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ABILITY OF GROWTH HORMONE FRAGMENTS TO COMPETE WITH ^{125}I-IODINATED HUMAN GROWTH HORMONE FOR SPECIFIC BINDING TO ISOLATED ADIPOCYTES OF HYPOPHYSECTOMIZED RATS

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Several noncovalent complexes of large fragments of human GH, which are less active than native human GH in stimulating glucose metabolism in adipose tissue of hypophysectomized rats, were tested for their ability to compete with ^{125}I-iodinated human GH for specific binding to isolated adipocytes of hypophysectomized rats. The complexes tested were A (residues 1–134 + residues 141–191; *S*-carbamidomethylated), B (residues 1–134 + residues 135–191; *S*-carbamidomethylated) and C (residues 1–134 + residues 135–191; *S*-carboxymethylated). When compared to native human GH, the complexes were less active in competing with ^{125}I-iodinated human GH for specific binding to adipocytes, and their order of potency in the binding assay (A > B > C) was similar to that of their respective activities in stimulating glucose metabolism in isolated adipose tissue of hypophysectomized rats.

Pituitary GH has several seemingly unrelated biological activities, including growth-promoting, diabetogenic and insulin-like activities and in the case of primate GHs, lactogenic activity. Recent work in our laboratory [1–3] on the structure-function relationships of human GH has demonstrated that noncovalent complexes of large fragments of human GH have markedly altered activity profiles, when compared to native human GH, and more particularly that the various activities have been altered in a nonparallel fashion. With all of the fragment complexes studied to date, insulin-like activity, as assessed by the in vitro ability of the substance to stimulate glucose uptake and metabolism by isolated adipose tissue of hypophysectomized rats, is attenuated. However, the fragment complexes vary in the extent to which their insulin-like activity is attenuated. Since it has been shown that there are specific binding sites for human GH on isolated rat adipocytes [4–6], the availability of human GH fragment complexes having varying degrees of insulin-like activity provided an opportunity to study the correlation between their insulin-like activity on isolated adipose tissue and their ability to complete with ^{125}I-iodinated human GH for specific binding to isolated adipocytes of hypophysectomized rats. It has not yet been possible to demonstrate in vitro effects of GH on isolated adipocytes from hypophysectomized rats [7,8], probably because the cells are altered in some way during the isolation procedure. Therefore, no attempt was made in this study to measured effects of the complexes on the metabolism of the cells used in the binding studies.

A highly purified human GH (A-type; 2.1

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Abbreviations: Complexes A, B and C refer to preparations Dal, R22079 and RCM-TD-hGH, respectively, from Refs. 2 and 3.

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I.U./mg) used for preparation of the radioactive ligand and as the reference standard in the binding assay was isolated as previously described [9]. ^{125}I-Iodinated human GH was prepared from this hormone by iodinating it with ^{125}I by the lactoperoxidase method of Thorell and Johansson [10]. Three human GH fragment complexes were studied. Complex A, consisting of a stable noncovalent complex of residues 1–134 and 141–191, was isolated from plasmin-digested *S*-carbamidomethylated human GH [11]. Complex A has growth-promoting [1] and diabetogenic [12] activity equivalent to that of native human GH, whereas its lactogenic activity is enhanced [1] and its insulin-like activity is about 30% that of the native hormone [1,13]. Complex B, consisting of a noncovalent complex of residues 1–134 and 135–191, was produced by noncovalent complementation of *S*-carbamidomethylated human GH peptides 1–134 and 135–191 [3]. It has approx. 35% of the growth-promoting activity of human GH, full diabetogenic activity, whereas its insulin-like activity is only about 20% that of native human GH [3]. Complex C, consisting of a stable complex of residues 1–134 and 135–191, was produced by digesting human GH with thrombin and then reducing and *S*-carboxymethylating its disulfide bridges [2]. This complex has approx. 10% of the growth-promoting activity of human GH, full lactogenic activity, but its insulin-like activity is only about 5% that of the native hormone.

Isolated adipocytes were prepared from the epididymal fat pads of male hypophysectomized rats of the Sprague-Dawley strain (90–100 g) from the Charles River Breeding Laboratories, Wilmington, MA. The animals were maintained on a high carbohydrate diet [14] for 4 weeks before being used in experiments. The rats were killed by decapitation, and the epididymal fat pads were removed, minced and incubated with collagenase (2 mg/ml; Type I, Worthington, Freehold, NJ) in Krebs-Ringer bicarbonate buffer containing glucose (1 mM) and bovine serum albumin (1%, w/v) for 60 min at 37°C, according to the method of Rodbell [15]. The cells were washed four times with buffer without collagenase and then resuspended and preincubated for 3 h at 37°C, since preincubation of the cells has been shown to increase their ability to bind ^{125}I-iodinated human GH [16]. After preincubation, the cells were washed once, and 1.8-ml aliquots of the cell suspension (approx. 10^6 cells) were added to siliconized scintillation vials containing 0.1 ml ^{125}I-iodinated human GH (2–4 ng; 200 000 cpm; spec. act. of 100–150 μCi/μg) and 0.1 ml buffer containing various concentrations of human GH (2–5000 ng/ml), Complex A (10–5000 ng/ml), Complex B (40–5000 ng/ml) or Complex C (100–5000 ng/ml). Incubation was carried out for 2 h at 37°C. Triplicate 300-μl aliquots of each cell suspension were then centrifuged through dinonylphthalate oil to separate the cells from their suspension buffer as described by Gliemann et al. [17]. The cell layer was excised and counted in an autogamma scintillation counter. Specific binding was determined by subtracting cell-bound radioactivity obtained in the presence of 5000 ng/ml of unlabeled human GH from total cell-bound radioactivity. Total binding was 2–3% of the total radioactivity in the incubation mixture, and nonspecific binding was 0.6% of total binding. Values are expressed as percent of total cpm specifically bound per mg of extractable lipid, determined according to Gliemann [18].

The ability of native human GH and the various fragment complexes to compete with ^{125}I-iodinated human GH for specific binding to isolated adipocytes of hypophysectomized rats is

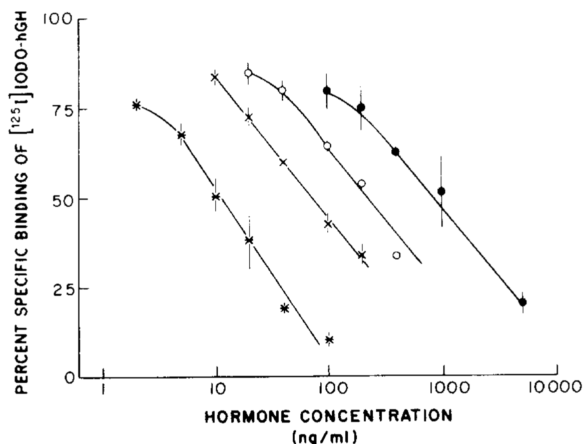

Fig. 1. ^{125}I-Iodinated human HG ([^{125}I]iodo-hGH) binding to isolated adipocytes in the presence of various concentrations of human GH (*), Complex A (×), Complex B (○) and Complex C (●). Values are expressed as percent of total specific [^{125}I]iodo-gGH binding and are given as means ± S.E. of three separate experiments.

shown in Fig. 1. It can be seen that, in each case, competition was concentration-dependent and that the complexes were considerably less active than native human GH in competing with ^{125}I-iodinated human GH for binding to the cells. The concentration of human GH required to inhibit binding by 50% ($B/B_0 = 0.5$) was 9.2 ± 0.9 ng/ml (mean $\pm$ S.E. of three experiments). In contrast, the concentration of Complex A required to inhibit binding by 50% was 67.4 ± 6.8 ng/ml, 198 ± 14 ng/ml for Complex B and 961 ± 96 ng/ml for Complex C.

These findings suggest that there is some correlation between the insulin-like activity of the several human GH fragment complexes tested and their ability to bind to adipocytes. All were less active than native human GH in vitro in stimulating glucose metabolism in isolated adipose tissue of hypophysectomized rats [1–3], and each was less effective than native human GH in competing with ^{125}I-iodinated human GH for binding to isolated adipocytes of hypophysectomized rats, their order of estimated potency in stimulating glucose metabolism in adipose tissue ($A > B > C$) being similar to their order of activity in the binding assay.

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References

1 Reagan, C.R., Kostyo, J.L., Mills, J.B., Moseley, M.H. and Wilhemli, A.E. (1978) Endocrinology 102, 1377–1386
2 Mills, J.B., Kostyo, J.L., Reagan, C.R., Wagner, S.A., Moseley, M.H. and Wilhelmi, A.E. (1980) Endocrinology 107, 391–399
3 Reagan, C.R., Kostyo, J.L., Mills, J.B., Gennick, S.E., Messina, J.L., Wagner, S.A. and Wilhelmi, A.E. (1981) Endocrinology 109, 1663–1671
4 Fagin, K.D., Lackey, S.L., Reagan, C.R., DiGirolamo, M. (1980) Endocrinology 107, 608–615
5 Grichting, G., Levy, L.K. and Goodman, H.M. (1981) Program of the 63rd Annual Meeting of The Endocrine Society, Cincinnati, OH (Abstr. 75)
6 Gavin, J.R., III, Saltman, R.J. and Tollefsen, S.E. (1982) Endocrinology 110, 637–643
7 Eastman, R.C. and Goodman, H.M. (1971) Proc. Soc. Exp. Biol. Med. 137, 168–174
8 Schoenle, E., Zapf, J. and Froesch, E.R. (1979) Endocrinology 105, 1237–1242
9 Mills, J.B., Ashworth, R.B., Wilhelmi, A.E. and Hartree, A.S. (1969) J. Clin. Endocrinol. Metab. 29, 1456–1459
10 Thorell, J.L. and Johansson, B.G. (1971) Biochim. Biophys. Acta 251, 363–369
11 Mills, J.B., Kostyo, J.L., Moseley, M.H., Reagan, C.R. and Wilhelmi, A.E. (1978) Endocrinology 102, 1366–1376
12 Reagan, C.R. (1978) Diabetes 27, 883–887
13 Goodman, H.M. and Kostyo, J.L. (1981) Endocrinology 108, 553–558
14 Goodman, H.M. (1965) Endocrinology 72, 216–225
15 Rodbell, M. (1964) J. Biol. Chem. 239, 375–380
16 Edén, S. and Schwartz, J. (1981) Fed. Proc. 40, 400 (Abstr.)
17 Gliemann, J., Østerlind, K., Vinten, J. and Gammeltoft, S. (1972) Biochim. Biophys. Acta 286, 1–9
18 Gliemann, J. (1968) Acta Physiol. Scand. 72, 481–491