Biologic Activity and Receptor Binding Properties of Biosynthetic Human Insulin in Isolated Rat Hepatocytes and Mouse Soleus Muscle In Vitro

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Studies from our laboratory have shown that freshly isolated hepatocytes from the adult rat represent a suitable system to measure insulin binding to cell surface receptors and insulin biologic effects. Biosynthetic human insulin (BHI) binding to isolated rat hepatocytes was measured under steady-state conditions at 20°C and 37°C, using ^{125}I-BHI and varying concentrations of unlabeled BHI. Control binding experiments were run in parallel with ^{125}I-pork insulin and varying concentrations of unlabeled pork insulin. No difference between BHI and pork insulin binding could be detected at either temperature. Similar results were obtained when BHI and pork insulin binding was studied using purified liver plasma membranes. These results indicate that BHI binds to hepatocyte receptors with the same characteristics as monocomponent pancreatic pork insulin. BHI's stimulation of α-amino ^{14}C-isobutyric acid (AIB) influx in rat hepatocytes was found to be indistinguishable from that induced by pancreatic human insulin or pork insulin: for the three insulins, maximal responses (about a twofold increase above basal) and concentrations producing a half-maximal response ($EC_{50} \cong 1$ nM) were identical. The isolated mouse soleus is a slow twitch, red skeletal muscle, which binds insulin, and responds to the hormone, in a dose-related fashion as regards glucose transport and metabolism. BHI stimulated 2-deoxyglucose uptake to the same extent as pancreatic human insulin or pork insulin. Maximal stimulation (a fourfold increase) and concentrations which produced a half-maximal stimulation ($EC_{50} \cong 0.5$ nM) were identical for the three insulins. These in vitro studies show that BHI has the same intrinsic activity (maximal effectiveness) and biologic potency (EC_{50}) as pancreatic human insulin and pancreatic porcine insulin in two major target tissues of insulin, liver and skeletal muscle. Moreover, receptor-binding properties of BHI are identical to those of pork insulin in isolated rat hepatocytes and liver plasma membranes. DIABETES CARE 4: 223–227, MARCH–APRIL 1981.

Studies from this laboratory have shown that adult rat hepatocytes, used in fresh suspensions or in monolayer cultures, and isolated mouse soleus muscles represent suitable systems to measure insulin binding and insulin biologic activity in vitro. Insulin has been shown to bind to specific receptors in liver plasma membranes[1,2] and in isolated hepatocytes.[3–5] In isolated rat hepatocytes, insulin rapidly stimulates (Na^+-K^+)ATPase transport activity[6] and enhances amino acid uptake by promoting the synthesis of new carrier proteins.[7] The isolated mouse soleus muscle, a slow-twitch red skeletal muscle, binds insulin and responds to the hormone in a dose-related fashion as regards glucose transport and metabolism.[8,9]

The objective of the present study was to investigate the receptor binding properties and the biologic activity of biosynthetic human insulin (BHI) in rat liver and mouse soleus muscle in vitro.

MATERIALS AND METHODS

Liver plasma membranes were prepared from adult female Wistar rats according to the method of Neville.[10] Partially purified membranes (Step 11 in ref. 10) were used. Hepatocytes were isolated from adult male Wistar rats by the collagenase method described elsewhere.[7] Hepatocytes were either used immediately after cell isolation or allowed to plate

on collagen-coated culture dishes and used after 24 h of culture in a serum-free Waymouth's medium. Soleus muscles were isolated from male Swiss albino mice as previously described.[8,9]

Insulin binding was measured using ^{125}I-BHI (A14 labeled) and ^{125}I-pork insulin (A14 labeled) from Lilly (Indianapolis), and monocomponent pork insulin from Novo (Gentofte, Denmark), iodinated according to our usual procedure.[5] Insulin biologic activity was measured with BHI and pancreatic human insulin from Lilly and with monocomponent pancreatic pork insulin from Novo.

RESULTS AND DISCUSSION

Insulin binding. The time course of total and nonspecific BHI binding to isolated rat hepatocytes at 20°C is shown in Figure 1. Total BHI binding reached a steady state after 2–3 h of incubation at 20°C; in these conditions, nonspecific binding did not exceed 10% of total binding. Control experiments were run in parallel with two different preparations of ^{125}I-pork insulin tracers. No difference could be detected between the rate and the amount of binding of ^{125}I-BHI and the two ^{125}I-pork insulins (Figure 1).

BHI binding to isolated hepatocytes was also measured under steady-state conditions at 20°C, using ^{125}I-BHI and varying concentrations of unlabeled BHI, and was compared with pork insulin binding using the two different ^{125}I-pork insulin tracers and varying concentrations of unlabeled pork insulin. In Figure 2, the competition curves for ^{125}I-BHI binding and ^{125}I-pork insulin binding are virtually superimposed (upper panel). Scatchard analysis of data (Figure 2, lower panel) confirmed that the binding properties of BHI and pork insulin in isolated rat hepatocytes are indistinguishable.

The binding of ^{125}I-BHI to liver plasma membranes was also found to be virtually indistinguishable from those of the two ^{125}I-pork insulin tracers, with regard to both the time course of total and nonspecific binding (Figure 3) and the inhibition of ^{125}I-hormone binding by unlabeled hormone (Figure 4). These data indicate that BHI binding to liver receptors has the same characteristics as the binding of monocomponent pancreatic pork insulin.

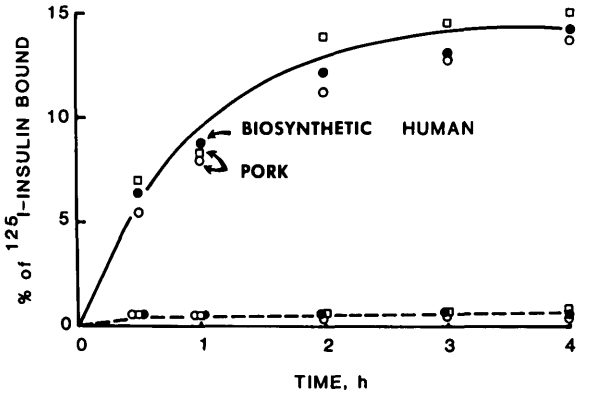

FIG. 1. Time course of ^{125}I-biosynthetic human insulin (BHI) and ^{125}I-pancreatic pork insulin binding to isolated hepatocytes. Hepatocytes (1 × 10^6/ml) were incubated at 20°C with 0.3 ng/ml (50 pM) of ^{125}I-BHI (A14 labeled, 2 ●) or ^{125}I-pork insulin (A14 labeled ○; or iodinated according to ref. 5, □) in the absence (——) or presence (– – –) of 5000 nM unlabeled hormone. At the indicated time points, cells were collected by centrifugation and counted for ^{125}I-radioactivity.

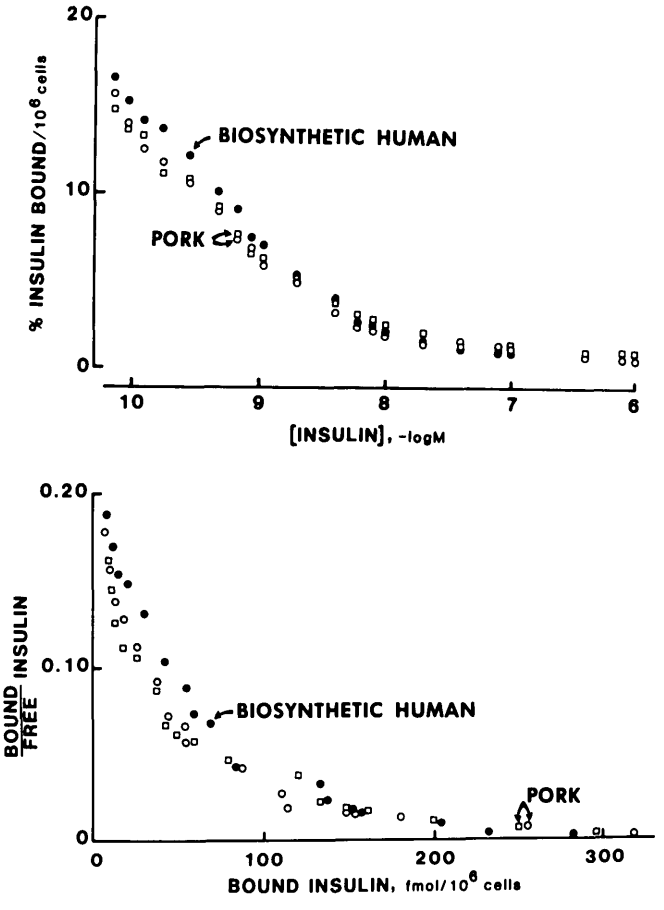

FIG. 2. Binding of BHI and pork insulin to isolated hepatocytes. BHI binding was measured with 0.3 ng/ml of ^{125}I-BHI (A14 labeled: ●) and varying concentrations of unlabeled BHI under steady-state conditions (3 h at 20°C). Control binding experiments were run in parallel with ^{125}I-pork insulin (A14 labeled, ○; or iodinated according to ref. 5, □) and varying concentrations of unlabeled pork insulin. Each point is the mean of three separate experiments. Lower panel: Scatchard analysis of the data.

Insulin biologic activity in hepatocytes. Insulin stimulates amino acid transport (measured with α-amino 1-^{14}C-isobutyric acid) (AIB) in isolated rat hepatocytes by inducing the emergence of newly synthesized carrier proteins.[7] This effect, which specifically involves system A, a Na^+-dependent, concentrative transport system, is maximally expressed after 2–3 h[7] and thus represents a relatively late effect of insulin in liver. The effects of BHI, pancreatic human insulin, and pancreatic pork insulin on AIB transport in isolated hepatocytes were investigated after a 2-h exposure of hepatocytes to varying concentrations of these hormones. As shown in Fig-

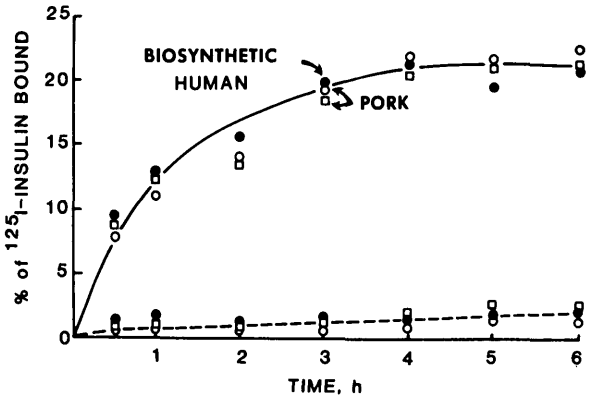

FIG. 3. Time course of ^{125}I-BHI and ^{125}I-pancreatic pork insulin binding to liver plasma membranes. Membranes (0.25 mg protein/ml) were incubated at 20°C with 0.3 ng/ml of each ^{125}I-hormone; all other conditions were identical to those indicated in the legend to Figure 1.

ure 5, the BHI's stimulation of AIB influx was identical to that induced by pancreatic human insulin or by pork insulin: For all three insulins, maximal responses (about a twofold increase above basal) and concentrations producing a half-

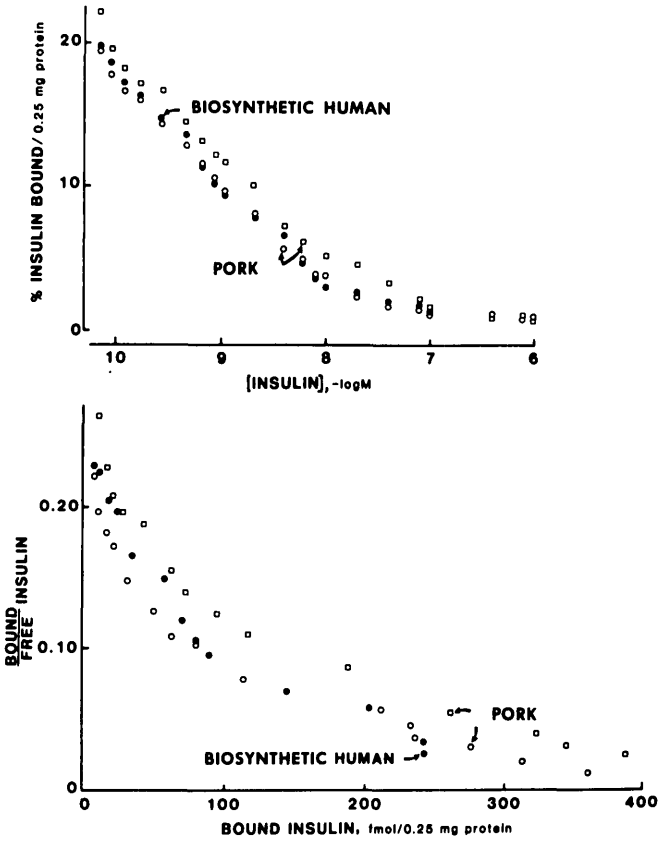

FIG. 4. Binding of BHI and pork insulin to liver plasma membranes. Membranes (0.25 mg protein/ml) were incubated for 4 h at 20°C with 0.3 ng/ml of each ^{125}I-hormone and varying concentrations of the corresponding unlabeled hormone as indicated in the legend to Figure 2. Each point is the mean of three separate experiments. Lower panel: Scatchard analysis of the data.

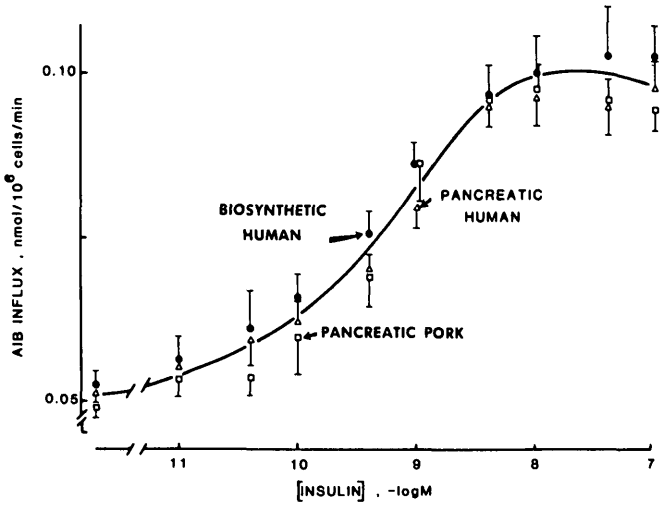

FIG. 5. Effect of BHI, pancreatic human insulin, and pancreatic pork insulin on AIB transport in isolated rat hepatocytes. Hepatocytes (1 × 10^6/ml) were incubated for 2 h at 37°C in the presence of varying concentrations of insulin. At the end of the incubation period, ^{14}C-AIB was added (0.1 μCi, 0.1 mM) and AIB influx was measured in 10-min assays as previously described.[7] Each point is the mean ± SEM of four separate experiments.

maximal response were identical. Thus EC_{50} (nM) were 0.65 ± 0.12, 0.67 ± 0.12, and 0.78 ± 0.15 for BHI, pancreatic human insulin, and pancreatic pork insulin, respectively. All three insulins were also found to stimulate AIB transport in primary cultured hepatocytes with the same intrinsic activity and the same potency (Figure 6).

We have recently reported[6] that in isolated hepatocytes, insulin stimulates (Na^+-K^+)ATPase transport activity, measured by the ouabain-sensitive uptake of $^{86}Rb^+$ (a K^+ tracer). This effect is detectable within 5 min of exposure of hepato-

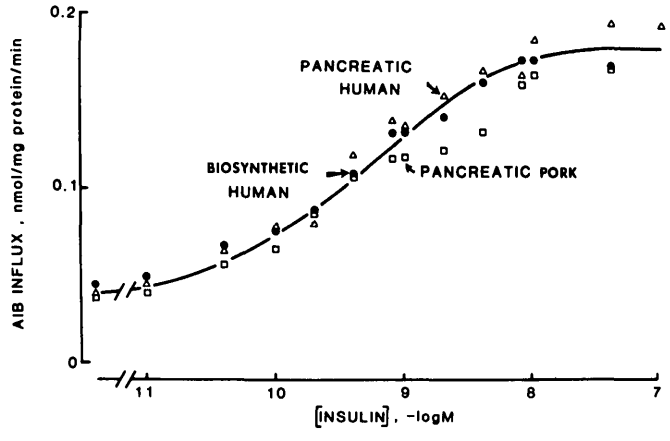

FIG. 6. Effect of BHI, pancreatic human insulin, and pancreatic pork insulin on AIB transport in primary cultures of rat hepatocytes. Hepatocytes (0.7 × 10^6/culture dish) were incubated for 3 h at 37°C in the presence of varying concentrations of insulin. At the end of the incubation period, ^{14}C-AIB was added (0.2 μCi, 0.1 mM) and AIB influx was measured in 5-min assays. Each point is the mean of three separate experiments.

TABLE 1
Effect of biosynthetic human insulin, pancreatic human insulin, and pancreatic pork insulin on (Na^+-K^+)ATPase transport activity in isolated rat hepatocytes

	Ouabain-sensitive Rb^+ uptake, N = 3 (nmol/10^6 cells/60 min)
Basal	11.3 ± 0.4
Biosynthetic human insulin (10^{-7} M)	14.6 ± 0.4
Pancreatic human insulin (10^{-7} M)	14.4 ± 0.3
Pancreatic pork insulin (10^{-7} M)	14.6 ± 0.5

cytes to insulin and is maximal (a 20–25% stimulation above basal activity) after 30 min. In three separate experiments, BHI was found to stimulate Rb^+ uptake by 29%. This was similar to the extent of stimulation of Rb^+ uptake observed with pancreatic human insulin (28%) and with pancreatic pork insulin (29%) (Table 1).

Insulin biologic activity in soleus muscle. As shown in Figure 7, BHI stimulated 2-deoxyglucose uptake (an index of the rate of glucose transport and phosphorylation) to the same extent as pancreatic human insulin or pancreatic pork insulin. Maximal stimulation (a fourfold increase) and insulin concentrations that produced a half-maximal stimulation ($EC_{50} \cong 0.50$ nM) were virtually identical for the three hormones. Similarly, when glucose metabolism was studied (Figure 8), no significant difference could be detected: glycolysis was stimulated by 200% and glycogen synthesis by

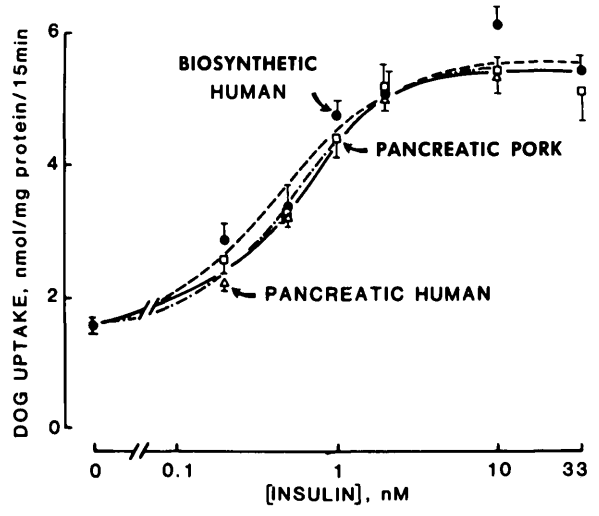

FIG. 7. Effect of BHI, pancreatic human insulin, and pancreatic pork insulin on 2-deoxyglucose uptake in isolated soleus muscle. Muscles were incubated for 60 min in 1 ml of Krebs-Ringer bicarbonate buffer, containing 20 mg/ml defatted bovine albumin and varying concentrations of insulin. ^{14}C-2-Deoxyglucose uptake (0.5 nM) was measured at the end of a 15-min incubation. Each point is the mean ± SEM of 6–10 individual muscles.

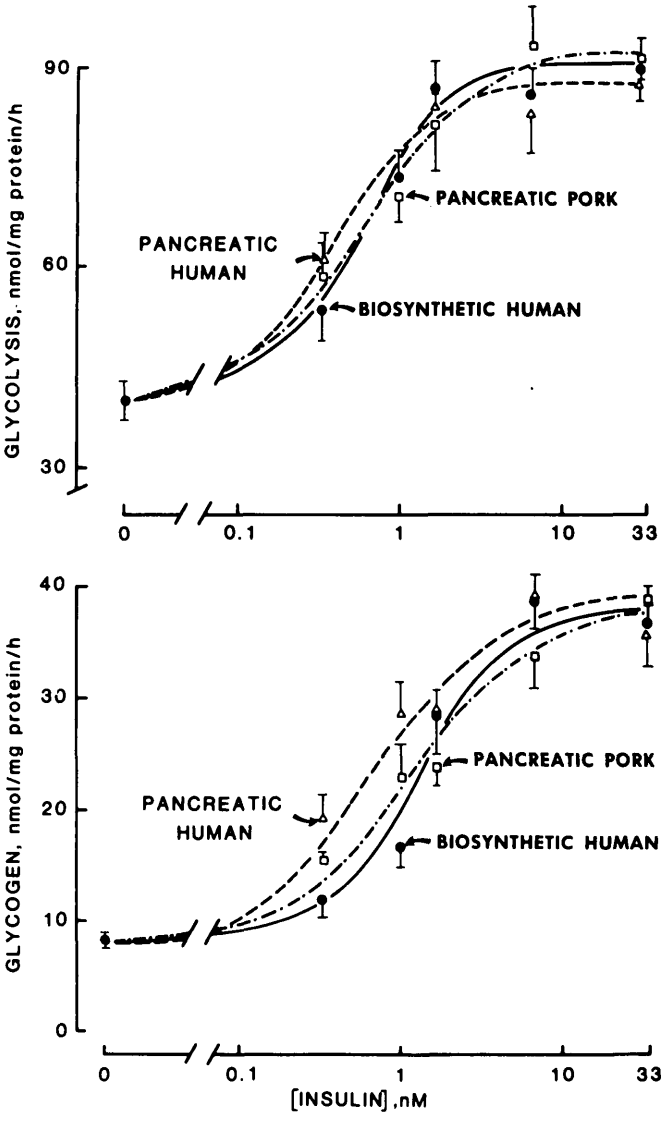

FIG. 8. Effect of BHI, pancreatic human insulin, and pancreatic pork insulin on glucose metabolism in isolated soleus muscle. Muscles were incubated for 60 min in 1 ml of Krebs-Ringer bicarbonate buffer, containing 5-^3H-D-glucose (5 mM) in the presence of varying concentrations of insulin. Data are expressed as nanomoles of glucose converted into ^3H_2O, an index of glycolysis (upper panel), or incorporated into muscle glycogen (lower panel). Each point is the mean ± SEM of 5–12 individual muscles.

450%, while the EC_{50} were $\cong$0.8 nM (glycolysis) and $\cong$1.0 nM (glycogen synthesis) for all three insulins.

CONCLUSIONS

These studies have shown that BHI has the same binding properties as pork insulin in rat liver. Moreover, BHI has the same intrinsic activity (maximal effectiveness) and biologic potency (EC_{50}) as those of pancreatic human insulin and pancreatic

pork insulin in liver and skeletal muscle, two major targets for insulin action.

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