Comparison of Pancreatic Human and Biosynthetic Human Insulin with Respect to Their Action on Adipocytes and Chick Embryo Fibroblasts

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Biosynthetic human insulin (BHI) was compared to pancreatic human insulin and sperm whale insulin in terms of ability to stimulate incorporation of glucose into isolated rat adipocytes and thymidine into DNA in chick embryo fibroblasts. The human insulins were identical in their effects in both assays. Sperm whale insulin was more potent than the human insulins in stimulating glucose incorporation into rat adipocytes. All three insulins showed identical stimulation of DNA synthesis in the fibroblast assay. That action, however, is mediated via the receptor for insulin-like growth factor (IGF-I). Therefore, both human insulins were evaluated in terms of binding to the IGF-I receptor in chick embryo fibroblasts. The two human insulins behaved identically (and agreed with previous findings for sperm whale insulin). All insulins, however, were approximately 200-fold less potent than IGF-I itself in this binding assay. DIABETES CARE 4: 257–259, MARCH–APRIL 1981.

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We have studied some effects and binding characteristics of BHI in two tissues: a classical insulin target tissue (rat adipose tissue) and a target tissue of insulin-like growth factors[1] (chick embryo fibroblasts).

MATERIALS AND METHODS

Incorporation of U-^{14}C-glucose into isolated fat cells. Isolated fat cells were prepared from epididymal fat pads of fed, normal, male Zbz-Cara rats, weighing 110–120 g, according to the method of Rodbell.[2] Crude collagenase (Worthington) was used for digestion of the tissue. Then, 2–3 × 10^5 cells were incubated for 60 min at 37°C in 1 ml of Krebs-Ringer's bicarbonate buffer containing 2 mg human serum albumin, 0.2 mg glucose, 0.1 μCi U-^{14}C-glucose (The Radiochemical Center, Amersham), and different amounts of sperm whale insulin (same amino acid composition as pork insulin: gift of Dr. R. E. Humbel, Zürich), human pancreatic insulin (Lilly, Indianapolis), or BHI (Lilly). Next, 0.3 ml of the cell suspension was centrifuged in 400-μl plastic tubes through a layer of dinonylphthalate (10,000 g, 20 sec) according to the method of Gliemann et al.[3] The tops of the tubes were cut off and the cell layer was counted in 5 ml of Instagel (Packard) in a liquid scintillation counter (Packard).

Thymidine incorporation into chick embryo fibroblasts. Primary cultures of fibroblasts (~10^6 cells/dish) from 12-day-old chick embryos were prepared as described previously.[4] They were made stationary during a 24-h period by exposing them to a culture medium (see below) devoid of serum or growth factors. The cells were preincubated for 8 h with sperm whale insulin, human pancreatic insulin, or BHI, or were incubated without hormone, in minimum essential medium Eagle (Difco), supplemented with 0.03% glutamine, 1 ml/100 ml of nonessential amino acids (Difco), and 350 mg/100 ml of human serum albumin (Swiss Red Cross, Bern, Switzerland). After that time, 6-^{3}H-thymidine (0.1 μCi/dish) was added for 2 more hours. The cell layers were then rinsed three times with ice-cold saline/phosphate buffer, pH 7.4, detached with 0.25% trypsin (dissolved in the same buffer), transferred to 15-ml plastic tubes, and precipitated with 10% trichloroacetic acid. The precipitate was washed twice with the same acid, digested in 1 ml 1 N KOH and neutralized with 1 N HCl; 0.5 ml was counted in 10 ml of Instagel (Packard) in a beta counter (Packard).

Competitive inhibition of the binding of ^{125}I-labeled IGF-I by different insulins. The binding experiment was performed in culture dishes (Lux, Ø 35 mm) with primary stationary cultures of chick embryo fibroblasts (see above). The cells were incubated for 1 h at room temperature in 1 ml of Dulbecco buffer, pH 7.4, containing 10 mg human serum albumin, ^{125}I-labeled IGF-I (~2 × 10^5 cpm, ~1 ng), and various amounts of unlabeled IGF-I, human pancreatic insulin, BHI, or no insulin. Next, the cells were rinsed three times in 2 ml

of ice-cold Dulbecco/albumin buffer (same as above), detached from the dishes with 0.25% trypsin (in Dulbecco/albumin buffer), and counted for radioactivity in a gamma counter (Packard).

RESULTS

The stimulation of U-^{14}C-glucose incorporation into isolated rat fat cells as a function of the concentration of human pancreatic insulin, BHI, and sperm whale insulin is shown in Figure 1. The amino acid sequence of sperm whale insulin is the same as that of pork insulin, and it is routinely used in our laboratory as an insulin standard. In the fat pad assay[5] and in the fat cell assay, pork and sperm whale insulin give identical dose-response curves. As can be seen from the results in Figure 1, human pancreatic insulin and BHI are equally potent, but they appear to be about two times less active than the sperm whale insulin.

In order to understand the meaning of the next experiment, which was carried out with chick embryo fibroblasts, knowledge of the following facts is important: Besides the insulin receptor, most cells have a second membrane binding site with a high affinity for the insulin-like growth factors (IGF).[1] Insulin-like growth factors also bind to the insulin receptor, but with an affinity that is about 1% that of insulin. In contrast, insulin does not bind to the IGF receptor with the exception of the chick embryo fibroblasts, where insulin

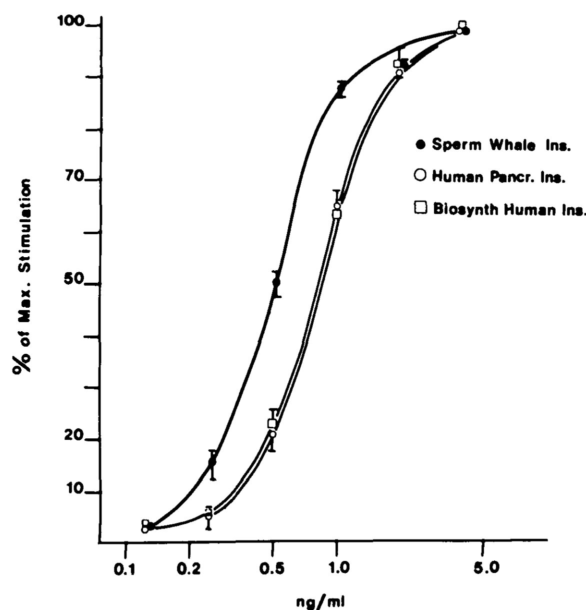

FIG. 1. Stimulation of the incorporation of U-^{14}C-glucose into isolated rat fat cells by sperm whale insulin (●), human pancreatic insulin (○), and BHI (□). The experimental procedure is described in MATERIALS AND METHODS. All points are the means of four incubations from two different experiments. Bars give the SEM. Stimulation of glucose incorporation is plotted as percentage of maximal stimulation in the presence of 100 μU/ml of sperm whale insulin. Maximal stimulation was fivefold to sevenfold the basal value in the absence of hormones.

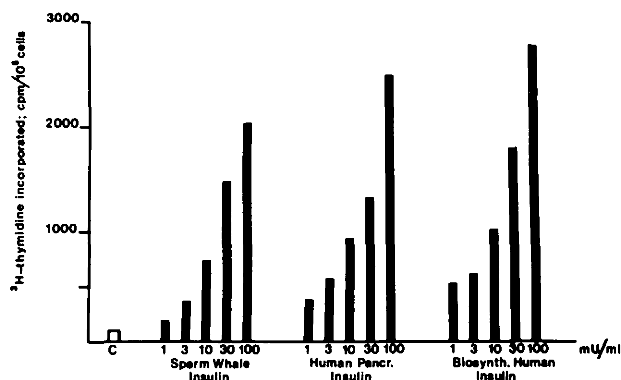

FIG. 2. Stimulation of ^{3}H-thymidine incorporation into primary cultures of chick embryo fibroblasts by sperm whale insulin, human pancreatic insulin, and BHI. The experimental procedure is given in MATERIALS AND METHODS. The height of the columns gives the mean of duplicates from one experiment. The variation was less than ±10% of the mean; C = control.

has an affinity of about 1% that of IGF.[1] Therefore, it was of interest to investigate (1) whether BHI stimulated DNA synthesis from ^{3}H-thymidine to the same extent as our standard sperm whale insulin, and (2) whether or not BHI displaces labeled IGF from the IGF receptor of chick embryo fibroblasts in the same way as our standard sperm whale insulin.

In Figure 2, the results are given of an experiment carried out with primary cultures of chick embryo fibroblasts: sperm whale insulin, human pancreatic insulin, and BHI were equally potent in stimulating ^{3}H-thymidine incorporation into DNA of the fibroblasts. Half-maximal effects are observed between 10 and 20 mU/ml ($0.7-1.4 \times 10^{-7}$ M), which is approximately 200 times higher than the IGF concentration required for half-maximal stimulation of this index (not shown; see ref. 1).

In Figure 3, the "displacement" of ^{125}I-labeled IGF-I from chick embryo fibroblasts by unlabeled IGF-I, pancreatic human insulin, and BHI is shown. Again, the two human insulin preparations compete with labeled IGF in a similar fashion, and the dose-response curves are identical. Both insulins are approximately 200 times less potent than IGF-I, which agrees with earlier findings obtained with sperm whale insulin in chick embryo fibroblast.[1]

DISCUSSION

The results of Figures 1 and 2 demonstrate that human pancreatic and biosynthetic insulin are indistinguishable with respect to (1) their biologic potencies in stimulating glucose incorporation into rat adipocytes, and (2) their biologic potencies in enhancing DNA synthesis in chick embryo fibroblasts. The finding that both human insulins are approximately two times less potent than sperm whale or pork insulin in rat adipocytes is unexpected and remains unexplained, but it was reproducible in two different experiments.

Whereas all the known biologic effects of insulin on the

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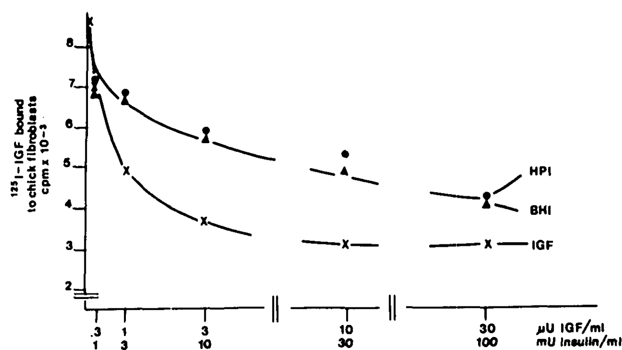

FIG. 3. *Competitive inhibition of the binding of* ^{125}*I-labeled IGF-I to primary cultures of chick embryo fibroblasts by unlabeled IGF-I, human pancreatic insulin (HPI), and BHI. The experiment was performed as described in* MATERIALS AND METHODS. *All points are the means of duplicates from one experiment. The variation was less than ±10% of the mean value.*

adipocyte are mediated by the insulin receptor, insulin stimulates DNA synthesis and multiplication of fibroblasts via the IGF receptor.[1,6] The two human insulins are equivalent with respect to their biologic actions mediated by these two different receptors. Furthermore, the results of Figure 3 show that the apparent affinities of the two human insulins toward the fibroblast IGF receptor are the same.

Thus, in all three different sets of experiments BHI behaved identically to human pancreatic insulin.

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