Comparison of Biologic Potency of Human Insulin (recombinant DNA) and Purified Porcine Insulin (PPI) on the Rat and Human Adipocyte Lipogenesis Model

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To assess whether human insulin (recombinant DNA) is superior to porcine insulin, we compared the biologic effect of these two insulin preparations on rat and human adipocyte lipogenesis in vitro. The biologic potency of these two insulin preparations was similar on both human and rat adipocytes. Iodination of human and porcine insulin with ^{125}I at A14 position led to a significant but similar loss of potency of both insulin preparations. DIABETES CARE 5 (SUPPL. 2): 102–103, 1982.

The recent biosynthesis of human insulin is a significant technological advance. Whether its availability will mark an advance in the treatment of diabetes is not clear. We have therefore conducted a series of experiments to establish whether human insulin (recombinant DNA) has a potency greater than that of purified porcine insulin (PPI) on rat and human fat cells. To our knowledge, this is the first report demonstrating the similarity of potency of purified porcine and human insulin on human adipocyte lipogenesis in vitro.

MATERIALS

Analytical grade sodium chloride, sodium hydroxide, sodium hydrogen carbonate, potassium chloride, calcium chloride, sodium dihydrogen phosphate, 4-[2-hydroxyethyl]1-piperazine (Hepes) and glucose were obtained from British Drug House (BDH). Collagenase from Clostridium histolyticym no. 4196 CLS 405239, specific activity 131 units/mg obtained from Millipore Corporation (Freehold, New Jersey). Human serum albumin (crystallized) was obtained from Curewell India. Both labeled and unlabeled human insulin (recombinant DNA) and PPI were gifts of Eli Lilly and Company, Indianapolis, Indiana. ^{3}H-glucose and ^{14}C-glucose were obtained from Radiochemical Centre, Amersham, U.K. Epididymal fat pads were obtained from cesarean-delivered male Sprague-Dawley rats weighing 100–150 g and kept on ad libitum diet (Grain Harvesters, Brand 41B). The composition of Krebs-Ringer bicarbonate buffer (KRB) pH 7.4. for rat adipocytes used in all experiments was as follows in mmol/L: NaCl 121, KCl 4.8, KH_2PO_4 1.2, $MgSO_4$ 1.0, $NaHCO_2$ 25, CaCl 1.3. Bovine serum albumin was present, 1% concentration.

Subcutaneous human abdominal fat was obtained at laparotomy. Adipocytes were prepared and suspended in Hepes buffer containing the following substances (concentration mmol/L): NaCl 135, KCl 4.8, $MgSO_4$ 1.70, $CaCl_2$ 2.5, $NaHPO_4$ 1.0 and Hepes 10; pH was adjusted to 7.4 at 37°C with 1 mmol/L NaOH. Human albumin was present at 10–50 g/L.

INSULIN BIOASSAY

Rat adipocytes were prepared from epididymal fat pads by collagenase digestion in KRB buffer pH 7.4 containing 1% albumin.[1] Human adipocytes were prepared from fat obtained from laparotomy by collagenase digestion in Hepes buffer pH 7.4 containing 25% albumin.[2] In both systems the cells were extensively washed in the appropriate buffer and resuspended in the same buffer. Insulin bioassay was set up according to the method of Moody et al, 1974.[3] Cells were incubated at 4×10^7 cells/L in the presence of two respective insulin preparations at 5–20 mU/L for rat adipocytes and 50–400 mU/L for human adipocytes. Radioactive glucose was added 0.2 μCi/ml in the form D-[3-^{3}H]glucose for rat adipocytes and D-[^{14}C] glucose for human adipocytes. The cells were gassed for 30 s. (O_2 95%, CO_2 5%) and incubated at 37°C in shaking water bath for 2 h. The reaction was terminated by the addition of toluene-based scintillant and the radioactivity in extracted lipid was measured in a Phillips PW 4540 β scintillation spectrometer.[3]

CALCULATIONS

Six incubation bottles containing adipocyte suspensions were used for each concentration of insulin and for basal counts (no insulin added). The six observations for each concen-

TABLE 1
The biologic potency of human and porcine insulin on rat and human adipocyte lipogenesis: percentage increase over basal

Rat adipocytes (mean ± SEM)			Human adipocytes (mean ± SEM)		
Insulin mU/L	Porcine insulin	Human insulin	Insulin mU/L	Porcine insulin	Human insulin
5	161 ± 8	258 ± 5	50	153 ± 6	183 ± 10
10	441 ± 9	418 ± 3	100	203 ± 8	215 ± 9
20	520 ± 9	456 ± 6	200	487 ± 13	431 ± 8
			400	541 ± 9	481 ± 5

tration of insulin were then divided by the mean of the basal counts. The results were finally expressed as percent enhancement over basal ± SEM.

RESULTS

Purified pork insulin and human insulin were equipotent in stimulating lipogenesis in rat adipocytes. Human adipocytes were less sensitive than rat adipocytes to both PPI and human insulin. Both of these insulins were also equipotent in their stimulatory effects on rat and human adipocytes (Table 1).

Monoiodinated derivatives of PPI and human insulin were also equipotent but both were less potent than their respective noniodinated insulins (Figure 1).

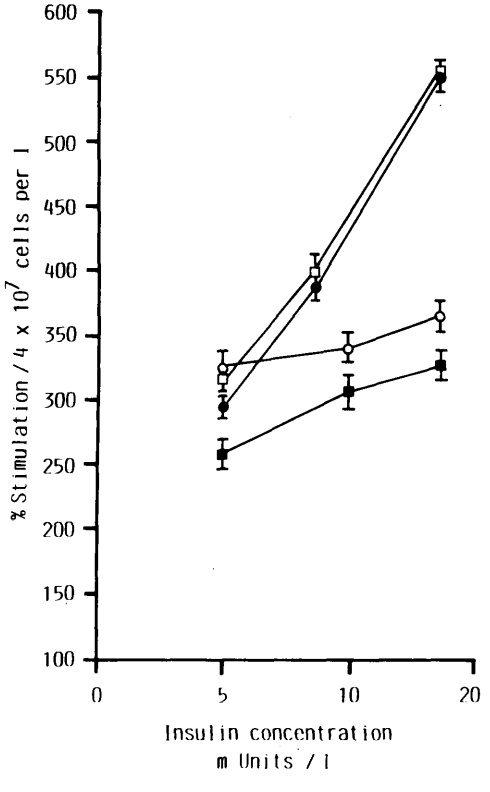

FIG. 1. The biologic potency of human insulin (HI) and PPI and their monoiodinated derivatives, ^{125}I labelled and unlabelled HI on rat adipocyte lipogenesis □—□ *HI;* ●—● *PPI;* ○—○ ^{125}I*-PPI;* ■—■ ^{125}I*-HI.*

CONCLUSION

Human insulin is similar in potency to PPI in the assay systems used in our experiments. These data are consistent with other reports about the hypoglycemic effects of these insulins both in vivo[4,5] and in vitro.[5] Monoiodinated insulins, though biologically active, are less potent than naturally occurring noniodinated insulin. The interpretation of data obtained from experimental work using these derivatives must hence carry reservations.

Thus, since human insulin is similar in potency to PPI in its biologic effects, it would appear not to offer any specific advantage over PPI as far as its biologic action and clinical use are concerned. Any advantages of the use of human insulin in diabetic patients must hence arise from the possibility that it may be less immunogenic than PPI.

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