Binding of Insulin to Rat Pancreatic Islets: Comparison Between Pancreatic Human Insulin and Biosynthetic Human Insulin

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Human pancreatic insulin, biosynthetic human insulin (BHI), and pork insulin were compared in terms of their binding characteristics to insulin receptors on rat pancreatic islets. There was no difference in binding or on biologic effect, i.e., ability to inhibit insulin secretion. DIABETES CARE 4: 252–253, MARCH–APRIL 1981.

Recently we have demonstrated the existence of insulin receptors in rat pancreatic islets. Criteria of this binding were high affinity ($K_d = 0.5 \times 10^{-9}$ M), saturability ($R_0 = 740$ binding sites/islet cell), reversibility, and displaceability.[1] Binding data were in harmony with biologic effects of insulin—i.e., inhibition of glucose-induced insulin release, decrease of pentose phosphate shunt activity, and decrease of NADPH/NADP ratio ($ED_{50} \sim 1.1 \times 10^{-9}$ M). Using rat pancreatic islets, displaceability of ^{125}I-insulin by pancreatic human insulin and by BHI was tested. In addition, the effect of the two insulins on glucose-induced insulin secretion was investigated.

METHOD

Details of the methods used have been reported recently.[1] Approximately 100 collagenase-isolated rat pancreatic islets per vial were incubated in 0.1 ml Krebs-Ringer's bicarbonate/albumin (2%) buffer at 37°C for 20 min in the presence of unlabeled pork insulin and radioactively labeled insulin (spec. act. 100–120 μCi/μg with 0.3 atoms of iodine per insulin molecule, provided by Amersham Buchler GmbH & Co., West Germany). Radioactivity of islets and of filtrates was counted after islets had been filtered on a cellulose acetate filter. "Specific binding" was calculated by subtracting nonspecific binding (excess of unlabeled insulin) from total binding. Data are not corrected for insulin degradation since it was less than 4% per 100 islets, as has recently been shown.[1] In parallel batches, five islets were incubated in 1 ml KRB/albumin buffer at 37°C for 90 min in the presence of 16.7 mM glucose and in the presence of various concentrations of exogenous insulin. Thereafter, insulin released into the medium was determined radioimmunologically.

RESULTS AND DISCUSSION

The effect of pork insulin, pancreatic human insulin, and BHI on isolated rat pancreatic islets is shown in Figure 1. Parameters being measured were (A) displacement of ^{125}I-pork insulin from its binding sites and (B) effect on glucose-induced insulin secretion. In (A), ^{125}I-insulin binding (ordinate) versus concentration of various insulins added to the incubation medium (abscissa) is shown. By increasing the concentration of unlabeled insulins from 10^{-10} to 10^{-7} M,

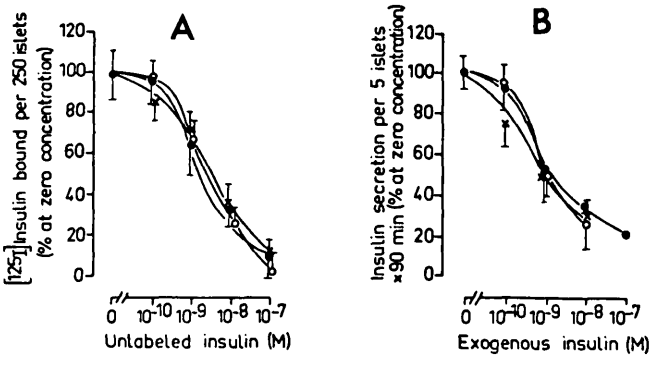

Fig. 1. Effect of pork insulin (●—●), pancreatic human insulin (×—×), and BHI (○—○) on isolated rat pancreatic islets. Parameters: (A) ^{125}I-insulin binding (displacement study); (B) inhibition of insulin secretion by exogenous insulin.

^{125}I-insulin was displaced by either substance in a concentration-dependent manner. There was, however, no statistical difference in displacement between pork insulin and both the human insulins. The dissociation constant of high-affinity site was in the range of 0.5×10^{-9} M (Scatchard analysis).

In Figure 1B, the effect of each of these insulins (concentrations in the abscissa) on glucose-induced insulin secretion is shown. It is obvious that added pork insulin, pancreatic human insulin, and BHI decrease insulin secretion in a concentration-dependent manner. The curves were statistically not different from each other, suggesting that as far as the inhibitory effect of exogenous insulin on glucose-induced insulin release is concerned, again there is no difference between pork insulin, pancreatic human insulin, and BHI.

We conclude that, using rat pancreatic islets, there is no difference in binding or biologic effect (as far as inhibitory effect on insulin release is concerned) between human pancreatic insulin, BHI, and pork insulin.

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REFERENCE

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