Different Counterregulatory Responses to Human Insulin (recombinant DNA) and Purified Pork Insulin

KLAUS J. SCHLÜTER, KARL-GEORG PETERSEN, JOACHIM SONTHEIMER, FRANZ ENZMANN, AND LOTHAR KERP

The biologic effect of human insulin (recombinant DNA) and purified pork insulin (PPI) was compared during insulin-induced hypoglycemia at two intravenous dosages 0.075 and 0.1 U/kg body wt in 12 healthy volunteers. Serum insulin concentrations and plasma glucose curves were identical. PPI induced a significantly ($P < 0.05$) higher output of epinephrine, growth hormone, and cortisol at both doses. Less inhibition ($P < 0.05$) of endogenous insulin secretion was observed for human insulin at 0.1 U/kg body wt. An elevated incidence of sweating during hypoglycemia was related to epinephrine secretion. The results indicate that homologous insulin produces in vivo effects which are different from those produced by heterologous insulin. DIABETES CARE 5 (SUPPL. 2): 78–81, 1982.

The aim of this investigation was to compare the effects of intravenous administration of human insulin (recombinant DNA) and purified pork insulin (PPI) in terms of hypoglycemic effect and counterregulatory response. In a previous study, it could be shown that fully synthetic human insulin in comparison to identically formulated pork insulin shows less inhibition of C-peptide secretion and less pronounced output of cortisol and growth hormone during hypoglycemia.[1]

MATERIALS AND METHODS

In 12 healthy subjects the response of neutral regular human insulin (regular-human insulin, Eli Lilly and Company, Indianapolis Indiana), and identically formulated purified pork insulin (Lilly) were compared. Six subjects (22.8 ± 2.6 yr (mean ± SEM); 75.8 ± 5.9 kg; 177 ± 7 cm) received 0.075 U/kg body wt and six (23.9 ± 2.4 yr; 73.9 ± 7.0 kg; 180 ± 6 cm) 0.1 U/kg body wt (i.v. bolus given within 15 sec). The sequence of human and pork insulin administration in each subject was carried out according to a randomization scheme. The tests were performed on two separate days 1 wk apart. All subjects were within ± 15% of ideal body weight and had given their informed consent. They were without family or personal history of diabetes, and with normal laboratory chemistry, ECG, and oral glucose tolerance test (oral glucose load 75 g, mean venous plasma glucose < 5.0 mmol/L after 2 h). Before each test, they were required to fast for 12 ± 1 h, and fluid intake was restricted during this period. For the 3 days before each test, subjects received a carbohydrate-rich diet. On the test day, an i.v. cannula (Abbocath 18-G) was inserted and attached to a saline drip via a tab to facilitate frequent blood sampling. The tests were standardized in that they began at the same time (8:00 a.m.), after a rest period of 30 min, each day. Blood samples for glucose, insulin, C-peptide, growth hormone, cortisol, and catecholamines were drawn at −5, 0, 5, 15, 20, 25, 30, 35, 45, 60, 75, 90, 120 and 180 min following insulin injection; an additional sample for glucose determination was taken at 50 min. All samples were centrifuged and stored at −28°C (except for catecholamines) until assayed.

Plasma glucose was determined with the Beckman Glucose Analyzer (GOD method). The following radioimmunoassays were carried out: serum insulin (Phadebas Insulintest, Pharmacia Diagnostics, AB, Uppsala, Sweden); the cross-reaction of human insulin and PPI with the antibody used in the radioimmunoassay was identical), serum C-peptide (Riamat, C-peptide assay, Byk-Mallinckrodt, Dietzenbach, FRG), serum cortisol (Cortisol-Ria, Travenol, Cambridge, Massachusetts), serum STH (Serono, Freiburg, FRG).

Interassay variations were reduced by using the same immunoassay for all samples of an individual subject. Intra-assay error, measured as coefficient of variation, was below 5.8% in all cases.

Plasma catecholamines were determined by the use of liquid chromatography with electrochemical detection (LCEC).

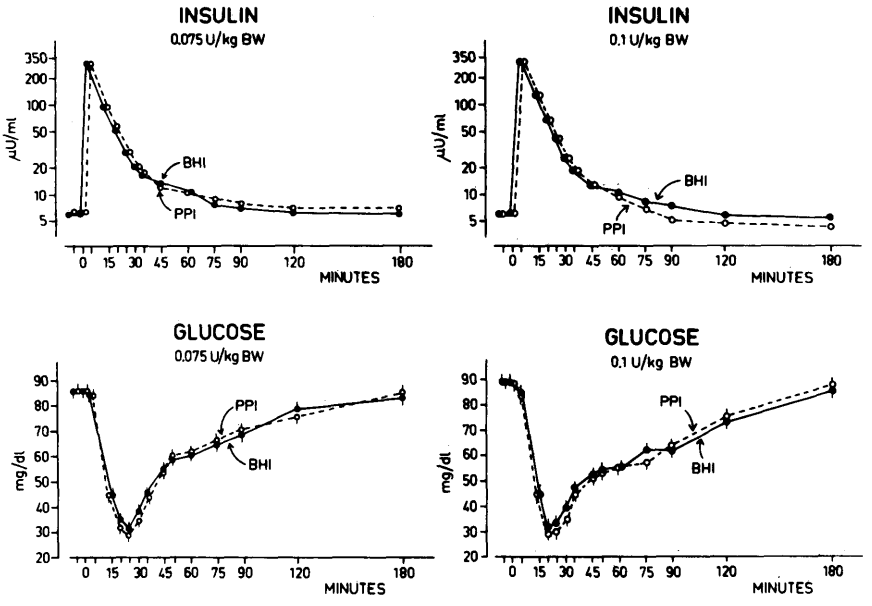

FIG. 1. Serum insulin (μU/ml) and plasma glucose (mg/dl) concentrations after an intravenous bolus (0.075 U/kg body wt or 0.1 U/kg body wt) of human insulin (BHI) and purified pork insulin (PPI) in 12 normal subjects (mean ± SEM).

Before liquid chromatography the catecholamines were preconcentrated by solid extraction onto alumina and then eluted with dilute acid. Details of the method will be published (A. Wehrli, J. Chromatography). Blood samples were collected with 2% of EDTA (90 mg/ml); glutathione (60 mg/ml, pH 6.5) was added, and they were kept frozen at −35°C.

Hypoglycemic symptoms were quantitated by means of a standardized questionnaire using a scale from 0 to 5 to describe the following symptoms: heat, sweating, palpitations, hunger, headache, tremor, jerks, feeling heavy, dizziness, and nausea. A final statement about the degree of symptoms was made at the end of the test.

Results are expressed as mean ± SEM. Areas under the concentration-time curves were calculated according to the equation

$$\sum_{n=1}^{n=N-1} \frac{(x_{n+1} - x_n)\,(y_{n+1} + y_n)}{2}$$

Wilcoxon's test for paired differences was used. Observed differences were judged to be statistically significant when the P value was less than 0.05.

RESULTS

The serum insulin concentrations and the plasma glucose response of the 12 subjects after the intravenous injection of 0.075 U/kg body wt and 0.1 U/kg body wt of neutral human insulin and purified pork insulin are shown in Figure 1. The mean basal fasting serum insulin concentrations ranged from 6.1 to 6.9 μU/ml. Five minutes after insulin injection, identical peak concentrations and identical rates of fall were measured in the two insulin tests. After administration of 0.075 U/kg body wt identical nadirs of plasma glucose (human insulin: 30.7 ± 2.9 mg/dl, PPI: 28.5 ± 1.9 mg/dl) were reached at the same time (human insulin: 24.7 ± 0.9 min, PPI: 25.1 ± 1.2 min) and after 0.1 U/kg body wt the nadirs (human insulin: 31.1 ± 1.5 mg/dl, PPI: 28.0 ± 1.1 mg/dl) were reached significantly ($P < 0.01$) 5 min earlier (human insulin: 18.4 ± 0.8 min, PPI: 19.8 ± 1.8 min). Identical recovery from hypoglycemia was observed for both insulins. Baseline concentrations were reached 3 h after insulin injection.

The counterregulatory output of epinephrine and the concentrations of plasma norepinephrine and plasma dopamine are shown in Figure 2. During hypoglycemia there was a more marked increase of epinephrine secretion after pork than after human insulin (area under plasma concentration/time curves of 0–180 min: 0.075 U/kg: human insulin 21,833 ± 1836 pg/ml/min, PPI 29,826 ± 2420 pg/ml/min, $P < 0.05$; 0.1

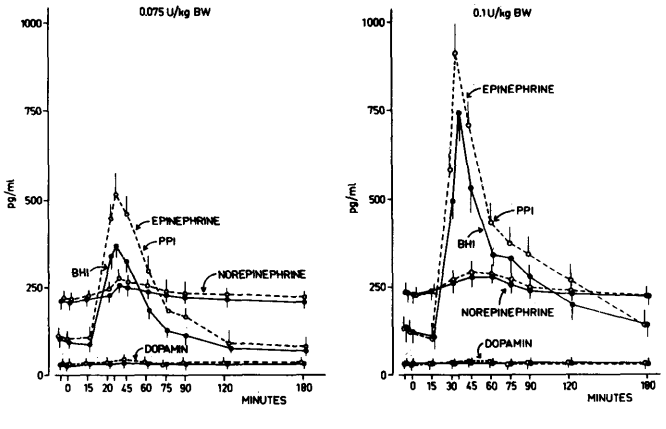

FIG. 2. Plasma epinephrine (pg/ml), plasma norepinephrine (pg/ml) and plasma dopamine (pg/ml) concentrations after an intravenous bolus (0.075 U/kg body wt or 0.1 U/kg body wt) of human insulin (BHI) and purified pork insulin (PPI) in 12 normal subjects (mean ± SEM).

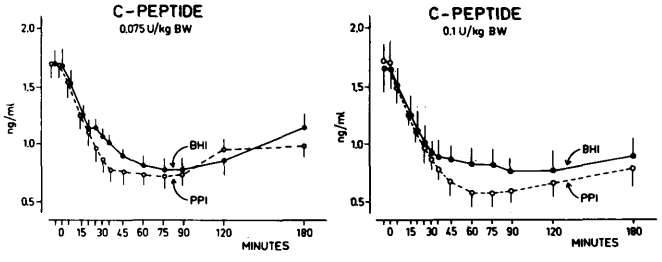

FIG. 3. Serum C-peptide concentrations (ng/ml) after an intravenous bolus (0.075 U/kg body wt or 0.1 U/kg body wt) of human insulin (BHI) and purified pork insulin (PPI) in 12 normal subjects (mean ± SEM).

U/kg: human insulin 64,420 ± 5719 pg/ml/min, PPI 86,951 ± 6814 pg/ml/min, $P < 0.05$). Plasma norepinephrine increased slightly during hypoglycemia, while dopamine remained constant. Differences between BHI and PPI were not observed (Figure 2).

The secretion of endogenous insulin represented by serum C-peptide concentrations (Figure 3) was inhibited by exogenous insulin 5 min after injection. No significant differences were detected following 0.075 U/kg body wt for human insulin and PPI (areas under concentration/time curves of 0–180 min: human insulin 174 ± 8 ng/ml/min, PPI 164 ± 11 ng/ml/min), however, following 0.1 U/kg body wt the inhibitory effect of PPI was significantly ($P < 0.05$) more pronounced (area under concentration/time curves of 0–180 min: human insulin 165 ± 10 ng/ml/min, PPI 130 ± 1.1 ng/ml/min).

Serum cortisol and serum growth hormone concentrations increased 30 min following intravenous insulin injection (Figure 4). The increases of cortisol and growth hormone were significantly ($P < 0.05$) less pronounced after human insulin than after PPI following both dosages (area under concentration/time curves of 0–180 min: growth hormone for 0.075 U/kg body wt: human insulin 2293 ± 177 ng/ml/min, PPI 2598 ± 183 ng/ml/min; for 0.1 U/kg body wt: human insulin 2144 ± 149 ng/ml/min, PPI 2659 ± 191 ng/ml/min; cortisol area for 0.075 U/kg body wt: human insulin 3465 ± 405 ng/ml/min, PPI 4896 ± 587 ng/ml/min; for 0.1 U/kg body wt: human insulin 5949 ± 1205 ng/ml/min, PPI 10687 ± 1453 ng/ml/min).

The hypoglycemic symptoms were recorded with the aid of a standardized questionnaire. Headache, tremor, nausea, and jerks were rarely observed and did therefore not allow statistical evaluation. The symptoms of hunger, palpitations, and feeling heavy exhibited similar frequencies after human and pork insulin.

Sweating, on the other hand, was markedly more pronounced following pork insulin. Estimates of the severity of hypoglycemic symptoms were given by the volunteers at the end of the test periods. Scores (maximum 5) of severity were significantly ($2 P < 0.01$) lower following human insulin (severity score for dosage 0.075 U/kg: human insulin 2.3 ± 0.4, PPI 3.5 ± 0.2; for dosage 0.1 U/kg: human insulin 2.6 ± 0.3, PPI 3.7 ± 0.2).

DISCUSSION

No differences between human insulin and pork insulin could be found for insulin concentrations and glucose response after intravenous administration of 0.075 and 0.1 U/kg body wt. This is in agreement with the findings of Galloway et al.,[2] who used dosages of 0.1 and 0.15 U/kg body wt. Although no differences in insulin concentrations and plasma glucose levels were found, there were significantly higher levels of epi-

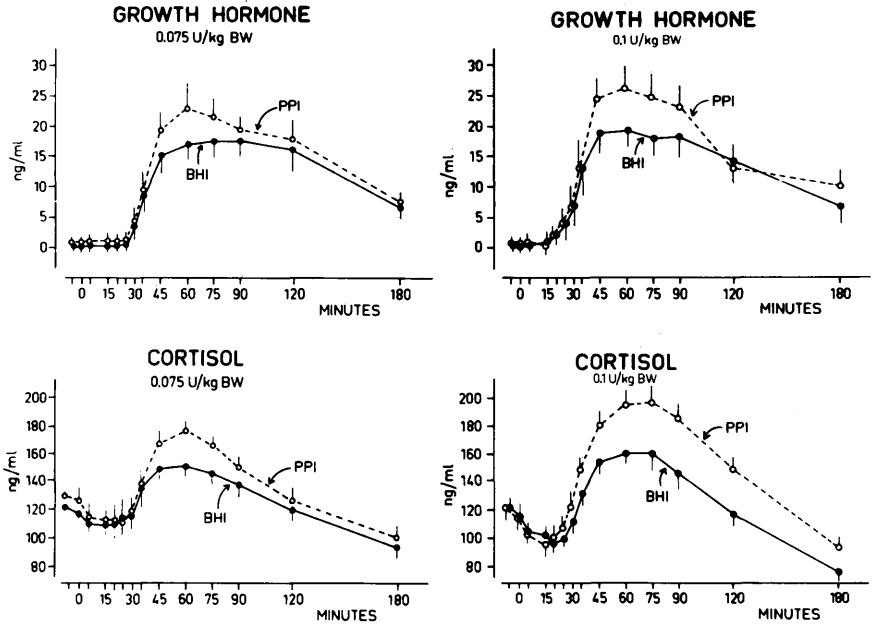

FIG. 4. Serum growth hormone (ng/ml) and cortisol (ng/ml) concentrations after an intravenous bolus (0.075 U/kg body wt or 0.1 U/kg body wt) of human insulin (BHI) and purified pork insulin (PPI) in 12 normal subjects (mean ± SEM).

nephrine secretion following for both dosages. These differences correlated with those found for severity of hypoglycemic symptoms. The acute counterregulation is primarily influenced by glucagon and catecholamine output. The plasma glucagon was not measured because the radioimmunoassay is not sensitive enough to detect differences in this range (coefficent of variation in glucagon radioimmunoassay is about 20%).

It is thus possible that the different secretion rates of cortisol and growth hormone are produced by different levels of epinephrine observed. The secretion of cortisol and growth hormone occurs at 30–35 min and is not responsible for the acute recovery of plasma glucose, which occurs after 25 min (0.075 U/kg body wt) or 20 min (0.1 U/kg body wt). On the other hand, it is possible that insulin acts directly on the central nervous system. If this is so, then our results indicate that pork insulin would have a stronger effect. Further evidence would be if, under euglycemic conditions achieved during clamp studies, this high insulin dosage (0.1 U/kg body wt) produced, independent of epinephrine secretion, a more pronounced increase of cortisol and growth hormone after pork insulin. Such a study was carried out and the differences were found.[3]

Epinephrine has been reported to inhibit endogenous insulin secretion.[4] The C-peptide levels observed in this study are not consistent with the hypothesis that the inhibition of C-peptide secretion is caused by the output of epinephrine since the differences between human and pork insulin are observed earlier. The pronounced suppression of endogenous insulin secretion is not necessarily caused by an interaction of exogenous insulin and the islets of Langerhans. This may well be the consequence of differences in sympathetic nerve activity that occur prior to epinephrine secretion.

ACKNOWLEDGMENTS: The authors are grateful to Heike H. Vorwerck for the radioimmunoassay.

From Abtl. für Klinische Endokrinologie der Medizinischen Universitätsklinik Freiburg i. Br. (K. J. S., K.-G. P., J. S., L. K.) and Bad Homburg, (F. E.) FRG.

Address reprint request to Dr. K. J. Schlüter, Medizinische Universitätsklinik Freiburg i. Br., Hugstetter Str. 55, D-7800 Freiburg i. Br., FRG.

REFERENCES

[1] Schlüter, K. J., Petersen, K.-G., Borsche, A., Hobitz, H., and Kerp, L.: Effects of fully synthetic human insulin in comparison to porcine insulin in normal subjects. Horm. Metab. Res. *13*: 657–59, 1981.

[2] Galloway, J. A., Spradlin, C. T., Root, M. A., and Fineberg, S. E.: The plasma glucose response of normal fasting subjects to neutral regular and NPH biosynthetic human and purified pork insulins. Diabetes Care *4*: 183–88, 1981.

[3] Schlüter, K. J., Petersen, K.-G., Enzmann, F., and Kerp, L.: Different potencies of biosynthetic human insulin and purified porcine insulin. Horm. Metab. Res. In press.

[4] Porte, D.: A receptor mechanism for the inhibition of insulin release by epinephrine in man. J. Clin. Invest. *46*: 86–94, 1967.