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Assays for human growth hormones*

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Abstract: Human growth hormone is, in effect, defined by its activity in an *in vivo* bioassay and the standard used with it, growth being measured as the increase in body weight in hypophysectomised immature rats. The assay reflects the hormone's survival and metabolism *in vivo*, its cell–cell interactions, the activation and effects of its secondary hormones, such as GF1 and GF2, and various feedback mechanisms. Although it is insensitive, imprecise, easily influenced by contaminants TSH and vasopressin, it is the only practical assay that reflects all the *in vivo* properties of "hGH". The *in vivo* tibial epiphysis bioassay is more sensitive and precise, but the response reflects only the elongation of bone. Both these bioassays are well established.

By contrast, *in vitro* receptor assays do not reflect *in vivo* properties; there may be different natural forms of receptor molecules, they may be altered during their extraction, and the measured response (like those of immunoassays) is not relevant to the biological action of the hormone. The validity of a bioassay depends on the use of a suitable standard. The collaborative study of the International Standard for human growth hormone (in 1984) revealed marked disparities between results with different assay methods. When a growth hormone protein (such as somatropin, 191 amino acids) is produced in quantity, reproducibly, and with physicochemical properties consistently related to *in vivo* bioassay results, it may then be reasonable to use physico-chemical tests for control purposes. Many such tests require international reference materials for comparison purposes.

Keywords: *Human growth hormones; assay systems; bioassays; rDNA products.*

Introduction

There is sometimes confusion as to what is meant by "human growth hormone", how its identity is defined, and why complex imprecise *in vivo* bioassays are required, instead of simpler receptor or immunoassays, for the control of the therapeutic product. The human pituitary secretes a mixture of growth promoting proteins, and gland extracts used for clinical replacement treatment for the past 20 years, have contained various mixtures of them together with small quantities of other potent pituitary hormones. *In vivo* bioassays were essential to quantify the growth hormone activity of such products.

With the introduction of recombinant-DNA products for clinical use, consisting of essentially pure 22 kDa growth hormone protein (Somatropin), the need for the *in vivo* bioassays has been questioned.

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Definitions

Human growth hormone (hGH) is, for a biologist, the mixture of proteins secreted by the anterior pituitary, having high body growth-promoting activity. Extracts from mixtures of pituitary glands generally contain about 75% of the 22 kDa protein, 20% of the 20 kDa protein and small amounts of three other related GH proteins, all derived from the same gene, (but not the so called growth factors secreted by the pituitary). The 22 kDa molecular species is given the INN "Somatropin" and is widely taken to be "the" growth hormone. A little is known of the differences of biological actions of somatropin and 20 kDa proteins. Less is known of the physiological circumstances in which the relative proportions of 22 and 20 kDa proteins secreted are changed, although their secretion is profoundly influenced by age, time, stress, metabolic state and other factors.

As with many "biological" substances, bioassays help to identify — and sometimes define — what we mean by "growth hormones": the proteins extracted from anterior pituitary glands, which have the highest biological activity (1) in a bioassay system generally accepted as "defining" the hormone (the increase in body weight in young hypophysectomised rats); and (2) when they are assayed against a biological standard generally accepted as suitable for measurement of the hormone, (the International Standard).

The International Standard for human growth hormone was established by WHO in 1982 [1]. Each ampoule of the Standard contains an identical quantity of a highly purified mixture of 22 and 20 kDa proteins. The results of the international collaborative study [2] showed a wide diversity of estimates of the potency, and content of the ampoules, made by various assay methods and compared with various "pure" and impure pituitary extracts used as local standards in several "expert" laboratories. Such extracts contained various amounts of 22 and 20 kDa proteins, denatured forms, and other impurities from the gland.

One must conclude that, before the use of the International Standard for human growth hormone and the International Unit it defined, "units of growth hormone" referred to in literature must have been imprecise measures of GH activity or growth hormone proteins [3].

The biological actions of "growth hormone"

The biological actions of "growth hormone" in the intact animal are very diverse and complex. They include the stimulation of growth of bone, muscle, organs and tissues; lipolysis; production of the somatomedins IGF1 and IGF2 and their effects, and it is not yet clear which activities are mediated by each molecular form; and the feedback mechanisms acting on the hypothalamus and the pituitary via somatostatin and the GH releasing hormones, the peptide hormones which, respectively, inhibit or stimulate the secretion of GH.

Assays

The two common *in vivo* bioassays for "GH" are: (1) the body weight gain; and (2) the tibial plate assay in hypophysectomised young rats. The assay consists of injecting preparations of GH s.c. or i.m. into groups of the rats 2 weeks after they have been hypophysectomised. Usually 2 or 3 dose concentrations of test and standard are used,

and the rats are treated once or twice daily for 6–8 days. The measured response (increase in body weight by the end of treatment) in the former reflects total body growth minus fat lost by lipolysis. The assay lasts 6–8 days and the response reflects the direct actions of GH, its metabolism, and the indirect actions due to the delayed secondary effects of somatomedins IGF1 and IGF2 produced by GH. Due to the destruction of the pituitary in the test animals, it does not reflect all the normal feedback mechanisms at the hypothalamus and pituitary of somatostatin and growth hormone-releasing hormones. The response is easily influenced by stress, metabolic state, and contaminants in pituitary extracts, especially TSH, and vasopressin (if it is present) inducing water retention. The assay is difficult, insensitive, slow, imprecise and costly.

In the other *in vivo* bioassay, the tibial plate assay in hypophysectomised young rats, the measured response (increase in thickness of the upper epiphysis of the tibia) reflects the effect of increasing bone length only. It is also influenced by stress, TSH and other factors, but not by vasopressin. It is also difficult, slow and costly, but it is more sensitive and precise.

Both assay methods are described in monographs of the EP and BP.

Receptor assays are usually based on preparations of receptor proteins from pregnant rabbit liver membranes. But receptors may be damaged during their isolation, and in this *in vitro* system the measured response does not reflect the activation of intracellular events, the long term *in vivo* metabolism of GH or the generation and effects of somatomedins. But the assay is more rapid, less costly, and useful for multiple samples.

Immunoassays, with polyclonal or monoclonal antibodies, are widely used in diagnostic clinical chemistry. But the specificity of many assay systems does not discriminate between 20 and 22 kDa proteins and the measured response does not relate to the biological activities of the intact hormone or to the intended clinical action of a product intended for treatment. Such assays are rapid, simple, cheap and useful for multiple samples.

Comparison of *in vivo* and *in vitro* assay systems

For *in vivo* systems the reagents (rats) are reproducible and widely available. The measured response relates to the intended clinical effect. The assays are well tried and well documented (in pharmacopoeias and scientific literature).

In vivo bioassays should be used when the exact molecular identity of the substance in the product is not known, or when unknown denaturation of the molecules may have altered biological actions; when the product contains an unknown or unreproducible mixture of substances with similar biological actions; when impurities may have related (agonist or antagonist) biological actions; or if the assay sensitivity is greater, if needed, than other assay methods.

They are thus appropriate for assays of growth hormone activity in extracts of growth hormones from pituitaries, since these virtually never consist of a single pure GH protein.

In contrast, batches of assay reagents used in *in vitro* receptor and immunoassays are variable and supplies of them are limited. The specificity and validity of each batch of reagents are often not well or widely documented, and the assay response does not reflect *in vivo* metabolism or the long term or secondary effects of the hormone or impurities often associated with it.

But when a product (such as a rDNA protein) is known to be pure, and its provenance known, batches of it are shown to be reproducible, its molecular structure thoroughly well known and characterised by physico-chemical methods, and its correlation with measured biological activity is consistent in quality and quantity, then bioassays may not be necessary.

Many physical and chemical tests of identity of proteins, as used in the control of proteins made by recombinant DNA technology, rely on comparisons with pure, stable reference preparations. Where possible, these should consist of the native substance, ampouled without denaturation and without the carrier substance (e.g. albumin) often used to stabilise the substance but which might interfere in analytical methods.

Conclusion

In vivo pharmacopoeial bioassays reflect the intended biological actions of a preparation of growth hormone more closely than *in vitro* receptor, cell, or immunoassays. They should be used when the molecular identity, heterogeneity or purity of a GH product is uncertain.

When the product is a consistently reproduced single protein, whose provenance and method of manufacture is known in detail, it may be appropriate to rely on a spectrum of tests of physicochemical properties of the protein to characterise its molecular identity, and to quantify it.

References

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