Receptor Binding of Biosynthetic Human Insulin on Isolated Pig Hepatocytes

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Biosynthetic human insulin (BHI) and pancreatic human insulin were compared with respect to receptor binding in a heterologous assay system: displacement of pork A14-^{125}I-monoiodoinsulin from receptors on pig hepatocytes. The concentrations of human insulin giving half-maximal displacement were identical for both preparations, i.e., 0.5 nM. Their relative potency was 1.01 ± 0.14 (SD, N = 5), suggesting that biosynthetic and pancreatic human insulin exert the same biologic activity. DIABETES CARE 4: 235–237, MARCH–APRIL 1981.

The main source of insulin for treatment of diabetes and for research has been extracts from bovine or pork pancreases. In addition, insulin from a large number of animal species, including man, has been purified and characterized with respect to its structure and biologic activity.[1] After the discovery of the amino acid sequence of bovine insulin by Ryle and co-workers,[2] the chemical synthesis of A- and B-chains was achieved and a synthetic insulin preparation obtained by chain combination.[3–5]

A recent development has been the biologic synthesis of human insulin by a bacterial system, *Escherichia coli*. Synthetic insulin A- and B-chain genes were linked to the β-galactosidase gene by recombinant DNA technology, inserted into the *E. coli*, and the two peptide chains were synthesized in bacterial fermentations.[6,7] This synthesis was followed by purification and chain combination to yield human insulin.[8] Eli Lilly and Company (Indianapolis) has prepared human insulin using the same procedures. This biosynthetic human insulin was found to be equivalent to the preparation of pancreatic human insulin in several in vivo and in vitro test systems.[9] These assays included the IM-9 lymphocyte radioreceptor assay.[10]

The purpose of this paper is to show that human insulins of different origin—pancreatic and biosynthetic—are identical with respect to their receptor binding affinity in a heterologous assay system (displacement of pork A14-^{125}I-monoiodoinsulin from receptors on isolated pig hepatocytes).[11] This suggests that the two insulin preparations have the same biologic activity.

MATERIALS AND METHODS

Pork A14-^{125}I-monoiodoinsulin (hereinafter ^{125}I-insulin) was a gift from Novo Research Institute (Copenhagen) and was prepared as described by Larsen and Jørgensen.[12] This preparation was indistinguishable from unlabeled pork insulin with respect to biologic activity.[13]

Human insulin, both biosynthetic and pancreatic, was obtained from Lilly and treated in the following way: Approximately 100 μg of each insulin preparation was dissolved in 1 ml 0.1 N HCl. The molar concentrations of the insulin solutions were calculated[14,15] from the uv absorbance at 276 nm using a molar extinction coefficient of 6100 M^{-1} cm^{-1}. The stock solutions were serially diluted at 4°C in sodium phosphate buffer (40 mM, pH = 7.4, containing NaCl 100 mM) and human serum albumin (Behringwerke, BRD) (1.5 g/L) to give concentrations in the range of 20 pM to 20 μM. These dilutions were used to give the final concentrations in the displacement assay (see Figure 1).

Hepatocytes were isolated from a piece of pig liver (100 g) by EDTA and collagenase perfusion, according to the principles described by Berry and Friend.[16] Binding of ^{125}I-insulin to receptors on hepatocytes was measured as described previously.[17] Pork insulin (Novo) (4 μM) was added to determine the nonspecific binding of ^{125}I-insulin on hepatocytes.

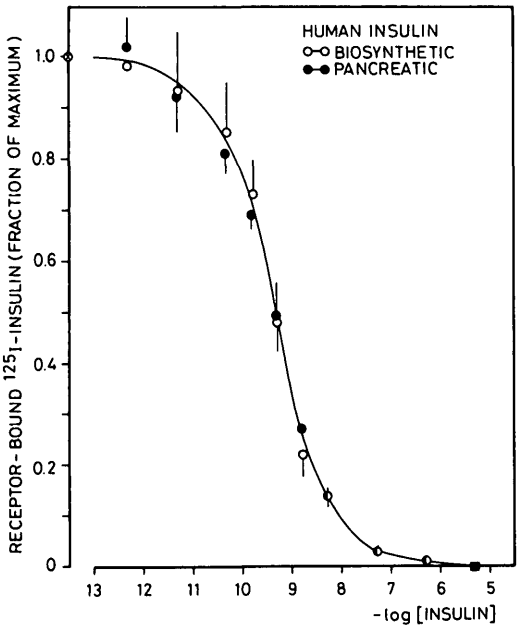

FIG. 1. Displacement of receptor-bound ^{125}I-insulin on hepatocytes by human insulin. Isolated hepatocytes (10^6 cells/ml) were incubated at 37°C in Krebs-Ringer's-Hepes buffer, pH = 7.4, containing human serum albumin (10 mg/ml) and bacitracin (0.7 mM) with 50 pM of ^{125}I-insulin and human insulin, either biosynthetic (○) or pancreatic (●) in the concentrations indicated. After 60-min incubation, the cells were separated from the buffer by centrifugation through silicone oil. The receptor-bound ^{125}I-insulin was calculated by subtraction of the nonspecific binding determined in the presence of 4 μM pork insulin (■). The value for ^{125}I-insulin alone (⊗) was considered as maximum, and the displacement by human insulin was plotted as the fraction of maximum versus the negative logarithm of the insulin concentration. The points represent mean values of five separate experiments with standard deviations as bars.

Bacitracin (Dumex, Denmark) was added to reduce proteolytic degradation of extracellular ^{125}I-insulin during 60-min incubation at 37°C.[11] The degradation was determined as the decrease in trichloroacetic acid precipitable ^{125}I-insulin.

RESULTS AND DISCUSSION

Isolated hepatocytes from pigs are equipped with a large number of specific receptors that bind pork ^{125}I-insulin with high affinity. The total amount of binding sites is equal to about 5×10^4 per cell, and the apparent dissociation constant is 0.4–0.8 nM.[18] These characteristics are similar to those of the conventional isolated rat hepatocyte preparation, when the difference in the ^{125}I-insulin preparation is taken into account.[17] The binding affinity of unlabeled insulin analogues is determined as their ability to displace ^{125}I-insulin from cell receptors and is expressed as the concentration of insulin giving half-maximal displacement.

The displacement curves for the two human insulin preparations (biosynthetic and pancreatic) are shown in Figure 1. The results represent the mean of five separate experiments, and the two curves are similar. The displacement by human insulin was expressed in fractions of maximum receptor binding. This was calculated from the values of total and nonspecific bound/free ^{125}I-insulin in fmol per 10^6 cells/fmol per μl medium. The mean value of total bound/free at 50 pM ^{125}I-insulin was 16.9 ± 2.9 (SD, N = 5), and the nonspecific bound/free value was 0.5 ± 0.1 (SD, N = 5). Consequently, the maximum receptor bound/free value was 16.4 ± 2.9 (SD, N = 5), and the receptor-bound ^{125}I-insulin at increasing concentrations of human insulin was calculated as fractions of this value. The insulin concentration giving half-maximal displacement was 0.5 nM. The mean relative potency of BHI compared with pancreatic human insulin in five experiments was 1.01 ± 0.14 (SD, N = 5).

Degradation of pork ^{125}I-insulin was measured by the appearance of trichloroacetic acid soluble ^{125}I-activity in the medium. After 60 min incubation at 37°C, a fraction (0.15 ± 0.05; SD, N = 5) of the extracellular 50 pM ^{125}I-insulin was degraded. This was due to degradation of receptor-bound ^{125}I-insulin and release of degradation products from the cells,[17,18] since the extracellular degradation of ^{125}I-insulin by the soluble insulin-specific protease was almost completely inhibited by bacitracin 0.7 mM.[11]

In the present study, the degradation of human insulin by hepatocytes was not measured. However, it is unlikely that different rates of degradation of the two human insulin preparations could influence the binding results. First, the receptor-mediated degradation of several insulin analogs was proportional to receptor binding in rat hepatocytes.[18] Second, the rates of degradation of human biosynthetic ^{125}I-insulin and semisynthetic ^{3}H-insulin in rats in vivo were identical.[19]

Insulin exerts important direct effects on liver metabolism,[20] but neither of these actions were measured in the present study. The identical receptor binding of the two human insulin preparations suggests that they exert the same biologic activity in vitro. Firstly, in isolated hepatocytes the concentration dependence of receptor binding of insulin is similar to that reported for several biochemical actions of insulin, as reviewed previously.[17] Secondly, the binding affinities of insulin analogs relative to pork insulin parallel their relative potencies on glucose metabolism in adipocytes.[17,21] Only two exceptions to this rule have been reported: Hagfish insulin[22] and coypu insulin (R. Horuk and S. Gammeltoft, unpublished), which show a discrepancy between binding and action. Third, the biosynthetic and the pancreatic human insulin preparations showed identical biologic activities with respect to stimulation of amino acid transport in isolated rat hepatocytes.[23]

The amino acid sequence of human insulin[24] is identical to that of pork insulin[25] apart from the substitution of threonine for alanine as the C-terminal amino acid of the B-chain. This suggests that the three-dimensional structures of the two insulins are similar.[26] The biologic activities of human insulins isolated from pancreas[27,28] or prepared by a complete chemical synthesis[29] are equivalent to that of pork insulin.

In conclusion, BHI is identical to insulin extracted from human pancreas with respect to receptor binding on isolated pig hepatocytes. We can interpret this to mean that the two insulin preparations exert the same biologic activity in vitro with respect to regulation of liver metabolism.

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