J. clin. Path., **30**, Suppl. (Ass. Clin. Path.), **7**, 12-15

Relationships of structure with function of the gonadotrophins

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The gonadotrophins are an interesting group of hormones in which to study relationships of structure with function. They are glycoproteins with carbohydrate components which confer important properties of biological activity and they consist of subunits which bear interesting relationships with one another. Furthermore, they are easier to study than some other hormones because of the specific biological assay methods available which are relatively simple to carry out.

Function

The action of a hormone generally consists of several parts. Firstly, there is the transfer of the hormone from the site of biosynthesis (or from injection site in experimental work) to the target site. The stability of the hormone in the circulation is important: the biological half life may need to be long or short according to the action of the particular hormone. Secondly, there is the recognition of the target and usually binding to it. Here subtle changes in structure may alter recognition and binding ability. Thirdly, there is action at the target site which may or may not be dependent on the intermediate action of a second messenger. *In-vivo* bioassays will usually give a measure of the total hormone activity but *in-vitro* assays will measure only one or two of the activities, such as binding or steroidogenesis. Another action, the immunological reactivity, may not correlate with the hormonal function. There are many instances where full immunological activity is retained in a chemically modified structure which possesses no hormonal activity.

There are well established *in-vivo* assays for the gonadotrophins. Follicle stimulating hormone (FSH) is usually measured by the ovarian augmentation method (Steelman and Pohley, 1953; Brown, 1955) and luteinizing hormone (LH) by the ovarian ascorbic acid depletion method of Parlow (1961) or the ventral prostate assay in hypophysectomised rats (Greep *et al.*, 1941). There are many radioreceptor assays using ovarian or testicular preparations *in vitro* for both FSH and LH (Catt *et al.*, 1971; Lee and Ryan, 1971; Leidenberger and Reichert, 1972; Gospodarowicz, 1973; Cheng, 1975). A further function of the hormones *in vitro* may be observed by measuring steroids produced by the gonads such as progesterone (Watson, 1971; Shirly and Stephenson, 1973) or testosterone (Dufau *et al.*, 1972, 1976). The placental gonadotrophin (human chorionic gonadotrophin, HCG) is usually assayed by the techniques used for LH.

Specific radioimmunoassays have now been described for each of the gonadotrophins and the immunological properties of the subunits have been studied extensively.

Structure

The pituitary gonadotrophins FSH and LH are glycoproteins with a molecular weight of about 30 000. HCG has a molecular weight of over 40 000. FSH and HCG contain up to 30% carbohydrate. LH contains rather less. Sequential studies on the carbohydrates in FSH (Butt and Kennedy, 1972) and in HCG (Bahl, 1969) have shown that sialic acid appears in terminal positions in both molecules and there are also terminal N-acetylglucosamine and fucose residues. The sugar chains are attached to the peptide chains through asparagine and serine linkages.

One of the most interesting features is the subunit structure. Each of the hormones consists of two non-covalently linked subunits (α and β). The α is similar in each but the β is hormone-specific. Sequences have been determined for each of the subunits and some microheterogeneity has been recognized at the N- and C-termini. There are up to 92 amino-acid residues in human α-FSH, α-LH, and α-HCG and the sequences are identical (Bellisario *et al.*, 1973; Shome and Parlow, 1974a; Rathnam and Saxena, 1975). Carbohydrates are attached to asparagine residues at positions 42 and 78. β-FSH and β-LH each contain up to 118 residues, but the sequences vary considerably and the sugars

are attached at different positions (Carlsen *et al.*, 1973; Shome and Parlow, 1974b; Saxena and Rathnam, 1976). β-HCG is interesting in that it contains some 30 extra amino-acids as an extension to the C-terminus. In view of the similar biological properties of LH and HCG, not surprisingly the first 118 residues in β-HCG show considerable homology with the same residues in β-LH and more than 80% are identical.

Effect of chemical modifications on activity

Even slight chemical modifications to the gonadotrophins may lead to loss of *in-vivo* biological activity (Butt, 1969, 1975). The FSH molecule is very sensitive to oxidation. Photo-oxidation, which affects histidine and tyrosine residues, or chloramine-T, which oxidises cysteine and methionine, both destroy biological activity but radioimmunological activity is preserved. Not all the disulphide groups seem to be essential, however, since activity is retained after reduction with mercaptoethanol followed by alkylation with iodoacetamide (Rathnam and Saxena, 1972).

Modifications to the sugar residues have been studied extensively and particularly for FSH and HCG. The removal of terminal sialic acid residues from both hormones results in loss of *in-vivo* biological activity, while immunological activity remains (Ryle *et al.*, 1970; Vaitukaitis and Ross, 1971). The loss of biological activity is related to the shortened half life of the molecule in the circulation. Intact FSH has a half life of about 90 minutes, but this is reduced to a few minutes after modification (Vaitukaitis and Ross, 1971) since the carbohydrate protects the hormone from binding to receptors in the liver (Morell *et al.*, 1971). Nevertheless, the desialylated hormone retains certain biological characteristics. Like intact FSH, it stimulates the incorporation of thymidine into mouse ovaries *in vitro* (Ryle *et al.*, 1970) and modified HCG binds to testicular receptors in the testis and stimulates steroidogenesis (Tsuruhara *et al.*, 1972).

Moyle *et al.* (1975) made a careful study of the effects of the sequential removal of carbohydrates from HCG by means of specific carbohydrases. When sialic acid residues were removed binding activity to rat Leydig cells was not impaired and synthesis of testosterone and the accumulation of cyclic AMP was stimulated. Removal of the next carbohydrate, galactose, actually increased the binding capacity, as judged by the ability of the derivative to compete with HCG itself for binding sites. Although the ability to stimulate cyclic AMP accumulation was decreased the derivative was still capable of stimulating maximum steroidogenesis. Further sequential removal of N-acetylglucosamine and mannose residues caused some loss of binding ability, but even these derivatives retained steroidogenic capacity. Moyle *et al.* (1975) discussed the possible function of the carbohydrates on the basis of these and other observations, and suggested that they may stabilise the molecule against attack by peptidases *in vivo*, that they may facilitate binding to receptors, and that they enhance the production of the second messenger. It is difficult to explain, however, why complete stimulation of cyclic AMP requires the full carbohydrate complement since LH, which contains considerably less carbohydrate than HCG, stimulates cyclic AMP maximally (Moyle and Ramachandran, 1973).

Subunit structure

Purified subunits of the gonadotrophins are biologically inert. Early reports of activity probably arose because of inefficient separation of the two subunits. When pure α subunits are incubated under conditions in which reassociation of α and β subunits occurs no biological activity is recovered (Reichert *et al.*, 1973). Likewise β subunits do not generate any activity on incubation. But when α is mixed with β biologically active molecules are produced, the type of activity being that of the β subunit.

Separate α and β sub-units bind only weakly to target sites *in vitro* (Canfield *et al.*, 1971; Catt *et al.*, 1973) and do not stimulate steroid synthesis (Braunstein *et al.*, 1972; Channing and Kammerman, 1973; Morgan *et al.*, 1974). This suggests that the binding sites of the intact molecules include part of both subunits or that they depend on the tertiary conformation. Combarnous and Hennen (1974) treated LH with carbodiimide to link the subunits covalently. When 0·01 M reagent was used there was no loss of activity. This suggests that after binding to the receptor no dissociation of the subunits is required to elicit biological activity.

Two of the seven tyrosine residues in intact LH are difficult to iodinate but when the subunits are separated they are readily iodinated. It may be that these two residues take part in the association of the subunits. Similarly, nitration of these groups with tetranitromethane occurs in only five of the seven residues in ovine LH but all seven are nitrated when the hormone is dissociated (Papkoff, 1973).

Cheng (1976a) studied the carboxymethylation of methionine in bovine LH. There are four residues in the α and three in the β subunits. Under the conditions of the experiments dissociation occurred but, since the modified subunits reassociated, methionine residues do not seem to be essential for

interaction between subunits. When a total of three residues were alkylated biological activity in a radioreceptor assay was reduced to less than 5% of the original. Further alkylation reduced the activity still more. Immunological activity of reassociated material was not affected, however, so that the methionine residues are unlikely to be involved significantly in immunologically important sites. Nevertheless, it seems that at least one or two methionine residues in each chain are essential for *in-vitro* biological activity. Further studies on bovine LH (Cheng, 1976b) indicated that residues 8 and 33 in the α subunit and 52 in the β subunit were preferentially modified by carboxymethylation. But when human α-LH, which contains valine not methionine at position 8, was combined with carboxymethylated bovine β-LH a considerable amount of activity was regenerated. This suggests that the methionine at position 8 is not essential for biological activity but that the residues at α-33 and β-52 are important for optimal binding by the LH to receptors.

Conclusions

The gonadotrophins are glycoproteins of about 30 000 molecular weight and quite small changes to their structures may profoundly affect their hormonal activity. Certain components of the complete biological activities may be retained, however, particularly the capacity to bind to receptors and to promote steroidogenesis. Immunological activity may survive quite drastic modifications. There is no evidence that smaller fragments of these hormones retain *in-vivo* biological activity, so that the possibility of synthesising small compounds with gonadotrophic action seems remote.

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