Comparative Studies on Intermediary Metabolism and Hormonal Counterregulation Following Human Insulin (recombinant DNA) and Purified Pork Insulin in Man

C. ROSAK, P.-H. ALTHOFF, F. ENZMANN, AND K. SCHÖFFLING

Human insulin (recombinant DNA) and purified pork insulin (PPI) were administered intravenously at a dosage of 0.075 U/kg in eight healthy men. Both insulins exerted the same hypoglycemic effect with the same restoration pattern to normal glucose levels at the end of the test. Differences were found with respect to a stronger antilipolytic and antiketogenic effect of human insulin; also the reactive rise of both compounds at the end of the test is less under human insulin in comparison with PPI. In spite of the same glucose nadir, the pattern of hormonal counterregulation is different under human insulin in comparison with PPI. There was less epinephrine and glucagon and practically no prolactin secretion following human insulin. Growth hormone secretion is augmented under human insulin. The clinical significance of these results under long-term treatment with human insulin has to be assessed.
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Insulin with the amino acid sequence of human insulin synthesized by *Escherichia coli* bacteria via A- and B-chain coupling has been introduced recently into the therapy of diabetes mellitus. It offers the possibility of using the homologous insulin in the treatment of the disease. Some of its biologic and immunologic properties have been described.[1]

Lowering of blood glucose in man by insulin application induces a sequence of metabolic and hormonal events and consequently a rise of the lowered blood glucose level.[2–5] The pattern of counterregulation mainly is dependent on the depth and rapidity of blood glucose decrease;[6] however, the metabolic and hormonal changes induced by the hormone insulin itself are not yet thoroughly defined.

The present study was conducted to characterize the effect of intravenously applied human insulin (recombinant DNA) and purified pork insulin (PPI) on a number of different metabolic and hormonal parameters measured simultaneously and to compare the specific pattern of hormonal and metabolic counterregulation as induced by both insulin preparations.

MATERIALS AND METHODS

Eight healthy male volunteers free of metabolic and endocrine disorders were investigated in this study after written consent was obtained. Experiments were performed in the fasting state in the morning between 8 and 9 a.m., food having been withheld 12 h before. An indwelling intravenous catheter was placed into the antecubital vein 30 min before the first blood sample was drawn. At 0 min, the intravenous injection of 0.075 U/kg of either human insulin or PPI was performed.

If one does perform the insulin test from an endocrine aspect, such as testing the function of the anterior pituitary or the adrenal gland, a maximal stimulus on lowering the blood glucose level has to be exerted and the usual dosage is between 0.2 and 0.15 U/kg. In the present study, we have aimed to objectify even discrete differences of both insulins, which is why we have chosen the rather submaximal dosage of 0.075 U/kg.

Blood samples were drawn at the time points 0, 15, 30, 45, 60, 90, 120, and 180 min for determination of glucose, lactate, free fatty acids, β-hydroxybutyric acid, potassium,

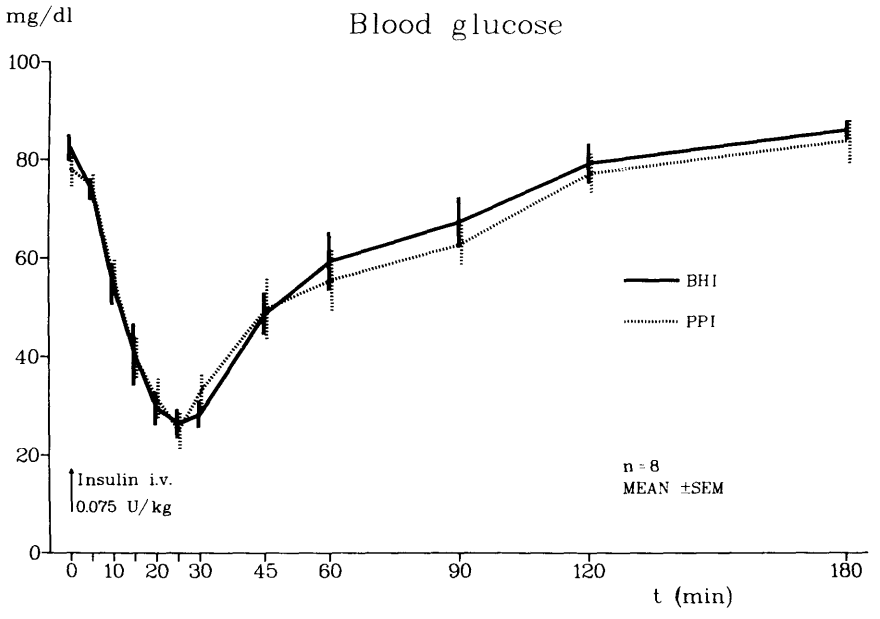

FIG. 1. Blood glucose levels in eight healthy volunteers following 0.075 U/kg of human insulin (BHI) and PPI, respectively.

phosphate, epinephrine, norepinephrine, glucagon, cortisol, growth hormone, and prolactin. For blood glucose determination, a 5-min interval has been performed during the first 45 min. The blood samples were then prepared according to the individual methodologic procedures. If demanded the blood samples were transferred immediately into prechilled tubes with appropriate ingredients, centrifuged in cold, and worked off until plasma or column effluent could be stored at −20°C.

RESULTS

The concentration changes of the parameters investigated are expressed in Figures 1–12.

With respect to the blood glucose levels, the nadir following both preparations is, at 25 min, 25.1 ± 3.7 mg/dl for PPI and 26.3 ± 2.9 mg/dl for human insulin (Figure 1). Starting point levels of free fatty acids are 0.501 ± 0.082 mmol/L for PPI and 0.523 ± 0.069 mmol/L for human in-

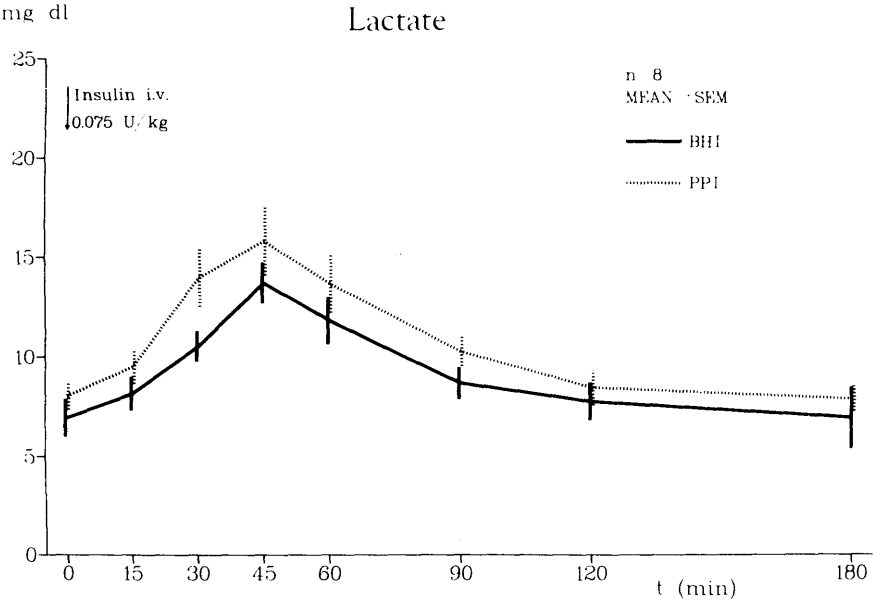

FIG. 2. Lactate serum concentration in eight healthy volunteers following 0.075 U/kg of human insulin (BHI) and PPI, respectively.

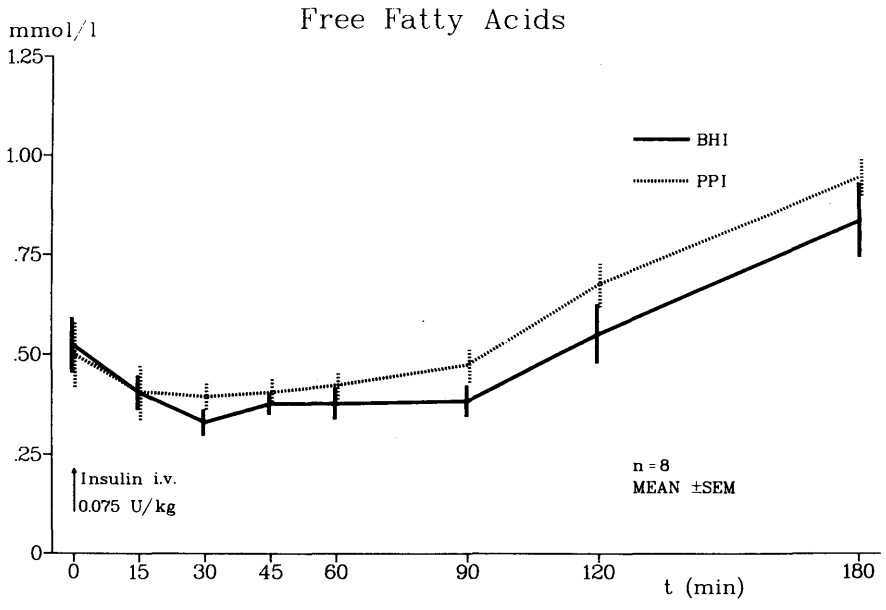

FIG. 3. Free fatty acid serum concentration in eight healthy volunteers following 0.075 U/kg of human insulin (BHI) and PPI, respectively.

sulin. After 30 min, a depression to 0.393 ± 0.031 mmol/L for PPI and 0.328 ± 0.031 mmol/L for human insulin is detected. At the end of the test, again different levels can be observed: 0.950 ± 0.046 mmol/L for PPI and 0.840 ± 0.093 mmol/L for human insulin. In good correlation are the β-hydroxybutyric acid levels at the end of the test with 2.7 ± 0.8 mg/dl for PPI and 2.1 ± 0.5 mg/dl for human insulin (Figure 4).

The peak levels of epinephrine at 30 min differ with 470 ± 73 ng/L for PPI and 420 ± 72 ng/L for human insulin. Following PPI, the decrease is much slower with 405 ± 109 ng/L for PPI and 273 ± 24 ng/L for human insulin at 45 min. A similar pattern can be observed for the glucagon levels (Figure 10). The 30-min concentration following PPI was 0.187 ± 0.017 ng/ml and following human insulin was 0.198 ± 0.038 ng/ml. The 45-min and 60-min serum concentrations are 0.210 ± 0.034 ng/ml and 0.147 ± 0.020 ng/ml, respectively, for PPI, and 0.174 ± 0.031 ng/ml and 0.141 ± 0.010, respectively, for human insulin.

The main differences are found in the secretion pattern of the hypophyseal hormones growth hormone and prolactin. Growth hormone is secreted to a minor degree following PPI,

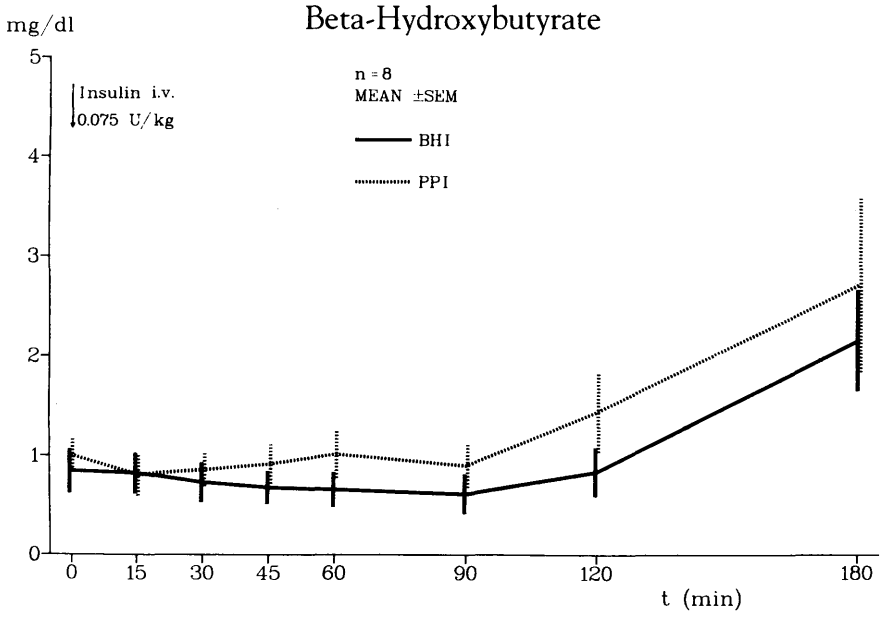

FIG. 4. β-hydroxybutyric acid serum concentration in eight healthy volunteers following 0.075 U/kg of human insulin (BHI) and PPI, respectively.

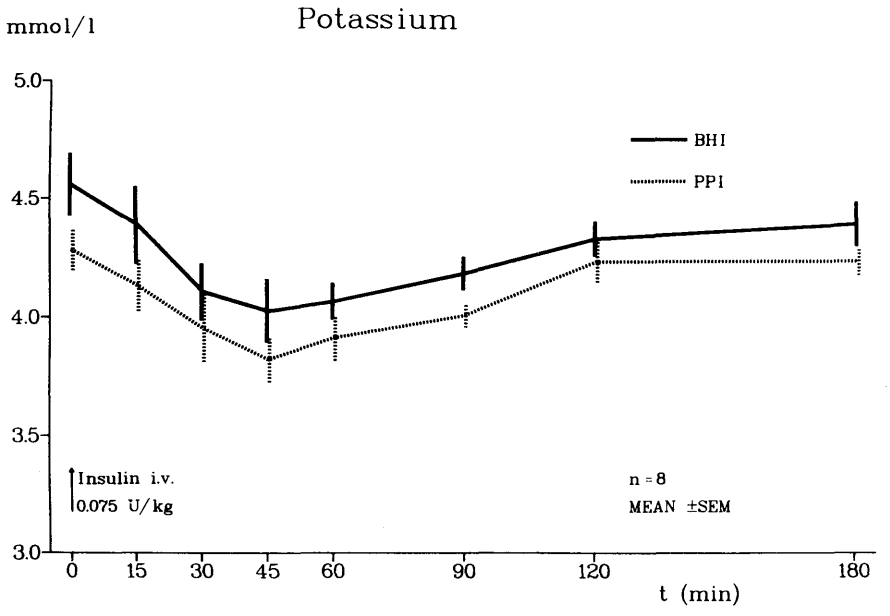

FIG. 5. Potassium levels in eight healthy volunteers following 0.075 U/kg of human insulin (BHI) and PPI, respectively.

the 60-, 90-, and 120-min serum concentrations of 27.6 ± 5.0 ng/ml, 24.5 ± 4.8 ng/ml and 13.1 ± 4.5 ng/ml in comparison with the serum concentrations following human insulin of 37.8 ± 9.7 ng/ml, 30.0 ± 6.4 ng/ml and 20.3 ± 3.8 ng/ml at the same time points (Figure 11). Regarding the prolactin levels following PPI, an increase of prolactin from 7.3 ± 0.7 ng/ml to a peak level of 13.7 ± 0.8 ng/ml at 45 min can be observed. Following human insulin, no increase of prolactin over starting point levels at any time of the test is observed (Figure 12).

DISCUSSION

The chronological sequence induced by the insulin bolus is the association of insulin to specific receptors on the cell membranes of the different target tissues and activation or blockade of those to the receptor linked enzyme systems. The result is lowering of blood glucose via stimulation of glucose utilization and inhibition of glucose production, antilipolysis, inhibition of ketogenesis as well as lowering of potassium and phosphate.

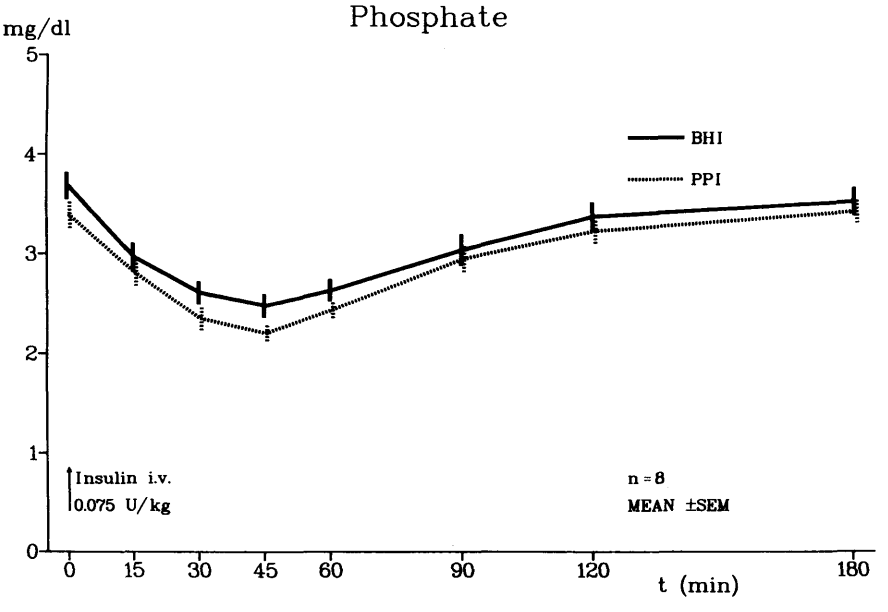

FIG. 6. Phosphate levels in eight healthy volunteers following 0.075 U/kg of human insulin (BHI) and PPI, respectively.

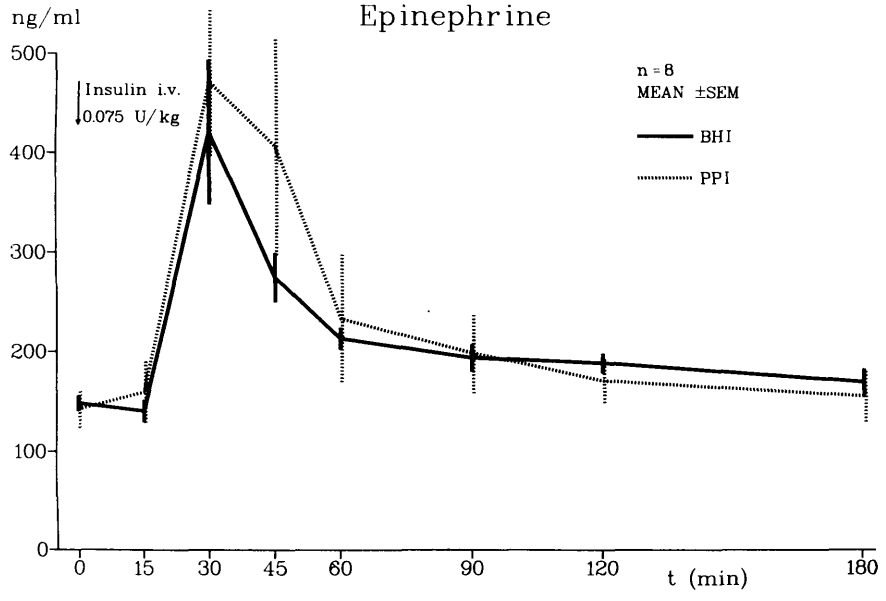

FIG. 7. Epinephrine serum concentration in eight healthy volunteers following 0.075 U/kg of human insulin (BHI) and PPI, respectively.

Influences on other substrates, such as amino acids, have not been investigated in the present study.

Our data show at the same hypoglycemic effect of both insulins, a more pronounced antilipolytic and antiketogenic effect of human insulin in comparison with PPI with less counterregulatory rise of both parameters at the end of the test. Similar results with respect to a more antiketogenic effect by human insulin have been demonstrated recently.[7] If these effects are due to a different influence of human insulin on enzyme systems regulating free fatty acid levels and ketone body production cannot be concluded from the presented data.

The induction of the hormonal counterregulation is triggered by glucose-sensitive areas in the hypothalamus. The different reaction of the sympathetic nervous system and the hypophyseal hormones prolactin and growth hormone to the same glucose nadir and recovery kinetics is difficult to understand. The hypothalamus is involved in the regulation of prolactin and growth hormone secretion, producing the prolactin inhibiting factor PIF, which is supposed to be dopa-

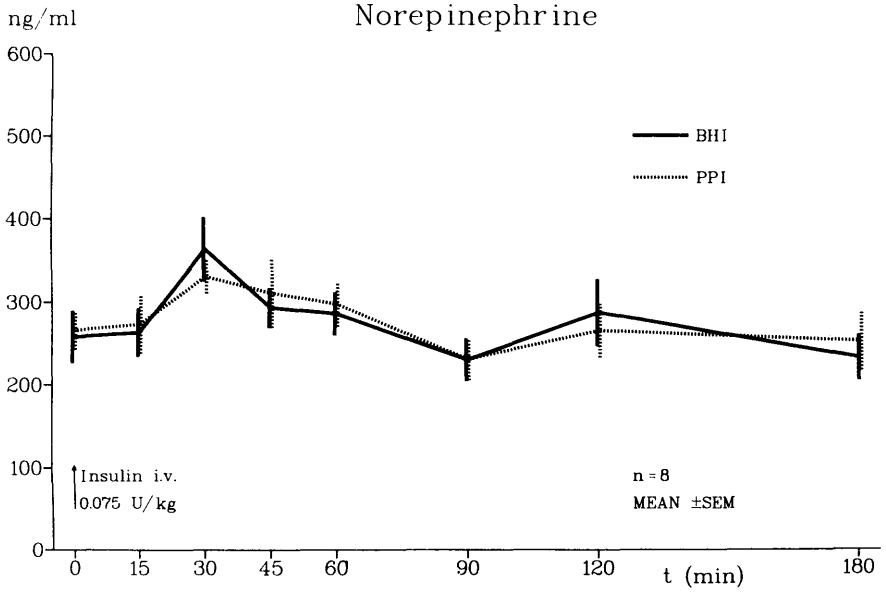

FIG. 8. Norepinephrine serum concentration in eight healthy volunteers following 0.075 U/kg of human insulin (BHI) and PPI, respectively.

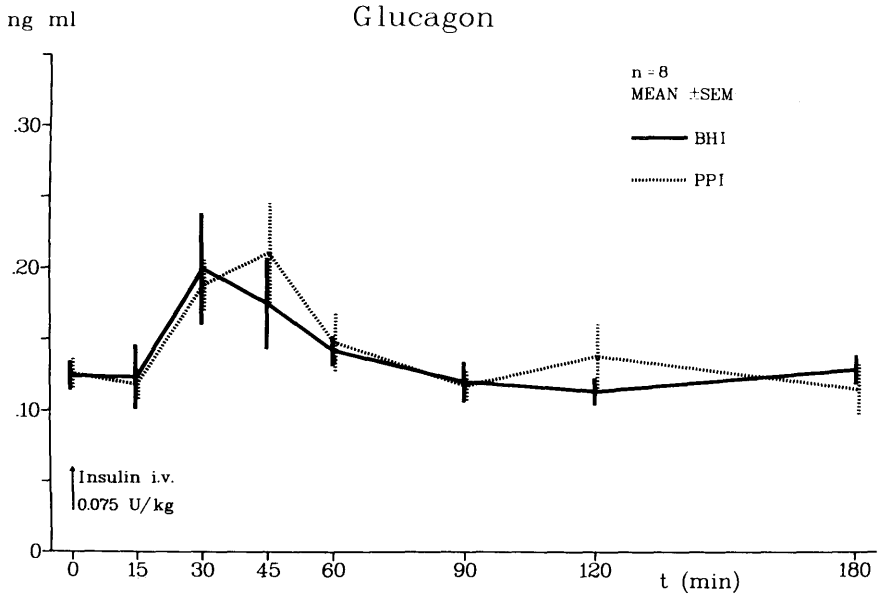

FIG. 9. Glucagon levels in eight healthy volunteers following 0.075 U/kg of human insulin (BHI) and PPI, respectively.

mine.[8–10] Central dopamine stimulation reduces prolactin secretion and on the other hand enhances growth hormone secretion. Our hypothesis to explain the presented data is by a different stimulation of the central dopaminergic system by human insulin and PPI, respectively, with the consequence of a different hypophyseal hormone secretion pattern. In good correlation with the hormone results are the reports of our volunteers that the side effects of hypoglycemia such as sweating, blurred vision, and palpitation have been less expressed under human insulin in comparison with PPI. The same observation has been reported by some of our diabetic patients under treatment with human insulin.

The clinical significance of the absence of prolactin secretion and the described differences of free fatty acid and β-hydroxybutyric acid levels following human insulin induced hypoglycemia are difficult to assess at present. Prolactin possibly has diabetogenic potency[11–13] and is secreted after different stress applications.[14] On the other hand the half-period of free fatty acids and ketone bodies is within minutes and again there are at present no data available on the re-

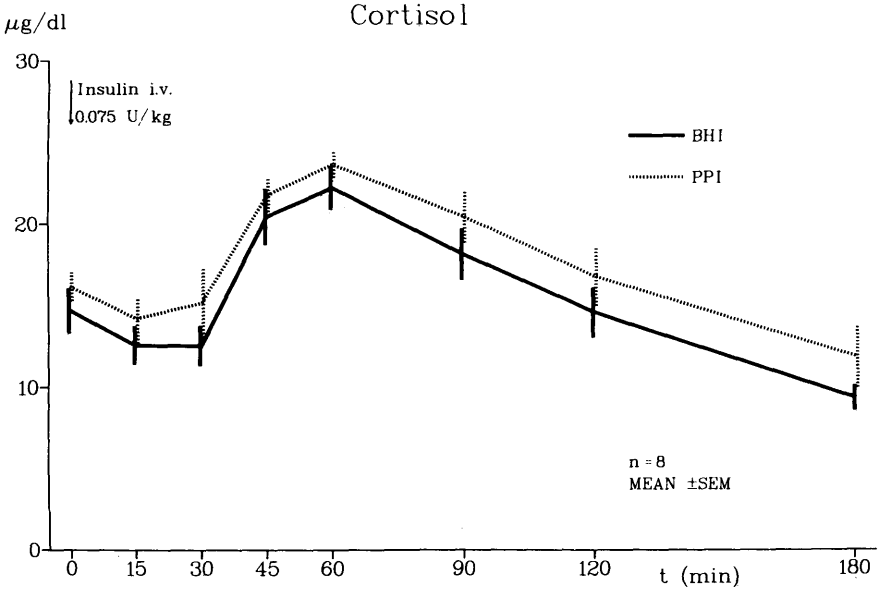

FIG. 10. Cortisol serum concentration in eight healthy volunteers following 0.075 U/kg of human insulin (BHI) and PPI, respectively.

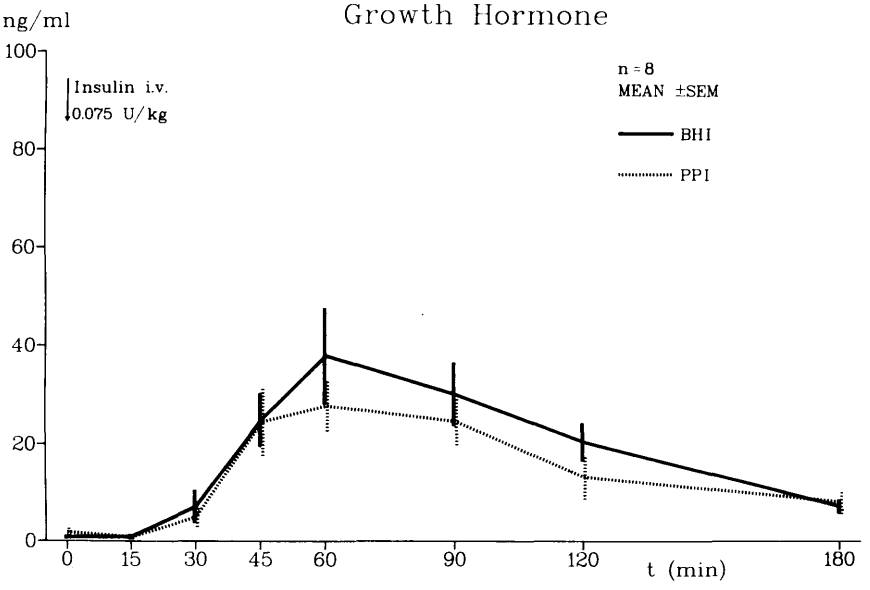

FIG. 11. Growth hormone levels in eight healthy volunteers following 0.075 U/kg of human insulin (BHI) and PPI, respectively.

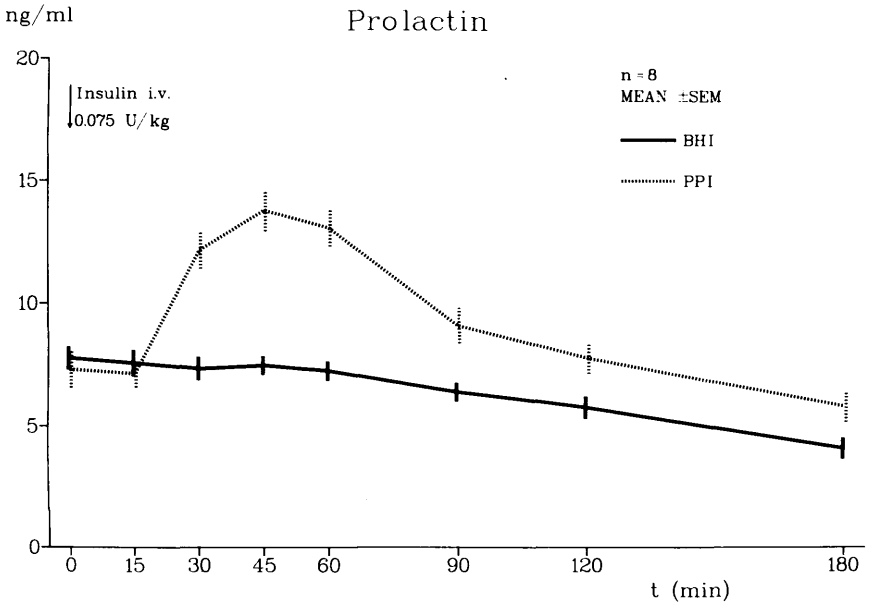

FIG. 12. Prolactin levels in eight healthy volunteers following 0.075 U/kg of human insulin (BHI) and PPI, respectively.

lation of changes in serum concentrations in the above mentioned range of those parameters and a better management of diabetes mellitus. Prospective studies during long-term application will be necessary to further elucidate the significance of these effects in the diabetic patient.

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From the Department of Endocrinology, Center Internal Medicine, Johann Wolfgang Goethe University, 6 Frankfurt am Main 70, Germany.

Address reprint requests to C. Rosak at the above address.

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