many instances ectopic production of these hormones or their subunits has been detected.

The glycoprotein hormones* from the anterior pituitary—lutropin, follitropin, and thyrotropin—and those of placental origin—human and equine choriogonadotropin—have two nonidentical subunits designated α and β, which are held together by noncovalent bonds. It has been shown that the α subunits in many mammalian species are common to all three pituitary glycoprotein hormones. This remarkable identity extends to the placental gonadotropins, as shown in human, but may also be true in other species in which such secretory capacity has been detected. Hybrid molecules, both interspecies and intraspecies, can be obtained by the appropriate recombination of subunits under suitable conditions. The biological activity of the recombinant is dictated by the selection of the β subunit. There is a high degree of conservation of structure of the α and β subunits of the hormones between different species. Because of the ability to generate hybrids and because of the similarity in structure, it has been concluded that the binding regions between the subunits must be of a similar nature, but as we shall note later, there are also subtle differences. Because the β subunits combine with a common α subunit, it has been further speculated that areas of the sequence among the different hormone β subunits that are similar must be responsible for interaction with the α subunit, but those regions that differ may be responsible for conferring hormonal specificity.

During the past decade workers have recorded significant advances in the determination of the structure of these hormones from several species including human. Although an understanding of nonmammalian gonado-

* The nomenclature of hormones used in this chapter follows the recommendations of IUB–IUPAC. Lutropin, LH (luteinizing hormone); follitropin, FSH (follicle-stimulating hormone); thyrotropin, TSH (thyroid-stimulating hormone); human choriogonadotropin, hCG; equine chorionic gonadotropin, eCG (formerly called pregnant mare serum gonadotropin, or PMSG); adrenocorticotropin, ACTH; deglycosylated, DG.

tropins is by no means complete, it is encouraging that progress is being made. We have also witnessed significant advances in studies of the structure and function of the gonadotropic hormones using the well-defined techniques of protein chemistry. In nonmammalian hormone preparations, we have unique structural variations (analogs) provided by nature that can be used to probe structure–function relationships.

Detailed investigations of the structure and function of many hormones have been responsible for the design of structural analogs having desired agonistic or antagonistic activities. In the case of several small and large peptides and reasonably small proteins with hormonal activity, it has been possible to chemically synthesize analogs having desired properties by using the modern methods of synthetic technology and purification. In numerous studies using gonadotropin-releasing hormone, oxytocin, vasopressin, adrenocorticotropin, melanotropin, enkephalin, and other hormonal peptides, an impressive array of analogs have become available. Similar studies of more complex hormones, such as the gonadotropins, which have 15–45% carbohydrate as an integral part of their molecule, are more difficult to carry out. Hence, all data pertaining to the structure and function of the gonadotropins are from studies involving modification of individual and functional amino acid side chains in the polypeptide backbone and the monosaccharide units in the covalently linked carbohydrate moiety. These have been achieved mainly by using either specific reagents capable of modification or specific enzymes that remove part of the molecule. The presence of an oligomeric structure in these hormones presents unique challenges for study, in addition to many problems that are normally investigated with other hormones or proteins. Thus, in the study of structure–function relationships of gonadotropins, one seeks to answer the following questions:

1. What is the effect on the quaternary structure? Is the hormone destabilized (biophysical studies)?
2. How does a specific modification affect subunit interaction (recombination)?
3. What is the effect on immunological activity?
4. What is the effect on interaction with the specific receptor(s) in target cells? Is affinity reduced or is hormone specificity altered?
5. Are cell responses such as cyclic AMP accumulation and steroidogenesis affected?
6. What are the resultant effects on half-life (metabolism) of the hormone and activity *in vivo*?
7. Are there any antagonistic effects *in vitro* and/or *in vivo*?
8. What are the specific residues/groups involved?

Many reviews since 1971 record the progress made in this field (Pierce, 1971; Sairam and Papkoff, 1974; Jutisz and Tetrin-Clary, 1974; Liu and Ward, 1975b; Ward, 1978; Sairam, 1978; Pierce and Parsons, 1981), and these should be consulted for more details. The purpose of this chapter is to highlight more recent data with special reference to structure–function relationships emphasizing the antagonists. Although the focus is on pituitary gonadotropins in particular, a discussion of the chorionic gonadotropins and thyrotropins is necessary for a better understanding of the subject.

II. Isolation and Characterization

Highly purified and well-characterized preparations of follitropin (FSH) and/or lutropin (LH) are now available from at least seven different mammalian species—human, porcine, ovine, bovine, equine, rat, rabbit (see Sairam and Papkoff, 1974; Liu and Ward, 1975b; Reichert, 1975), hamster (Glenn *et al.*, 1982), and whale (Takahashi and Ui, 1977). Purified gonadotropins from baboon (Shownkeen *et al.*, 1973) and dog pituitaries (Hartree *et al.*, 1972) have been obtained, but their properties have not been thoroughly investigated. Their general properties and behavior in different fractionation systems are now well known, and thus purification procedures should not be too difficult to devise for other species. However, the purification of FSH continues to present problems despite the fact that its structure from several species is known. Its low content in the pituitary, its heterogeneity, and its lability are mainly responsible for this difficulty.

Chorionic gonadotropin of placental origin in human is also excreted in the urine in large quantities during the first trimester of pregnancy; it can be extracted from the urine fairly easily and purified by simple conventional techniques (Bahl, 1973; Birken and Canfield, 1978). The same cannot be said of human pituitary gonadotropins, which are also excreted into the urine of normal men and women and of postmenopausal subjects. Although there has been an interest in urinary FSH and LH for almost 40 years, few significant advances have been made during the past decade since the structure of gonadotropins of several species including human became known. However, they have been partially purified and characterized (Van Hell *et al.*, 1972), but reliable data on their amino acid and carbohydrate composition are yet to come. A detailed study of their structure would be interesting with respect to structure–function relationships. A knowledge of their structure would reveal changes that may occur during their metabolic clearance, and because they are biologically active, the changes would reflect the extent and effect of enzymatic modifications in the polypeptide and/or the carbohydrate portions of the molecule.

During the last decade, considerable attention has also been focused on the gonadotropic hormones of nonmammalian species within such groups as birds, fishes, amphibians, and reptiles. These can be viewed as natural hormone analogs created during the course of evolution. In general the techniques employed for the purification of mammalian gonadotropins are also applicable to those of nonmammalian species (Papkoff *et al.*, 1976). The elucidation of their structures would be very valuable. Partial sequence data are available on fish gonadotropin (see Section IV,A). Although two separate gonadotropins, FSH and LH, have been identified in birds, amphibians, and some reptiles, this question has not been unequivocally answered for fish (Fontaine, 1980; Farmer and Papkoff, 1979; Ng and Idler, 1979) and for snakes (Licht *et al.*, 1979a). The snake hormones are apparently unique among the tetrapod gonadotropins with respect to their biological, immunological, and biochemical properties. It has been suggested that snakes may have only one gonadotropin molecule and that it does not exhibit a clear homology to the known FSH and LH of other nonmammalian species.

In normal mammals, gonadotropins are also found in tissues other than the pituitary, although in much lower concentrations (Yoshimoto *et al.*, 1977; Braunstein *et al.*, 1979). Interestingly, immunoreactive and bioassayable lutropin activity has been detected in regions of the rat brain (Emanuelle *et al.*, 1981). As for many other hormones that have also been found in several regions of the mammalian brain, its physiological significance remains to be established. The fact that the brain extract can stimulate the interstitial cells inducing testosterone secretion suggests that it may be related to pituitary LH.

III. Subunit Nature

Follitropin and lutropin from all species are glycoprotein hormones containing two noncovalently bonded dissimilar subunits. Evidence is emerging to show that this generalization extends to the nonmammalian gonadotropins as well. This has been documented at present for turtle (Papkoff *et al.*, 1976) and fish (Fontaine, 1980). Thus the subunit nature of the gonadotropic hormones is an ancient feature of evolution that has been preserved.

The early developments that led to the postulation and eventual demonstration of subunit structure have been reviewed in detail (Pierce, 1971; Sairam, and Papkoff, 1974; Liu and Ward, 1975b). To recapitulate, the gonadotropic hormones, including those from the placenta, consist of a common subunit designated α and a hormone-specific β subunit. The determination in 1971 of the complete amino acid sequences of the α and

Table I—Amino Acid Sequence of the Gonadotropins

Hormone	References	
	α	β
Ovine lutropin	Sairam *et al.* (1972a) Liu *et al.* (1972a) Papkoff *et al.* (1973)	Sairam *et al.* (1972b) Liu *et al.* (1972b) Papkoff *et al.* (1973)
Bovine lutropin	Ward and Liu (1971) Pierce *et al.* (1971)	Rogister and Hennen (1973) Ward and Liu (1971)
Porcine lutropin	Rogister *et al.* (1973)	Rogister and Hennen (1973)
Human lutropin	Sairam *et al.* (1972c)	Sairam and Li (1975b) Keutmann *et al.* (1979) Shome and Parlow (1973) Closset *et al.* (1973)
hCG	Bahl *et al.* (1972)	Carlsen *et al.* (1973) Morgan *et al.* (1973) Birken and Canfield (1978)
eCG	Moore *et al.* (1980)	Moore *et al.* (1980)
Human follitropin	Shome and Parlow (1974a) Rathnam and Saxena (1975)	Shome and Parlow (1974b) Saxena and Rathnam (1976)
Equine follitropin	Rathnam *et al.* (1978)	Fujiki *et al.* (1978)
Porcine follitropin	—	Closset *et al.* (1978)
Ovine follitropin	Sairam (1981)	Sairam *et al.* (1981)

β subunits of bovine thyrotropin (TSH) and ovine LH catalyzed work on other species. By the end of the decade complete data on LH and FSH from four species have become available. In three species, human, ovine, and porcine, the sequence of the α and β subunits of both hormones have been elucidated. The references for the various structural data are given in Table I. For the sake of completeness, work on the placental gonadotropins hCG and eCG is also included. Contributions from various laboratories around the world have complemented each other and have served to clarify discrepancies of some of the earlier data on amino acid sequences.

The primary structure of the gonadotropins should be considered in two parts: (1) the polypeptide backbone and (2) the oligosaccharide moiety.

IV. The Polypeptide Structure

A. Structure of the α and β Subunits

Within a given species the amino acid sequences of the α subunits of LH, FSH, and TSH are identical (Fig. 1). In human the sequences of the

pituitary and placental glycoprotein hormones have been established thus enabling structural comparisons. Thus the amino acid sequences of the α subunits of urinary hCG (of placental origin) and human pituitary TSH are both identical to those of LH and FSH. In compiling data from different laboratories, two difficulties have arisen. First, the amide/acid assignments are either not available in all cases, and where reported discrepancies still persist. Second, the positioning of some residues is not in

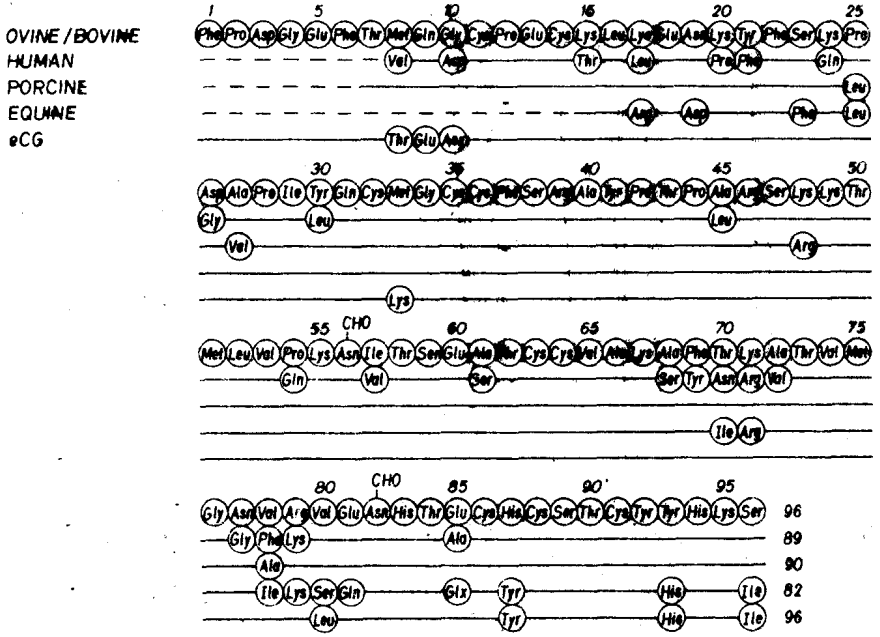

FIG. 1. Amino acid sequence of the α subunit of either LH and/or FSH from different species. The references to the various structures are given in Table I. CHO stands for carbohydrate moiety. The numbering of residues corresponds to the ovine/bovine hormones. Discontinuous lines show that the chains are shorter in the species as compared to ovine; solid lines show that the sequences are identical to ovine subunit. Only those residues that are different from the ovine are shown in the circles. As presently reported, equine pituitary FSH α (Rathnam *et al.*, 1978) and eCG α (Moore *et al.*, 1980) are different in four residue positions, and FSH α lacks a tetradecapeptide at the N-terminus. Note that in these two α subunits the His and Tyr at positions 87 and 93 are transposed as compared to other species. In the partial structure reported for the carp gonadotropin I subunit (Jollès *et al.*, 1977), there is about 63% homology with the ovine α subunit sequence.

Carp GTH I: Tyr-Pro-Arg-Asn-Asp-Met-Asn-Asn-Phe-Gly^{10}-Cys-Glu-Glu-Cys-
Ovine: Phe-——-Asp-Gly-Glu-Phe-Thr-Met-Glu-————-Pro-————-
(*C*) Lys-Leu-Lys-Glu-Asn-Asn^{20}-Ile-Phe-Ser-Lys-Pro-Gly-Ala-Pro-
(*O*) ————————-Lys-Tyr-————————-Asp-————
(*C*) Val-Tyr^{30}-Gln-Cys-Met^{33}-·····-Tyr-Tyr-His-Lys-Ser^{96}-
(*O*) Ile-——-Gly-————

complete agreement. Thus, the data shown in all the structural comparisons throughout the chapter represent the best possible assessment at the present time. The sequence of ovine and bovine α subunits appears to be the longest, consisting of 96 amino acid residues. Considering the four mammalian species for which the structures of LH and/or FSH α subunits have been determined, one can find approximately 60% over all identity, emphasizing the strong structural homology. Furthermore, approximately 80% of the differences in amino acid residues at various positions can be explained by a single base change in the codon. The most glaring difference in the structure of the α subunit appears to be that from the equine, for which data on FSH have been reported (Rathnam *et al.*, 1978). The equine has a long NH_2-terminal fragment of 14 amino acids missing as compared to the ovine hormone. This region consists of two half-cystine residues, which are present in all other species including nonmammalian (fish). If these are involved in the formation of —S—S— bridges with other half-cystine residues located elsewhere in the chain, as many reports apparently indicate (see Section IV,B), then the absence of two half-cystines in equine FSH α subunit would be inconceivable. However, the same workers that reported the sequence of equine FSH α have now proposed a link between the Cys-11 and Cys-14 (or 7–10 in their human FSH α sequence) (Fujiki *et al.*, 1980). If this is further substantiated in equine FSH α,* then it is likely that the preparation of FSH α studied could have this —S—S— pair as part of the NH_2-terminal piece that could have been cleaved during handling. We should await data from equine LH α subunit for clarification of this major difference.* The sequence of α subunit of eCG (Moore *et al.*, 1980), a hormone that has both gonadotropic activities in the rat, is in conformity with other known sequences and has 96 amino acids. Thus, pending further studies of the horse, present data show apparent differences in the structures of the α subunit of the pituitary and placental gonadotropins (see Fig. 1 legend). Another major difference between equine and other species (and in this respect data from the two laboratories on equine FSH α and eCG α agree) is the interchange in the positioning of His and Tyr at positions 87 and 93 of the sequence. These residues are located near the COOH-terminus, which is highly conserved, and in an area known to be important for interaction with receptor(s).

The point of attachment of the two carbohydrate moieties in all α subunits are identical and both linkages are *N*-glycosidic. In almost all of the

* Preliminary data of other workers who have reexamined equine FHS α do not confirm the lack of an N-terminal 14-residue sequence. It has been reported that equine LH α is also similar to the α subunit of other species (Bousfield and Ward, 1982).

α subunits examined to date, heterogeneity has been found to occur invariably at the amino terminus of the α subunit. Because they all give rise to biologically active recombinants with the β subunit, such heterogeneity is apparently of no consequence for subunit interaction or biological activity. Quantitative data on the extent of heterogeneity in the α subunits of the human hormones (Keutmann *et al.*, 1978) (Fig. 2) are available. Heterogeneity is minor in the hLH α and about 95% of the preparation has the 89 amino acid polypeptide chain. In both hFSH α and hCG α the longer 92 amino acid peptide chain predominates. This raises the question of whether or not the processing of the α subunits for the synthesis of FSH and LH might be slightly different. In ovine lutropin α subunit, data from our own laboratory indicate that more than 95% of the polypeptide chains begin with the Phe, and thus the 96 amino acid residue molecule predominates.

The similarity in the structure of the α subunit extends to the nonmammalian species also. In the partial structure reported for carp gonadotropin subunit I, 58% of the NH_2-terminal amino acid sequence is identical to that of ovine α subunit. In addition, its COOH-terminal sequence is the same as in ovine α subunit. Thus, subunit I of this species can be equated with the mammalian α subunit (Fig. 1).

	%	N-Terminal Sequence
hLH α	95	VAL-GLN-ASP-..
	5	Asp-Val-Gln-Asp-..
hFSH α	60	ALA-PRO-ASP-VAL-GLN-ASP-..
	30	Asp-Val-Gln-Asp-..
	10	Val-Glh-Asp-..
hCG α	60	ALA-PRO-ASP-VAL-GLN-ASP-..
	10	Asp-Val-Gln-Asp-..
	30	Val-Gln-Asp-..
hTSH α	100	VAL-GLN-ASP-..

FIG. 2. Amino terminal heterogeneity of the human α subunits. The sequence in larger type predominates.