NOTE
Biosynthetic Met-hGH:
Lack of Immunoreactivity for Other Pituitary Hormones

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Abstract
The immunoreactivities of 5 hGH preparations were examined in RIA systems for hPRL, hACTH, hLH, hFSH and hTSH. All preparations derived from pituitary extracts showed distinct displacements in almost all RIA systems when added 10³ to 10⁴ times larger amounts than the proper hormones. On the other hand, biosynthetic methionyl-hGH did not manifest any immunoreactivity in any system with comparable amounts. These results indicate that the displacements caused by hGH preparations in RIA systems for other pituitary hormones are solely due to contamination, and not due to intrinsic cross reaction. In in vitro and in vivo studies using extractive preparations of hGH one should be cautious about contamination of other pituitary hormones.

Several methods for the extraction and purification of hGH from pituitary glands have been described (Li and Papkoff 1956; Raben 1957; Wilhelmi 1961; Rose et al., 1963; Lewis et al., 1969). However, trace amounts of other pituitary hormones cannot be eliminated from these preparations (Holmström and Fholenhag 1975), even though GH is the most abundant hormone in the pituitary. These contaminated hormones are thought to be innocuous in the clinical use of hGH preparations, but cause some problems when used in relatively large amounts in in vitro and in vivo studies or in the examination of cross reactivity in immunoassays. In the latter cases, it is sometimes difficult to determine whether the displacement evoked by a “pure standard material” is due to contaminated material or to intrinsic cross reaction in immunoassay systems. Parallelism of the displacement curves to the standard strongly suggests contamination, but denatured or heterogeneous contaminated materials may cause displacement curves not parallel to the standard.

Biosynthetic hGH (Goeddal et al., 1979; Olson et al., 1981) is thought to be free from any contamination with other pituitary hormones. Therefore we thought it worthwhile to confirm whether the displacement in RIA systems for hPRL, hACTH, hLH, hFSH or hTSH with hGH preparations is fully due to contamination or is partly due to intrinsic cross reaction. The recent report about the artifactual rise in plasma lipo-tropin levels due to contamination after in vivo administration of extractive preparations of hGH (Kuhn et al., 1983) made us think that the publication of these results might be beneficial.

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Materials and Methods

The immunoreactivities of hGH preparations, Crescormon (Kabi, Sweden; Lot HN964), Nanormon (Nordisk, Denmark; Lot 294-2), Corpormon (Nikken Chemical, Japan; Lot 0101X), NIH GH (HS2243E) and Methionyl(Met)-hGH (Genentech, U.S.A.; Lot GH011Bl), were examined in the RIA systems for hPRL, hACTH, hLH, hFSH and hTSH. Since hGH preparations are distributed in Japan solely for the treatment of patients with pituitary dwarfism and are available only in limited quantities, vials of the first three preparations were retained at 4°C after injection to patients and the residual solutions were collected by centrifugation. Usually each vial contained 0.05 to 0.10 ml of the solution of hGH in physiological saline at an approximate concentration of 1 mg/ml. As the solution of hGH was made in the clinic and with limited accuracy, the hGH concentrations of combined solutions were determined by RIA using rabbit antiserum raised in our laboratory and NIH GH HS1934D as standard and all calculations were made on the basis of their immunoreactivity. All preparations showed dilution curves identical with or parallel to the standard hGH. NIH hGH and biosynthetic Met-hGH were generously supplied by NIAMDD and Genentech, respectively.

The hGH solutions were serially diluted from 1 mg/ml through 3.9 μg/ml and 100 μl each of these solutions in duplicates was added to the RIA systems of hPRL, hACTH, hLH, hFSH and hTSH. The RIA kit of hPRL was purchased from Dainabot Radioisotope Laboratories, Tokyo, Japan, that of hACTH from CEA, France, those of hLH, hFSH and hTSH from Daiichi Radioisotope Laboratories, Tokyo, Japan. Synthetic hACTH supplied by NIAMDD was used as standard in RIA of hACTH instead of the attached standards.

Results and Discussion

The displacement of 5 hGH preparations in the RIA systems for hPRL, hACTH, hLH, hFSH and hTSH are presented in Figures 1 to 5.

Several hundred nanograms to micrograms of pituitary-derived hGH preparations showed distinct immunoreactivities in each RIA system, except in the cases of Corpormon for hFSH and hTSH, although the amounts were 10^3 to 10^4 times larger than the proper hormones. Some of the dilution

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Fig. 1. Displacement curves in RIA system of hPRL with standard hPRL and five hGH preparations; 1. Crescormon, 2. Nanormon, 3. Corpormon, 4. NIH hGH, 5. met-hGH.

Fig. 2. Displacement curves in RIA system of hACTH with standard hACTH and five hGH preparations; 1-5. as in Figure 1.
Fig. 3. Displacement curves in RIA system of hLH with standard hLH and five hGH preparations; 1-5. as in Figure 1.

Fig. 4. Displacement curves in RIA system of hFSH with standard hFSH and five hGH preparations; 1-5. as in Figure 1.

Fig. 5. Displacement curves in RIA systems of hTSH with standard hTSH and five hGH preparations; 1-5. as in Figure 1.

curves were not parallel to the standards. This could be explained by denaturation and/or heterogeneity of contaminated hormones in these hGH preparations. We observed no immunoreactivity with biosynthetic met-hGH in any of these RIA systems for pituitary hormones except hGH.

The biosynthetic hGH used in the present study had an extra methionine at the NH2-terminus and this could interfere with intrinsic cross reaction. However, complete freedom from displacement in any RIA system for five other pituitary hormones indicates that the displacements caused with natural hGH preparations are not due to intrinsic cross reaction, but fully due to contamination with other pituitary hormones and their related peptides. In in vitro and in vivo studies using extractive preparations of hGH one should be cautious about these matters.

References


