Immunological Techniques and the Assay of Human Pituitary Glycoprotein Hormones

C. N. HALES and J. S. WOODHEAD

Department of Medical Biochemistry, The Welsh National School of Medicine, Cardiff CF4 4XN, U.K.

The application of radioimmunoassay techniques to the measurement of protein hormones in biological fluids has contributed greatly to progress in endocrinology. However, as knowledge of the physiology of hormonal regulation has increased it has become apparent that interpretation of immunoassay data may be difficult, since discrepancies can occur between the biological and immunological reactivity of protein hormones. It is now clear that the metabolism of these molecules in vivo may lead to polymorphism in the form of the circulating peptide and that different antisera may recognize the various forms to a greater or lesser extent (Franchimont et al., 1972). In addition, the pituitary glycoprotein hormones present a number of unique problems in terms of immunological measurement, which derive primarily from their structural similarities. In this communication we shall outline the methodology that has been applied to the determination of protein hormones, and show how developments of the basic techniques can be applied to the specific problem of the measurement of the pituitary glycoprotein hormones in blood.

The methodology employed in the conventional type of radioimmunoassay system has been reviewed extensively (Sonksen, 1974). It involves the competitive binding of the hormone to be assayed and a radioactively labelled derivative of that hormone by an antiserum raised to that molecule or a partial sequence of it. At the end of the assay the ratio of bound to unbound radioligand is an inverse function of the amount of unlabelled hormone, present as standard or unknown, in the incubation. The same principle has been applied to the assay of several hormones with receptor proteins instead of antibodies. This method has the advantage that it recognizes only biologically active forms of the circulating molecules. Thus LH (luteinizing hormone) has been assayed by using preparations of ovarian (Lee & Ryan, 1972) or testicular tissue (Catt et al., 1971) and FSH (follicle-stimulating hormone) by using a rat testis tubular preparation (Reichert & Bhalla, 1974). In addition to the problem of discriminating between LH and HCG (human chorionic gonadotrophin) activity, this type of system has the disadvantage of relatively low sensitivity, though one exception is the ACTH (adrenocorticotropic) assay of Lefkowitz et al. (1970). The problem has been further complicated by the difficulty of producing iodinated preparations of labelled hormones which retain their biological activity. It would seem that both the presence of radioiodide in a hormone molecule and exposure of the molecule to oxidizing agent during the iodination procedure are detrimental to hormonal binding.
The only biological system which has a sensitivity capable of measuring physiological concentrations of peptide hormones is the histochemical redox bioassay described by Chayen et al. (1972). Holdaway et al. (1973) were able to demonstrate good agreement between amounts of plasma ACTH obtained in this system with values obtained by radioimmunoassay and conventional bioassay procedures. The method has also been applied to the measurement of LH (Rees et al., 1973), though the results obtained for plasma samples taken during a menstrual cycle show significant differences from those obtained by radioimmunoassay (Holdaway et al., 1974).

A non-competitive immunological assay system was described by Miles & Hales (1968) which involves the use of iodinated antibodies and was called the immunoradiometric assay. The system involves the use of an immunoadsorbent which is prepared by coupling the peptide hormone to a diazonium derivative of powdered cellulose. The immunoadsorbent is used to extract antibodies from an immune serum and the antibodies can be iodinated while they are bound to the solid phase, thereby protecting immunological binding sites from damage. After washing the samples to remove the iodination medium, low-affinity antibodies can be removed by washing the complex at pH 3. High-affinity antibodies can be eluted by further decreasing of the pH to 2. In the assay system, the eluted antibodies are incubated with solutions of hormone. At the end of the incubation period unreacted labelled antibody is removed by reaction with an excess of immunoadsorbent. Radioactivity remaining is thus a function of the concentration of hormone in the incubation medium. A number of practical advantages have been encountered in using this system for certain protein-hormone immunoassays (Woodhead et al., 1974).

An extension of this system has been described (Beck & Hales, 1974) where the antibody is not labelled directly with iodine, but rather with a preparation of iodinated anti-IgG (immunoglobulin G). This double-antibody immunoradiometric assay, which avoids the problem of direct iodination, may prove advantageous where a particular antiserum is in short supply. In addition it could be of value since a single antibody iodination (e.g. of anti-rabbit IgG) could provide the label for a variety of peptide-hormone assays.

A third type of assay system which may prove to have considerable importance in the glycoprotein-hormone field is the two-site assay (Addison & Hales, 1971; Readhead et al., 1973). This system involves the extraction of hormones from plasma or standard solutions on to an immunoadsorbent consisting of a solid-phase preparation of antibody. The hormone bound during this incubation is measured by a second incubation with a labelled antibody preparation. The uptake of radioactivity during this second incubation is proportional to the concentration of hormone in the first incubation. One important advantage of this method is that by using antibodies directed against selected antigenic determinants a high degree of assay specificity can be achieved (O'Riordan et al., 1972).

The problem of specificity has in fact proved to be the major stumbling block in the assay of the pituitary glycoprotein hormones, and this is due primarily to the high degree of molecular homology between these peptides. It is now known that a single chain (α-chain) is common to LH, FSH and TSH. The biological and immunological specificity of these molecules resides in the β-chain, though regions of homology occur also in this subunit. This isolation of LH and FSH in pure form is difficult and a low amount of contamination of a preparation of either with the other hormone must be expected. Another source of error in assays of the glycoproteins stems from alterations which can occur during extraction and purification procedures. Whereas deamidation may have little effect on the subsequent reactivity of the molecules, desialylation of LH and FSH decreases their biological activity in systems in vivo (Braunstein et al., 1971), though the extent to which activity is decreased seems to vary from one bioassay system to another. No effect of desialylation on immunological activity has been reported, though it is not known whether or not carbohydrate chains are involved in antigenic determinants. If this is so it could add further to the problem of discrimination between the different glycoproteins.

Isohormone variants have been noted in the case of bovine PTH (parathyroid hormone) (Keutmann et al., 1971) where three types of PTH molecule have been isolated.
from pooled bovine parathyroid glands and which have minor sequence variations. Although the existence of isohormones might account for some of the apparent heterogeneity of glycoprotein-hormone preparations (Franchimont, 1972) it is also likely that the variations seen in electrophoretic mobility of these preparations could arise as a result of the degradative changes referred to above.

A further complication in the assay of the pituitary glycoprotein hormones arises from the secretion of the placental hormones. For example HCG possesses the α-chain common to the other glycoprotein hormones and differs from the structure of LH only in the possession of an extra 30 amino acid residues at the C-terminus of the β-chain (Canfield, 1974). It has only recently been possible to distinguish this hormone immunologically from LH for this reason (Vaitukaitis et al., 1972).

The methods by which specificity has been achieved in conventional RIA systems have followed three main approaches. First, since the main problem of cross-reaction arises as a result of the shared α-chains it has been common practice to saturate assays with an excess of this chain and make use of the more specific antibodies which are directed against the immunologically distinct β-chains. The close homology between LH and HCG has been used to advantage in that the latter is considerably easier to obtain in pure form as a result of its placental origin. Thus many assays have been developed for LH with antisera raised to HCG which show no apparent cross-reactivity with FSH.

An interesting approach has been adopted for the measurement of LH (Niswender et al., 1968), FSH (Midgley et al., 1971; Hendrick et al., 1971) and TSH (Franchimont, 1972) which involves the use of antisera raised against the corresponding hormones from different species. The increased specificity of these ‘heterologous’ systems seems to derive from the fact that the β-chains show a much higher degree of conservation than the α-chains in the various species. Thus an antiserum to ovine FSH which showed poor discrimination between ovine FSH, LH or TSH could yield a useful assay for human FSH with little unwanted cross-reactivity.

A third approach has involved the development of assays with antisera raised to the individual β-chains (Vaitukaitis et al., 1972; Pierce et al., 1972). The individual chains can be dissociated by using urea or high salt concentrations and separated by counter-current distribution or gel filtration. In this way the problems of specificity should be resolved before immunization. However, this technique also has its problems. The first is that it may prove difficult in practice to isolate completely the individual chains. Franchimont (1972) has noted this difficulty with respect to the purification of HCG α- and β-chains with Sephadex G-200. Jacobs & Lawton (1974) have shown that immunization with a preparation of LH β-chain which has a 5% contamination with α-chain results in the production of an antiserum which shows considerable cross-reactivity with the contaminant. The work of Jacobs & Lawton (1974) also demonstrates a second problem arising from the use of individual chains as immunogens, namely that the antibodies produced react much better with the immunogen than with the native hormone, particularly if their results are interpreted in terms of the molar concentrations of hormone used. Clearly, immunization with fragments of a three-dimensional structure may result in the production of antibodies which do not recognize the antigenic determinant present in the native molecule. Jacobs & Lawton (1974) were, however, more fortunate in their production of antibodies to TSH β-chain, where reactivity with the intact molecule was as good as that with the individual β-chain. A problem which has received little attention is the question of altered immunoreactivity of the labelled hormones. Jacobs & Lawton (1974) have reported that whether TSH assays are seriously affected or not depends on the fraction of purified iodinated hormone which is used in the assay.

The immunoradiometric assay described above has been successfully applied to the assay of parathyroid hormone, where alterations in the stability and behaviour of iodinated peptide have been encountered (Addison et al., 1971). Miles (1971) has used the immunoradiometric system to develop a highly specific assay for human LH with a non-specific antiserum. This was achieved by treating the antibody with immunoadsorbent containing the cross-reacting antigen. By subsequently reacting this treated antibody with a limiting amount of highly purified immunoadsorbent, Miles (1971) was able to
select those antibodies with the highest affinity for LH. In this way both the assay precision and specificity were greatly improved.

An extension of the use of immunoadsorbents to produce further assay specificity has been used in the measurement of FSH by using the two-site assay system (Readhead et al., 1973). The immunoadsorbent used to extract hormone from plasma was prepared by coating polyethylene tubes with a non-specific antiserum. This technique relies on the strong physical adsorption of antibody molecules to the plastic and was developed as a solid-phase system by Catt & Tregear (1967). Antibodies specific for FSH were prepared by absorbing the antiserum with LH immunoadsorbent and free TSH. These specific anti-FSH antibodies were iodinated and used in the two-site system. In addition to the specificity for FSH achieved with this system the sensitivity of the assay could also be improved by repeated extraction of samples of plasma on to the immunoadsorbent. It is conceivable that the two-site system could provide a convenient method for assaying a range of glycoprotein hormones. Tubes coated with non-specific antibody could be used for extraction of plasma samples, since these can be stored for long periods at -20°C without loss of immunological reactivity (Readhead et al., 1973). Only a small amount of highly purified glycoprotein is necessary for extraction of antibodies which would then provide the required specificity.

Another important advantage of the use of this type of system is that it precludes the necessity of raising antisera to isolated β-chains. It is clear from the work of Jacobs & Lawton (1974) that the isolation of chain-specific antibodies from antisera raised to the native hormone molecules would be better than looking for suitable cross-reactivity with antibodies to the isolated chains.

A final reason for stressing the development of more specific assays relates to the problem concerning the nature of the circulating hormone. It is clear from the work of Habener et al. (1972) that parathyroid hormone is rapidly metabolized in the peripheral circulation so that much of what is measured by radioimmunoassay is biologically inactive (Potts, 1974). The presence of circulating fragments representing hormone degradation products could account for the sort of discrepancies noted by Cargille et al. (1968) where different antisera to FSH yielded different values for the same serum samples when the same standard was used. A similar problem could arise owing to the secretion of hormone fragments by ectopic gonadotrophin-secreting tumours (Rabson et al., 1973). By using a two-site assay system it has been found possible to measure native bovine parathyroid hormone in the presence of molecular fragments (O'Riordan et al., 1971). In the long term such approaches may be of considerable value in assessing the physiology of pituitary glycoprotein hormones and provide closer agreement between immunological and biological determinations of hormone activity.


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ENERGY COUPLING IN MICROBIAL TRANSPORT: a Colloquium organized on behalf of the Bioenergetic Organelle Group of the Biochemical Society and the Microbial Cell Surfaces and Membranes Group of the Society for General Microbiology by W. A. Hamilton (Aberdeen) and P. D. Mitchell (Bodmin)

Editor’s note. Please see pages 1023–1025 for the paper entitled ‘The Phosphoenolpyruvate-Sugar Phosphotransferase System’ by S. Roseman, also presented at this Colloquium.

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Respiration-Linked Transport in Bacterial Membrane Vesicles

WIL. N. KONINGS

Laboratorium voor Microbiologie, University of Groningen, Kerklaan 30, Haren (Gr.), The Netherlands

The use of isolated membrane vesicles (Kaback, 1971) permits the investigation of the biochemical properties of transport processes in cytoplasmic membranes. The preparations consist almost exclusively of intact unit-membrane-bound sacs. Freeze-etch electron-microscopy studies showed that the orientation of the membranes of the vast majority of the vesicles is the same as in protoplasts (spheroplasts) and whole cells (Kaback, 1971; Konings et al., 1973). In addition, since membrane vesicles of Escherichia coli retain 70% or more and those of Staphylococcus aureus even 100% of the transport...