The Biologic Potency and Binding Affinity of Biosynthetic Human Insulin in Isolated Rat Adipocytes

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Using the isolated rat adipocyte system, we could not detect any difference in binding affinity or biologic potency of biosynthetic and pancreatic human insulin. DIABETES CARE 4: 250–251, MARCH–APRIL 1981.

The aim of the present study was to investigate whether the biologic properties of biosynthetic human insulin (BHI) could be distinguished from that of pancreatic human insulin.

MATERIALS AND METHODS

Unlabeled insulin. The unlabeled human pancreatic insulin (Lilly Lot 615-1054B-214-1) and biosynthetic human insulin (Lilly Lot 615-7ON-174-10) were dissolved directly in the vials in 1 ml of 0.1 mM HCl to give a concentration of 100 μg/ml according to the manufacturer. Further dilutions were done in the assay buffer.

^{125}I-labeled insulin. The human ^{125}I-monoiodoinsulin (Lilly Lot J84-02N-146) and the pork ^{125}I-monoiodoinsulin (Lilly Lot J84-02N-140) were dissolved in 1 ml water. Further dilutions were done in the assay buffer. For comparison, cells were incubated in parallel with pork A14-Tyr-^{125}I-monoiodoinsulin iodinated by Dr. S. Linde, Hagedorn Research Laboratory, as described elsewhere.[1,2]

Preparation of isolated adipocytes. Adipocytes were prepared from male Wistar rats weighing about 160 g using collagenase (Worthington type I, 0.5 mg/ml).[3]

Binding studies. In binding studies, 25 μl of packed cells (about 2.5 × 10^5 cells) were incubated in 0.5 ml buffer with tracer for 45 min. The cells were recovered after the addition of 10 ml chilled (10°C) 0.15 M NaCl, followed by centrifugation through silicone oil (0.99 kg/L) as described elsewhere.[4] Nonspecific binding was determined in the presence of 1 μM pork insulin and was about 3% of the total binding of tracer.

Biologic potency. The biologic activity of the insulins was determined using the enhancement of glucose conversion into lipids in isolated rat adipocytes, as described elsewhere.[5] In brief, 1-ml aliquots of adipocyte suspension (0.5% packed cell volume) were incubated for 120 min with 0.1 μCi 3-^{3}H-glucose, 0.12 mM D-glucose, and insulin (3 pM–25 nM), followed by the addition of 10 ml toluene-based scintillation fluid. The samples were allowed to extract overnight and the incorporation of ^{3}H-label into lipids was determined.

RESULTS AND DISCUSSION

Binding affinity. The displacement of pork ^{125}I-monoiodoinsulin by human pancreatic and biosynthetic human insulin, respectively, is shown in Figure 1. As can be seen, the concentration of the two insulins giving half-maximal binding was identical.

To test the relative binding affinity of the ^{125}I-labeled BHI directly, the maximal specifically bound/free (B/F) ratio was determined and compared with that obtained with the supplied labeled pork insulin. We used concentrations of 5–10 pM (according to manufacturer), which are very much below the K_d for binding in this system.[4] The number of cells and, therefore, the number of receptor sites were the same in incubations with the two tracers. Under the assumption that none of the receptors is able to bind only one of the tracers, the ratio between the maximal B/F values for the two tracers will give the ratio between the association constants. As can be seen from Table 1, the binding affinity of the Lilly biosynthetic human tracer was almost the same as that of the Lilly pork tracer. The same result was obtained with hepatocytes (data not shown).

We have previously described the properties of pork A14-Tyr-^{125}I-monoiodoinsulin prepared by Nordisk Insulin (Gentofte, Denmark).[1,2] This tracer had on the average a slightly higher affinity than that of the Lilly pork tracer, although statistical significance was not achieved (Table 1). In this connection it should be noted that the experiments were performed 45–60 days after iodination of the Lilly tracers, which had been stored lyophilized until receipt in our laboratory. We have shown that long-term storage of lyophilized

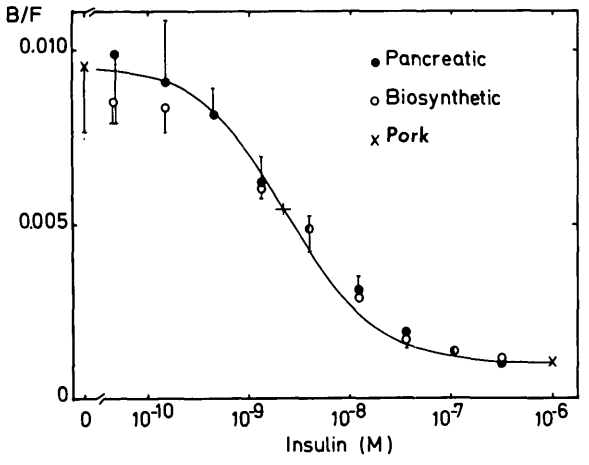

FIG. 1. Binding affinity of human insulins. Isolated rat adipocytes (4.8%, vol/vol) were incubated for 45 min at 37°C in the presence of 18 pM pork ^{125}I-monoiodoinsulin alone (x) or in the presence of the indicated concentrations of biosynthetic (○) or pancreatic (●) human insulin. The highest concentration used (1 μM) was obtained by adding pork insulin (monocomponent quality from Novo). The incubation was stopped as described in MATERIALS AND METHODS. *Bars represent SD when exceeding size of symbol. N = 4.*

monoiodoinsulin causes an increase in the fraction of ^{125}I-activity eluting in the void volume on a Sephadex G-50 Fine column.[2] Thus, the Lilly biosynthetic human tracer eluted from a Sephadex G-50 Fine column (1.5 × 100 cm, eluted with 0.1% wt/vol bovine serum albumin in 0.5 M acetic acid) with 11% of the radioactivity in the void volume and

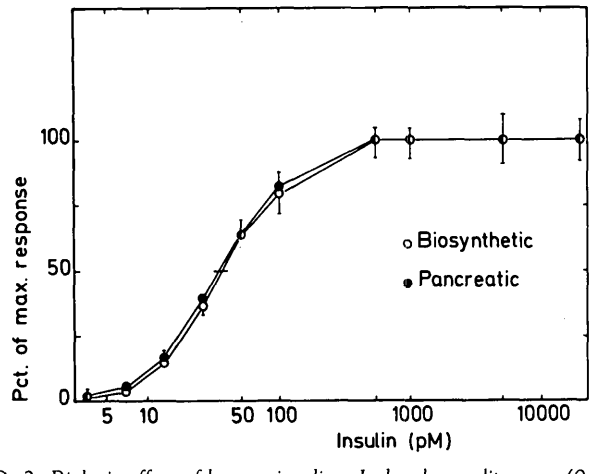

FIG. 2. Biologic effect of human insulins. Isolated rat adipocytes (0.5% vol/vol) were incubated in a volume of 1 ml for 2 h at 37°C in the presence of 0.1 μCi [3-^{3}H]glucose, 0.12 mM D-glucose, and the indicated concentrations of biosynthetic (○) or pancreatic (●) human insulin. The incubation was stopped as described in MATERIALS AND METHODS. *Bars represent SD when exceeding size of symbol. N = 3.*

TABLE 1

Comparison of binding affinity to isolated rat adipocytes of biosynthetic human ^{125}I-monoiodoinsulin (Lilly), pork ^{125}I-monoiodoinsulin (Lilly), and pork A14-Tyr-^{125}I-monoiodoinsulin (Nordisk). The binding studies were performed as described in MATERIALS AND METHODS

Tracer 1	Tracer 2	K_a^1/K_a^2*
Lilly human	Lilly pork	1.05 (0.99–1.23)
Lilly human	Nordisk pork	0.98 (0.96–1.04)
Lilly pork	Nordisk pork	0.92 (0.79–1.05)

* Mean (range) of five experiments. Employing Wilcoxon's test for pair differences, none of the ratios was significantly different from 1.00.

the remaining 89% at the position of iodoinsulin (data not shown).

Biologic potency. As can be seen from Figure 2, there was no detectable difference between the human insulin of pancreatic or bacterial origin.

CONCLUSION

The binding-affinity and biologic potency of biosynthetic and pancreatic human insulin were indistinguishable in the isolated rat adipocyte system. Likewise, no difference was observed in binding of ^{125}I-tracers prepared from the biosynthetic human and the pork pancreatic insulin, respectively.

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