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7

Standardization of hormone determinations

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Standardization of hormone determinations is important because it simplifies interpretation of results and facilitates the use of common reference values for different assays. Progress in standardization has been achieved through the introduction of more homogeneous hormone standards for peptide and protein hormones. However, many automated methods for determinations of steroid hormones do not provide satisfactory result. Isotope dilution-mass spectrometry (ID-MS) has been used to establish reference methods for steroid hormone determinations and is now increasingly used for routine determinations of steroids and other low molecular weight compounds. Reference methods for protein hormones based on MS are being developed and these promise to improve standardization.

Introduction

The aim of standardization is to obtain identical and correct results with different methods. The expression “harmonization” is often used as an alternative to standardization. However, while different harmonized methods may provide similar results, they may all be biased. Therefore, the goal should be to achieve standardization, but if standards are not available, harmonization is the only option. WHO-approved International Standards (IS) for most clinically important hormones are available from the National Institute for Biological Standards and Control (NIBSC) in the UK. However, only a few standards are available for cytokines and growth factors [1].

Standardization is feasible if a standard and a reference method are available. The reference method should preferably be a definitive method, in practice mass spectrometry (MS), which presently can be used for relatively small molecules like steroid and thyroid hormones and also for some peptide hormones [2–4]. Most hormone determinations are performed by immunoassay but MS is increasingly used for routine determination of steroid hormones. Standardization of immunoassays is challenging and results from quality assessment schemes show that for many methods, between-method variation is not yet on an adequate level [5].

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Development of hormone immunoassays

Routine determination of hormones became possible with the development of the first radioimmunoassay for insulin by Yalow and Berson in 1959 [6] and an assay of thyroxin by use of thyroxin binding globulin by Ekins in 1960 [7]. During the following decade, immunoassays for most peptide and protein hormones were developed using antisera produced in-house. Some of these antisera were widely distributed and when purified protein hormone preparations became available from NIH and WHO, fairly well standardized assays could be established. When commercial assays gradually became available, the need for standardization became obvious. Assay manufacturers used different antisera, calibrators and assay conditions and this caused substantial variation in the results obtained. Thus twenty years ago the results obtained by different assays for human chorionic gonadotropin could vary by more than 10-fold [8]. Presently, hCG assays give results that differ by less than 50% from the mean [9].

Initially, binding inhibition assays were used to measure both small and large hormones. In these, the hormone to be measured compete with radiolabeled hormone for binding to the antiserum. Binding inhibition assays are still used to measure small hormones. In these, assay sensitivity increases with decreasing concentrations of antiserum and increasing incubation time. Optimal detection limits requires assay times of hours or even days. However, presently, most hormone immunoassays are performed with automated analyzers, in which incubations times have been minimized, often to less than 10–15 min, in order to optimize throughput. Unfortunately this has lead to impaired quality of steroid hormone assays [10–12]. Thus, it has been decided that steroid hormone determinations have to be performed by mass spectrometry in studies to be published in the Journal of Clinical Endocrinology and Metabolism [13].

Hormones with an MW larger than 3 kD can determined by immunometric, also called sandwich assays [14]. In these, a capture antibody (or antiserum) attached to a solid phase, i.e., a tube wall or a particle, binds the hormone through one epitope and a detector antibody, which is labeled with a radioisotope, an enzyme, a fluorophore or a luminescent molecule, binds to another epitope. A polyclonal antiserum containing antibodies to many epitopes on the antigen and can be used both as capture and tracer antibody. This approach is seldom used in commercial assays, but combinations of monoclonal and polyclonal antibodies are used. The detection limit of sandwich assays improves with increasing antibody concentrations. Thanks to this and the use of sensitive detection methods, very low detection limits can be achieved in spite of short incubation times.

Problems for standardization

Hormone concentrations

Standardization of hormone determinations is complicated by a number of problems. First, the concentrations of hormones in circulation are very low in comparison to those of other similar substances. In healthy subjects, the concentrations of most peptide and protein hormones are in the range 1–50 pmol/l. The concentrations of the most abundant serum protein, albumin, is about 0.7 mol/l, which is 10–100 million-fold that of typical protein hormones. The concentrations of steroid hormones are in the range 20–300 pmol/l for estradiol to 50–500 nmol/l for cortisol. These should be compared to the concentration of cholesterol, 5 mmol/l, which is 10,000–100,000,000–fold higher than those of steroid hormones. It is inevitable that the presence of a huge excess of similar substances affects hormone determinations by causing nonspecific interference. Considering these facts, it is amazing how specific and sensitive immunoassays can be, but it explains some problems with steroid hormone assays with automatic analyzers [13,15].

Hormone heterogeneity

Most peptide and protein hormones occur in different forms in circulation, e.g., 22 and 20 kD growth hormone, intact hCG and its free β subunit (hCGβ) or intact paratahormone and hormonally inactive, truncated forms. In order to standardize assays for such hormones, it is necessary to define which forms should be measured and to use antibodies that detect relevant epitopes. Genetic polymorphism of protein hormones is fairly common and a variant of luteinizing hormone (LH), which differs with respect to two amino acids (Trp8Arg and Ile15Thr) is not detected by some antibodies [16].