Pharmacodynamics of Human Insulin (recombinant DNA)—Regular, NPH, and Mixtures—Obtained by the Gerritzen Method in Healthy Volunteers

KURT WEINGES, MANFRED EHRHARDT, GERFRIED NELL, AND FRANZ ENZMANN

The study was concerned with the comparison of the action profile of regular and NPH preparations of human insulin (recombinant DNA) and of PPI (pork purified insulin). In addition, the action profiles of some mixtures (10:90, 15:85, 20:80, 25:75, 30:70) of regular and NPH human insulin were evaluated. The comparisons were based on the Gerritzen test. There was no statistically significant difference in the time-course of the blood glucose levels after administration of NPH human insulin and NPH PPI. A tendency was noted, however, that NPH human insulin has a faster onset of action and that the serum glucose minimum for NPH human insulin lasts longer. The serum glucose curves after the application of regular and NPH human insulin initially lie closer together than after the respective PPI preparations. In the later phase, regular human insulin interferes less with the NPH curves. This means that combinations of regular and NPH human insulin may have a clinically useful action profile. No skin reaction or other adverse reaction was detected after application of human insulin and no antibodies against human insulin and *Escherichia coli* protein were found. DIABETES CARE 5 (SUPPL. 2): 67–70, 1982.

In the last years, progress in recombinant DNA technology[1] allowed the production of human insulin biosynthetically on an industrial scale. Preliminary clinical evaluations have been published.[2–5] In particular, it has been shown that the time-course of the action of regular human insulin and PPI on serum glucose levels did not differ significantly.[2] However, there was a tendency for human insulin to lower the blood glucose level somewhat faster during the first 30 min and, during the later phase, somewhat less than pork insulin. In extension of these investigations in this study, the effect of NPH human insulin and NPH PPI preparations on the time-course of serum levels of glucose was compared. Since it is customary to administer fixed-ratio intermediate insulins (in Germany in 65% of the patients), the effect of several mixtures of regular human insulin and NPH human insulin (10:90, 15:85, 20:80, 25:75, 30:70) on serum glucose level was investigated and compared with the action profile of a well-known depot insulin (Hoechst-Depot CS). In some experiments, the effect on the time-course of serum levels of insulin and C-peptide was also measured. These investigations have been carried out using the Gerritzen test.[6] This is a standardized method for the evaluation of the action profile of insulin in man.

MATERIALS AND METHODS

Seventeen healthy male volunteers, aged 22–41 yr, were studied. All signed consent forms and were investigated according to the protocol outlined below, which had been approved by the ethical committee of the Medical Faculty, University of the Saarland. All volunteers were within 10% of their ideal body weight. Before admission, a health assessment program focused on blood count, creatinine, urea, GOT, GPT, γ-GT, cholesterol, triglycerides, and electrolytes. In addition, before the first application of human insulin and 4 wk after the clinical investigation, serum samples were collected to assess the presence of *Escherichia coli* protein or insulin antibodies. An intradermal skin tolerance test was carried out as described by Weinges et al.[2]

The Gerritzen test was performed in the following manner. The volunteers came to the metabolic ward the evening before the test and fasted there overnight. Beginning at 6:00 a.m., they received 10 g of carbohydrate in the form of two Leibniz cookies every hour and water ad libitum until 4:00 p.m. (after application of the regular insulin preparations) or until 6:00 a.m. of the following day (after administration of the NPH preparations and the mixtures of regular and NPH insulin). At 8:00 a.m., the insulins were administered subcutaneously. The insulin dose was either 10 IU for regular

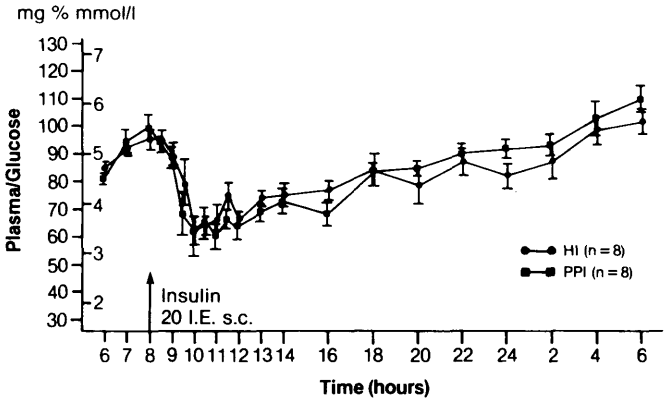

FIG. 1. Mean plasma glucose response of 8 healthy volunteers after subcutaneous administration of 20 U NPH human insulin or NPH purified pork insulin under Gerritzen test conditions. The bars represent standard errors of the mean.

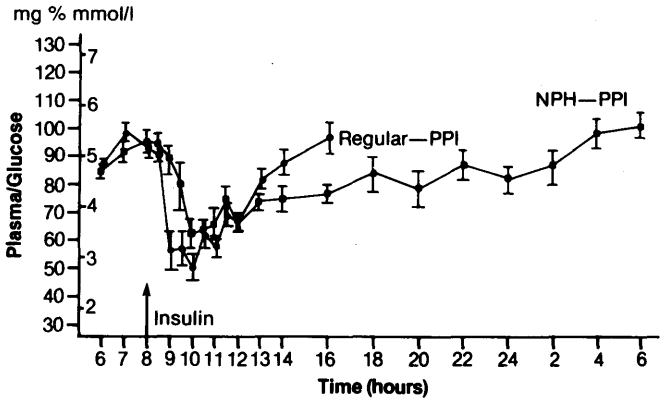

FIG. 3. Mean plasma glucose response of 8 healthy volunteers after subcutaneous administration of regular or NPH pork insulins under Gerritzen test conditions. The bars represent standard errors of the mean.

or 20 IU for intermediate- and long-acting preparations. Blood sugar was measured at 6:00, 7:00, and 8:00 a.m., then every 30 min from 8:00 to noon, then at 1:00 and 2:00 p.m., and every 2 h from 2:00 p.m. to 6:00 a.m. In the case of the application of regular insulin, the trial was finished at 4:00 p.m.

The serum levels of insulin and C-peptide were measured after the administration of mixtures of regular and NPH human insulin (20:80, 25:75, 30:70) and Depot Hoechst CS every 30 min from 8:00 until 12:00 a.m. Serum glucose levels were measured according to the glucose-dehydrogenase method (Merck, Darmstadt, FRG), serum C-peptide by the radioimmunoassay RIAMAT-C-peptide (Byk Mallinckrodt, Steinberg, FRG), and serum insulin by radioimmunoassay Phadebas (Deutsche Pharmazia, Frankfurt/Main, FRG). The experiments were performed in randomized crossover order. Each insulin was tested in four or five volunteers on each day of experimentation. Between each trial, there was an interval of at least 1 wk. The results are presented as means (N = 8–9) ± the standard error of the mean (in some cases).

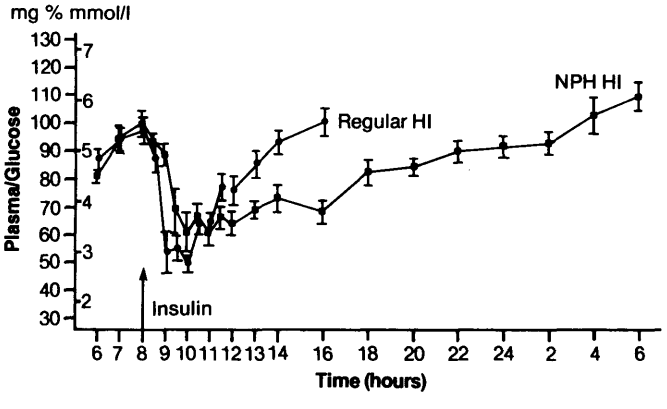

FIG. 2. Mean plasma glucose response of 8 healthy volunteers after subcutaneous administration of regular or NPH human insulins under Gerritzen test conditions. The bars represent standard errors of the mean.

The statistical evaluation was carried out using the paired *t* test.

RESULTS

During all the Gerritzen test periods, not a single skin reaction nor any other adverse effects had been observed. No antibodies against human insulin or *E. coli* protein were detected.

Figure 1 shows a comparison of the effects of NPH human insulin and NPH PPI on the time course of serum glucose levels. There are no statistically significant differences between the two curves.

In regard to a combination therapy with regular and NPH insulins, the effect of the respective preparations of human insulin and PPI were compared. Figure 2 shows the action of regular human insulin and NPH human insulin and Figure 3 that of the corresponding PPI preparations. As described by Weinges et al.[2] and as already shown in Figure 1, there is a tendency for both human insulin preparations toward a faster onset of action. The action of regular human insulin ceases somewhat earlier than the effect of regular PPI. The glucose minimum for NPH human insulin lasts longer than that of NPH PPI. In summary, initially both curves of serum glucose for the human insulin preparation lie closer together and in the later phase human insulin interferes less with the NPH curve.

It was therefore of interest to study the effect of combinations of regular and NPH human insulin on the time-course of serum glucose. First, it was tested which combination would mimic the action profile of that depot insulin which is most widely used in Germany, namely Hoechst Depot CS (Figure 4).

The onset of action of the combinations is similar to depot CS. However, over the next 12 h, the human insulin combinations develop a more pronounced blood sugar lowering effect than depot CS. The differences between the three combinations are statistically not significant during the same

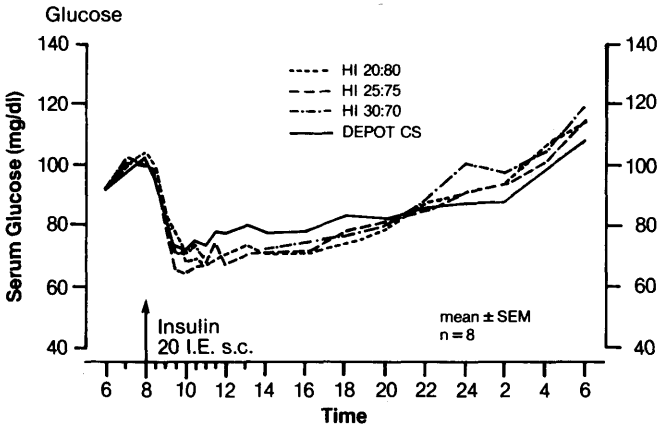

FIG. 4. Mean plasma glucose response of 8 healthy volunteers after subcutaneous administration of 20 U of three mixtures of regular and NPH human insulins (20:80, 25:75, 30:70) and of Depot CS insulin under Gerritzen test conditions.

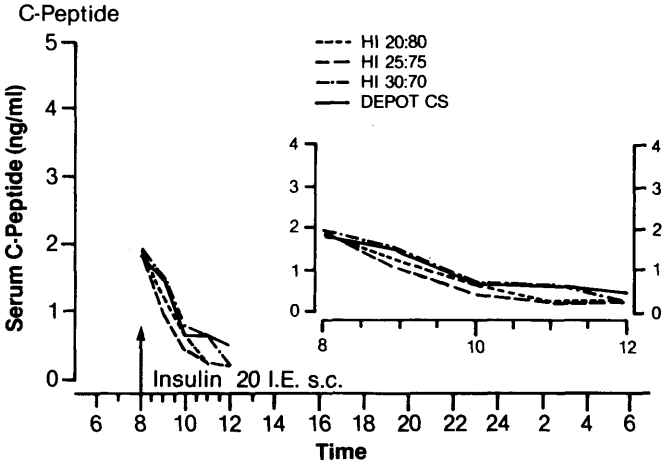

FIG. 6. C-peptide levels corresponding to the plasma glucose curves shown in Figure 4 and serum insulin levels shown in Figure 5.

period. There is some difference in the duration of the effects. The action of 30:70 fades after 16 h and 25:75 and 20:80 work for about 20 h, whereas depot CS works longest and still strongest during the last hours. Figure 5 shows that the behavior of the serum glucose levels during the first 4 h is reflected by the serum insulin levels. Human insulin provides higher insulin levels within a shorter time, especially the combination 30:70.

Figure 6 shows that, although the volunteers ate carbohydrates, the endogenous insulin secretion is suppressed and does not interfere with the exogenous insulin. The time courses of serum C-peptide are statistically not different.

To mimic the action profile of the reference depot insulin as close as possible, combinations with less regular insulin were investigated. The effect of the mixtures 10:90, 15:85, and 20:80 is shown in Figure 7. Even the admixing of only 10% regular human insulin speeds up the onset of action of NPH human insulin. The duration of action for these combinations lies between 18 and 19 h.

DISCUSSION

It has been shown (e.g., by Weinges et al.[2]) that the action of regular human insulin is comparable to the effect of regular PPI on serum glucose levels but somewhat faster in onset and with a tendency of less action in the late phase. In this study, it was demonstrated that NPH human insulin also exhibits a faster onset of action on blood glucose levels than NPH PPI and the minimum of serum glucose lasts longer after NPH human insulin application. Though these differences were statistically not significant, it seems that the time curves of serum glucose of

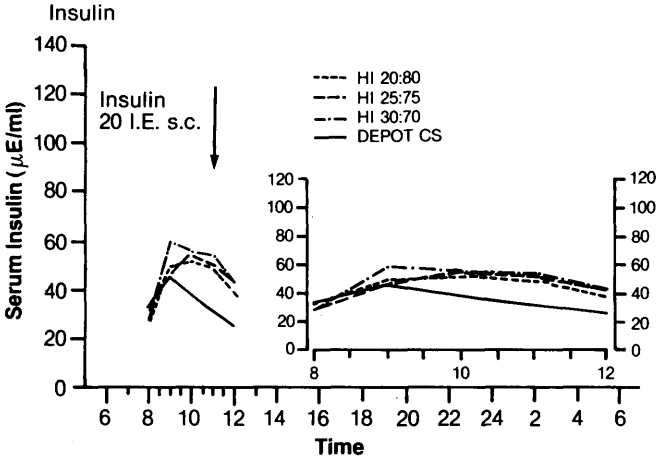

FIG. 5. Serum insulin levels corresponding to the plasma glucose curves shown in Figure 4.

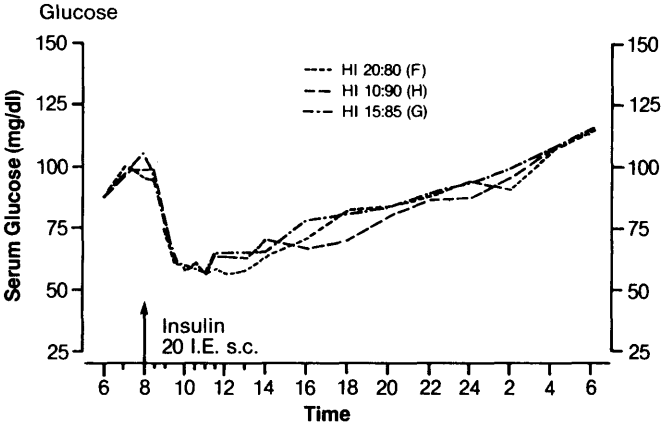

FIG. 7. Mean plasma glucose response of 8 healthy volunteers after subcutaneous administration of 20 U of three mixtures of regular and NPH human insulins (20:80, 10:90, 15:85) under Gerritzen test conditions.

the two human insulins lie closer together initially than the curves for the respective PPI preparations, whereas later regular human insulin interferes less with the NPH human insulin curve. Thus, a combination of both human insulin preparations has potentially useful properties with regard to clinical application. Therefore, the effect on serum glucose of a series of mixtures was tested against Depot Hoechst CS as a reference.

The quantitatively different effect on serum glucose levels after application of several insulins corresponds to the respective serum insulin levels. The fall in C-peptide levels demonstrates the suppression of endogenous insulin secretion.

This study corroborates the advantages of the Gerritzen test[6] in characterizing the action profile of insulins. When used in an appropriate manner, the test allows a rather realistic evaluation of the action profile.

It should be noted that no pathologic skin reactions or other adverse reactions occurred. In addition, no antibodies against human insulin or *E. coli* proteins could be detected.

In conclusion, human insulin preparations have proven to be effective and safe in humans. It is to be expected that combinations of regular and NPH human insulin will be useful in controlling diabetes.

From the Department of Internal Medicine, University of Homburg-Saar, Homburg-Saar, Germany (K.W., M.E., G.N.), and Lilly GmbH, Bad Homburg, Germany (F.E.).

Address reprint requests to Kurt Weinges, Department of Internal Medicine, University of Homburg-Saar, Homburg-Saar, Germany.

REFERENCES

[1] Chance, R. E., Kroeff, E. P., and Hoffmann, J. A.: Clinical, physical and biological properties of recombinant human insulin. FDA Conference on Insulin and Growth Hormone, held at the National Institutes of Health. In press.

[2] Weinges, K., Ehrhardt, M., and Enzmann, F.: Comparison of biosynthetic human insulin and pork insulin in the Gerritzen test. Diabetes Care *4:* 180–82, 1981.

[3] Teuscher, A., and Diem, P.: Skin sensitivity to synthetic human insulin. Diabetologia *13:* 435, 1977.

[4] Teuscher, A.: Die biologische Wirkung von vollsynthetischem humanem Insulin bei Patienten mit Diabetes mellitus. Schweiz. Med. Wochenschr. *109:* 734–47,1979.

[5] Diem, P., and Teuscher, A.: Immunologische Untersuchungen mit vollsynthetischem humanem Insulin bei Diabetikern. Schweiz. Med. Wochenschr. *109:* 1814–16, 1979.

[6] Gerritzen, F.: The duration of the action of different insulins. Br. Med. J. *1:* 249, 1952.