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RELATION OF STRUCTURE AND BIOLOGICAL ACTION OF GONADOTROPHINS

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SUMMARY

It is well known that exposure of gonadotrophins and of pituitary thyrotrophin to appropriate incubation conditions results in their dissociation into two dissimilar subunits. Within species specificity of these hormones is largely determined by a hormone specific (β) subunit. This has been shown in biological systems by generating hybrid molecules and in immunological systems by raising antisera to individual subunits. These observations are discussed in relation to more recent data concerning cross-reactions of subunits with antisera raised against the parent hormone, which suggest that specificity of this type of antiserum is conferred more by conformational features of the intact hormone than by those of the hormone specific subunit.

The *in vivo* biological potency of a hormone is determined by the extent to which metabolic processes allow it to reach the target tissue, and the extent to which the hormone, on arrival at the target, can activate specific receptors. In the case of glyco-peptides an important role for the sialic acid component has been described in determining the rate of metabolic clearance and this helps to explain discrepancies in potency estimates obtained with different bioassays. However, desialilated preparations of gonadotrophins lose none of their biological (or immunological) activity *in vitro*. In contrast, subunits are not only metabolized more rapidly *in vivo* than the parent hormone, but have little or no intrinsic activity in *in vitro* biological systems. The implications of these data for structural requirements of the hormone for receptor recognition are discussed.

INTRODUCTION

In this review I propose to deal with two aspects of the structure of gonadotrophins which have received intensive investigation in recent years. Advances in our knowledge of, firstly, the subunit structure of the hormonal glycopeptides and, secondly, the role of their carbohydrate moiety, have contributed immensely, *inter alia*, to our understanding of biological-immunological relationships. There is no doubt that those of us engaged predominantly in physiological studies need to be keenly aware of advances in this field, since the hormone concentrations upon which we base our physiological conclusions are determined by methods whose interpretation varies with the structural requirements of the particular hormone action being used as the end point for the determination. The present rather daunting chaos in assay interpretation which faces physiologists in the gonadotrophin field is gradually yielding to a kind of order, thanks largely to the efforts of scientists working in this area.

In this talk I shall be concerned largely with studies of human chorionic gonadotrophin (HCG) and luteinizing hormone (LH), partly because of personal experience and partly because HCG, because of its plentiful supply and relative freedom from contamination with pituitary glycopeptides, has to a great extent served as a model for the study of other hormonal glycopeptides. I shall deal firstly with the effects of structural modifications on immunological activity and then turn to the effects of such changes on biological action.

It is well known that LH and HCG cross-react in the majority of assays, both biological and immunological, and indeed this cross-reaction, first suspected over 30 years ago by Ostergaard[1] and demonstrated by Wide, Roos and Gemzell (1961)[2] has been widely exploited in developing radioimmunoassays (RIA) for LH[3]. The recent demonstration that the hormonal glycopeptides can be dissociated into subunits, and that thyrotrophin (TSH), LH, HCG and follicle stimulating hormone (FSH) contain a common (α) and hormone specific (β) subunit[4] has provided a conceptual framework for understanding the immunological cross-reactions of these hormones. It appears that while antisera developed against α-subunits are not hormone specific, those generated against the β-subunit cross-react to only a small extent with glycopeptides other than the parent hormone.

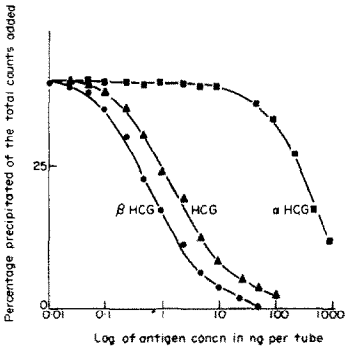

Fig. 1. Inhibition binding of ^{125}1-HCG B-subunit to anti-HCG B-subunit serum by HCG and subunits.

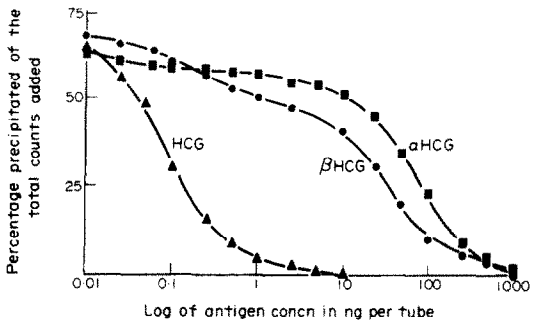

Fig. 3. Inhibition binding of ^{125}1-HCG to anti-HCG serum by HCG and subunits.

It is generally considered that the cross-reactions of the undissociated native hormones are in large part due to their possession of a common α-subunit. Figure 1 shows the inhibition of binding of I-125-βHCG to anti βHCG antiserum by HCG and its subunits (supplied by Dr. R. E. Canfield), using the system developed by Dr. Ross and his colleagues[5]. In this system βHCG was 500 times more potent than αHCG. Figure 2 shows data obtained with an antiserum to LHβ prepared in our laboratory[6] using as immunogen a preparation of LHβ supplied by Dr. A. S. Hartree. Shown also in this figure is the inhibition of binding of labelled LHβ to LHβ antiserum produced by HCG: the plateau of inhibition of binding indicates that with appropriate adsorption this antiserum is suitable for measurement of LH in the presence of HCG. With this and with Dr. Ross' antiserum it is of interest to note that the β-subunit inhibited binding with greater potency than the native hormone.

However, in contrast to these results, we have found that when an antiserum raised against native HCG (and which cross-reacts with LH and HCG) is used, the native hormone then inhibits binding with greater potency than its subunits[7]. Figure 3 shows the inhibition curve generated by HCG and its subunits with an antiserum to HCG (supplied by Dr. W. D. Odell) and labelled HCG. Here it is of interest to note that about 500 times more βHCG than native HCG was needed to produce 50% inhibition of binding of labelled HCG to the antiserum. Twice as much again of the α-subunit was required to produce the same effect. Considering now the inhibition of binding of labelled LH to the anti-HCG serum produced by LH and its subunits—that is the system used in the majority of radioimmunoassays for LH—Figure 4 shows that the native hormone once again inhibited binding with greater potency than its subunits, and again that the β-subunit was more potent than the α-subunit.

The results of these experiments may be summarized as follows:

1. In both the homologous labelled βLH-anti βLH, and the labelled βHCG-anti βHCG systems, the hormone specific subunit inhibited binding with greater potency than the native hormones.

2. Using the cross-reacting anti-HCG serum raised against the native hormone

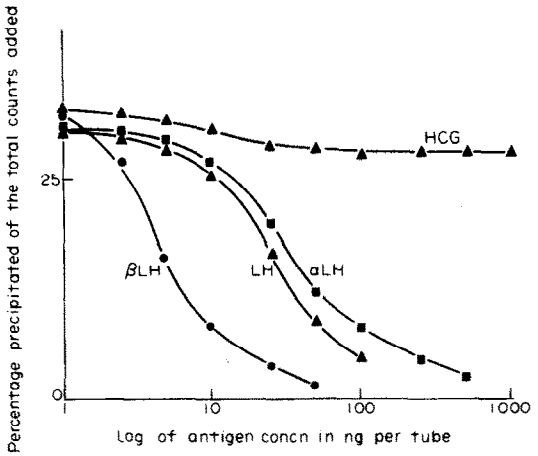

Fig. 2. Inhibition binding of ^{125}1-LH B-subunit to anti-LH B-subunit serum by LH and subunits. The inhibition curve of HCG is shown for comparison.

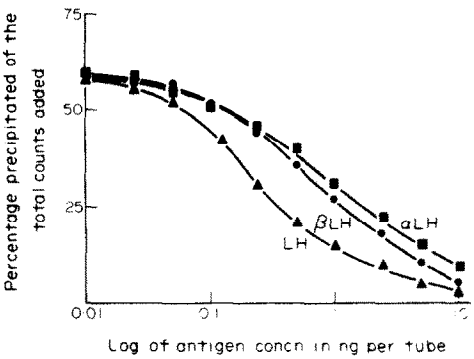

Fig. 4. Inhibition of binding of ^{125}1-LH to anti-HCG serum by LH and subunits.

(i) with labelled LH, LH inhibited binding with greater potency than βLH
(ii) with labelled HCG, HCG inhibited binding with greater potency than βHCG.

3. In all the systems tested, the β-subunits inhibited binding with greater potency than the α-subunits. The relatively greater potency of LHα compared to HCGα can be attributed to greater contamination of the LH subunit with native hormone.

Similar results to these have recently been obtained by Dr. A. S. McNeilly using an anti serum to LH supplied by Dr. Wilfred Butt[8] and we may conclude that these data indicate that the cross-reaction of LH and HCG with antisera raised against the native hormones cannot be explained by the possession of a common subunit. We therefore speculate that this cross-reaction results from conformational similarities of the native hormones rather than the accepted similarities of their α-subunits[7]. Furthermore the subunits are immunologically impotent in terms of their ability to inhibit binding of labelled native hormone to antisera raised against native hormones.

We turn now from considerations of immunological specificity and potency to structural features relating to the biological activity of gonadotrophins. It appears that specificity of biological action is also conferred by the β-subunit. This has been demonstrated in experiments in which hybrid molecules have been generated: for instance, incubation of LHα with TSHβ subunits produces a hybrid molecule which *in vivo* has thyrotrophic rather than gonadotrophic activity[4]. Parenthetically we may note that immunologically too hybrid molecules carry the specificity features of their β-subunits[9]. Biologically however, the subunits themselves appear, with the exception of a few reports[10], to be largely without activity. This lack of *in vivo* potency is unlikely to be due to damage to the subunits incurred during preparation, since activity is readily restored if the subunits are allowed to reassociate.

There appear to be two main reasons for this lack of *in vivo* biological potency of subunits. The studies of Braunstein, Vaitukaitis and Ross (1972)[11] indicate that after intravenous injection there is a significant increase in the rate of disappearance from the plasma of dissociated compared to undissociated HCG. In the same experiments these workers also demonstrated a difference of distribution of native from dissociated HCG, there being no gonadal concentration of radioactively labelled α or βHCG. Data from Dr. Channing's group[12] indicate that, using porcine granulosa cells as a binding agent for *in vitro* studies, the subunits of HCG had about 0·1% of the potency of native HCG, a result quite consistent with the known degree of contamination of the subunit with the intact hormone. We may conclude then that *in vivo* the subunits find difficulty in reaching their target: in addition it appears from *in vitro* studies that even if they do get to the gonad, they bind poorly to the target tissue.

We turn now to another area where structural modification appears to alter biological activity, though here we are almost entirely concerned with potency rather than specificity. It has been known for some time that removal of terminal sialic acid residues from HCG and the pituitary gonadotrophins results in marked loss of biological activity[13], and Van Hall and colleagues[14] have shown that this effect is quantitative in that biological potency varies in a reciprocal fashion with residual sialic acid content. Associated with the loss of biological activity of HCG there is a progressive reduction in its time of survival in the circulation. It has been suggested that the role of sialic acid is to protect the hormone from metabolic destruction in the body. However, in contrast to the results with subunits, desialilated HCG retains its immunoreactivity, its ability to compete *in vitro* in a radioreceptor assay using homogenates of rat testis as the binding agent and its ability to stimulate testosterone production by the testis[15].

More recently, Channing and Kammerman (1973)[16] have shown that *in vitro* asialo HCG has a potency equivalent to native HCG in causing luteinization of granulosa cells obtained from Simian ovaries. It therefore appears that the sialic acid content contributes to the metabolic fate of gonadotrophins and in this way only it modifies the biological activity of glycopeptide hormones. How it does this remains a matter for speculation, but Ashwell and his colleagues[17] consider that desialilation "reveals" the penultimate galactosyl residues and it is these which determine the hormone's persistence in the circulation. Thus exposure of these residues in other glycopeptides leads to hepatic accumulation and rapid disappearance of the compound from the circulation. Subsequently removal of the galactosyl residue however results in an increased plasma survival time[17]. Whether this hypothesis is sufficient to explain the data obtained with the gonadotrophins remains to be seen[15]. Nevertheless it is important to appreciate the powerful effect of variations in the carbohydrate content of highly purified gonadotrophin preparations in determining *in vivo* biological potency. Indeed, it appears that the desialilation affects the results of some bioassays—for instance, the ventral prostate weight assay of LH and HCG, which depends upon initiating and maintaining a response over several days—more than, say, the ovarian ascorbic acid depletion assay, which requires only brief exposure to the hormone. Since as a result of extraction procedures, different preparations of these compounds may contain different amounts

of sialic acid[18], one may readily appreciate how disparities in potency estimates develop between different bioassays, and between bioassays and radioimmunoassays.

In conclusion, structural modifications to the gonadotrophins have been shown to affect their biological activity in at least two ways. Enzymatic removal of sialic acid causes progressive attenuation of biological potency, an effect which is related to an alteration of the metabolic disposal of the hormone within the body. The effect therefore is largely upon transport of the hormone, since in *in vitro* systems there is retention of the ability to bind to homogenates of testis, to bind to membrane preparations and indeed to stimulate luteinization of granulosa cells or testosterone production by the testis[15]. In the case of dissociation of the gonadotrophins into subunits, the loss of *in vivo* activity is in part due to an effect on clearance of the hormone from the circulation. However, in addition, subunits have impaired potency in *in vitro* systems. They bind poorly to biologically relevant tissue components and it has been of great interest to us to observe a similar impairment of potency of the subunits in inhibiting binding of the native hormone to antisera developed against the native hormone, a result which may suggest comparable structural requirements in the native hormone for both antibody and receptor recognition. Alternatively, since the potency of subunits in both types of systems is of the order of 0·1% one may question whether there is any intrinsic potency of the subunits and whether the results cannot be better explained by contamination of subunit preparations with native hormone to the extent of 1 part in 1000. Whichever of the two explanations one favours, one further conclusion may be drawn and that is that subunits, generated *in vivo* either as part of the secretion of the native hormone or as part of its catabolism, are unlikely to play a regulatory role in the control of gonadal activity.

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DISCUSSION

Lindner:

In our hands, also, (Koch, Zor, Chabsieng and Lindner unpublished data) the β subunit of LH is completely inactive in *in vitro* systems, both with respect to generation of cyclic AMP and induction of ovum maturation with cultured follicles. But I meant to raise this question: you and others have found that the β subunit as well as the α subunit are very inefficient in inhibiting the binding of intact hormone to antibodies generated with native hormone. On the other hand, we find that antibodies raised to the β subunit are very effective in neutralizing the biological activity of the intact hormone. We have used this (*J. Endocr.* **58** (1973) 677) as a tool to knock out LH-like contamination present in thyrotrophic hormone or in FSH preparations when trying to show whether the biological activity observed is intrinsic to that hormone. The antibodies generated with this subunit recognize the intact hormone very well, whereas the subunit is not expressed as well when you immunize with the intact hormone. Can you explain this?

Jacobs:

Your biological data is quite consistent with our previously reported immunological findings that antisera generated against sub units cross react well with the parent hormones. The explanation lies in the nature of the major differences in the antibodies generated against parent hormones as against sub units. It is presumably related to conformational

changes associated with association and dissociation of the hormones used as antigens.

McKerns:

Figure 1 shows the effect of stimulation by LH and an interference effect of the β subunit of LH. I might say we have the same reaction with HCG and the β subunit of HCG. This is a cytosol preparation from homogenates of bovine corpus luteum and actually what we are measuring here is the activity of glucose-6-phosphate dehydrogenase as expressed by the metabolism of [1-C^{14}]-glucose-6-phosphate. As you can see in the middle line, the LH β has no activity but the LH has a marked stimulatory effect on the metabolism of glucose-6-phosphate. As seen in the lower line the β subunit has a marked antagonistic effect. In spite of what you said about the subunits having no or very little binding capacity to cellular structures or to membranes I think these experiments indicate that one possible target might be this enzyme in the cytoplasm (McKerns, *Endocrin.* in press).

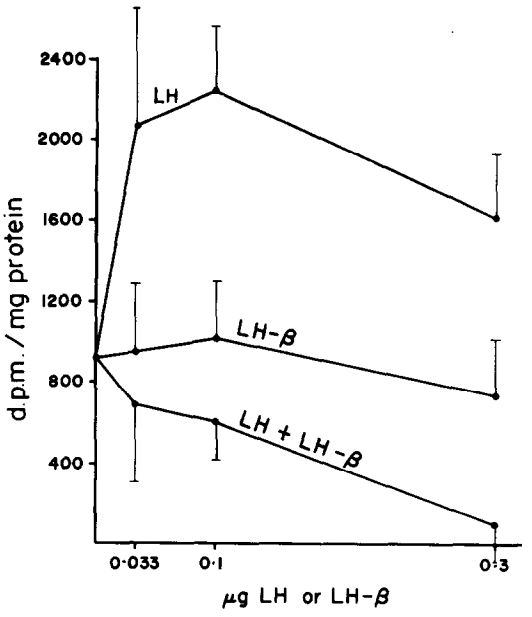

Fig. 1. (McKerns).

Jacobs:

They are very fascinating data and there are, of course, other ways by which these subunits might be working. I'm sure you know there's a recent report suggesting that although it's hard to show stimulation of adenyl cyclase by subunits guanyl cyclase stimulation has been demonstrated. May it be that, perhaps, what you are describing could be mediated by cyclic GMP?

McKerns:

No, there is no effect of cyclic AMP or GMP on this system. May I add another note? In the intact purified nucleus from these corpus luteum preparations, we also have a stimulatory effect of HCG and LH on RNA synthesis and a general expression of ribosomal and messenger RNA which seems to be coupled to the activation of the pentose phosphate pathway for an increased provision of PP ribose-P for the synthesis of nucleotides that are subsequently incorporated into the RNA.