Inhibition of Pancreatic Glucagon Responses to Arginine by Human Insulin (recombinant DNA) and Purified Porcine Insulin in Normal and Diabetic Subjects

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This study investigates and compares human insulin (recombinant DNA) and purified porcine insulin (PPI) in healthy volunteers and in type II diabetic patients, in terms of whether both these insulins were capable of influencing in a different manner pancreatic glucagon, C-peptide, and free fatty acids (FFA) concentrations. The findings reveal that the β-cell of human pancreas apparently recognizes human insulin more readily than PPI, as assessed by the inhibition of C-peptide, and a similar conclusion follows for the α-cell; this conclusion is underscored by the inhibited glucagon values. The delayed increments of glucagon under human insulin following arginine stimulation may be the result of a more rapid insulin absorption from subcutaneous tissue and a greater biologic action of this insulin in comparison with the PPI. Finally, human insulin has additional properties as demonstrated by its stronger antilipolytic effects. DIABETES CARE 5 (SUPPL. 2): 93–101, 1982.

In previous studies we were able to demonstrate[1] that both the hypoglycemic effects of human insulin (recombinant DNA) and purified porcine insulin (PPI) and the requirements of these two insulins as calculated via the artificial endocrine pancreas (Biostator) were almost identical.

Furthermore, it is well known that diabetes mellitus is a bihormonal disorder characterized by excessive glucagon secretion as well as diminished insulin release[2,3] and that an antagonism exists between insulin and glucagon in regard to their biologic actions.[4] Comparative studies[5] in healthy volunteers showed that both human insulin (recombinant DNA) and semisynthetic human insulin are absorbed from the site of subcutaneous injection more rapidly as well as in a greater amount compared with PPI.

The aim of the present study was to elucidate whether the apparent different absorption rates of human insulin and PPI were able to influence in a different manner arginine-stimulated glucagon, C-peptide, and free fatty acids (FFA) concentrations in man.

MATERIAL AND METHODS

Subjects. Informed consent[6] was obtained from six metabolically healthy male volunteers and six male type II diabetics. The normal subjects (aged 23–58 years) were not obese and had no family history of diabetes. The diabetic subjects (aged 43–64 yr) were ambulatory patients free of acute illness or ketosis and had never received insulin, but were treated with oral agents. All diabetic patients were overweight (+20–30% of ideal body weight). Their renal function was normal, as determined by creatinine clearance. Signs of microangiopathy were absent according to examination performed by fundus camera.

Experimental protocol. All studies were performed between 7 and 10 a.m., after an overnight fast. In diabetics, treatment with oral agents had been discontinued at least 24 h before the experiment. Both the patients and the healthy volunteers were kept on a constant diet (30 cal/kg ideal body weight: 40% carbohydrates, 20% protein, 40% fat) for at least 1 wk before the experiment and until its termination. Upon the arrival of the patients one small intravenous catheter was inserted in each forearm (plastic catheter, 18 g, Abbot Laboratories, North Chicago, Illinois). One served for arginine infusion and the other for blood sampling. A 60-min equilibration period was allowed before initial basal samples were collected. During the whole period of the experiment, patients remained recumbent in a climate-controlled room with environmental temperature of 23°C.

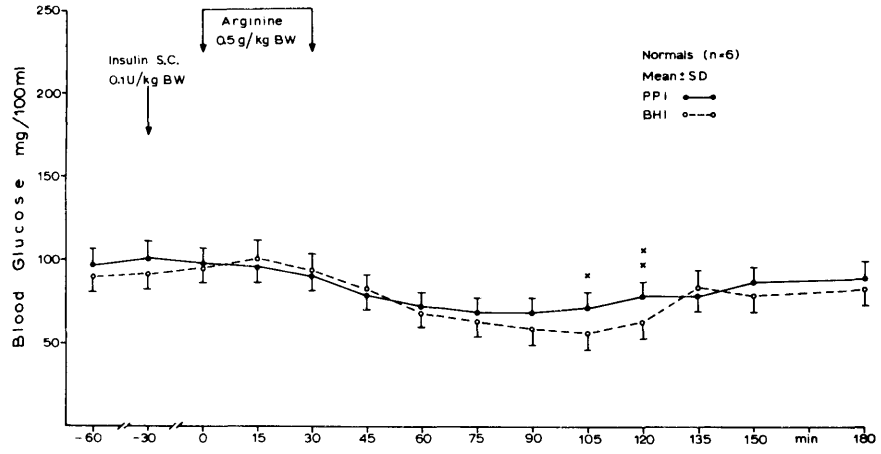

FIG. 1. Blood glucose in six healthy volunteers after the intravenous infusion of arginine and after previous subcutaneous administration of purified porcine insulin (PPI) or human insulin (BHI) (x: $P < 0.01$; xx: $P < 0.005$ or 0.001).

In diabetic and healthy volunteer patients, before any insulin administration, intradermal tests with human insulin or PPI (in doses of 0.01 U–0.1 U and 1 U) as well as with the solvent of the insulin preparations, were performed. The volume of intradermally injected solution was 0.05 ml.

Arginine (arginine-hydrochloride 21%; H^+ 1 mmol (meq)/ml, Cl^- mmol (meq)/ml, L-arginine-1 (meq)/ml; Braun-Melsungen, W. Germany) was administered in a dose of 0.5 g/kg body wt. The total amount of arginine was completed up to 300 ml with sodium chloride 0.9% and was administered via a special pump intravenously (Infusomat-Braun Melsungen, W. Germany) in exactly 30 min. Thirty minutes before the start of the arginine infusion, regular human insulin or PPI was injected subcutaneously above the deltoid muscle in a randomized fashion. Five days intervened between the two experiments. The volunteers received 0.1 U/kg body wt, and the diabetic patients 0.2 U/kg/body wt. Insulin was supplied by Eli Lilly and Company, Indianapolis, Indiana: lot no. for the human insulin CT-4969-1F; lot no. for the PPI: CT-5323-1A. Blood was drawn before and during the experimental procedures in regular intervals up to min 180. The following determinations were carried out: blood glucose by the hexokinase method of Schmidt,[7] radioimmunologically measurable insulin according to the method of Melani et al.,[8] and radioimmunologically measurable C-peptide by the method of Beischer et al.[9] In addition, radioimmunologically measurable pancreatic glucagon was determined by the method of Heding,[10] and free fatty acids according to the method of Dole and Meinertz.[11]

Data were evaluated statistically using the paired *t*-test, and surface areas calculated as described by Thompson et al.[12] Results are given as mean ± SD. In the figures one "x" stands for significance of <0.01 and "xx" for significance <0.005 or 0.001.

RESULTS

As can be shown, neither the normal individuals nor the diabetic patients experienced any skin or other reaction in the 24-h period following the intradermal test with human insulin as well as PPI.

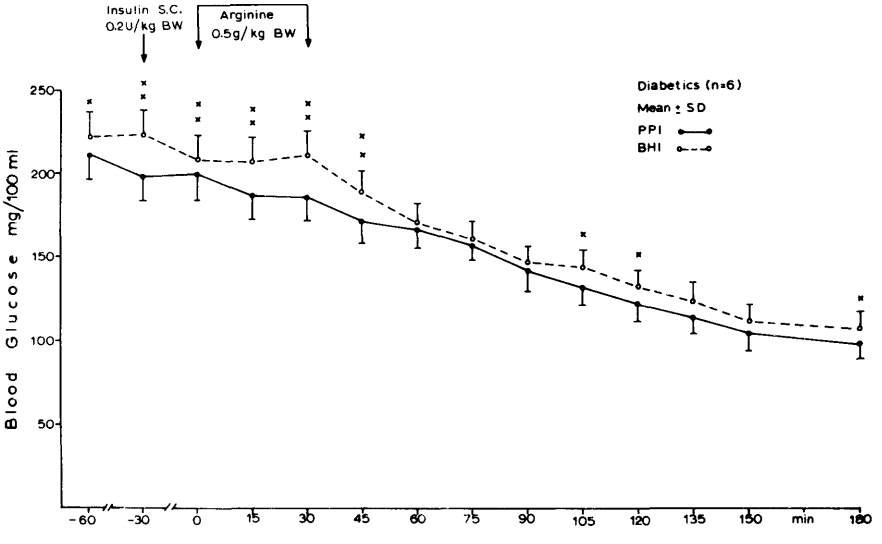

FIG. 2. Blood glucose in six maturity-onset diabetic subjects after the intravenous infusion of arginine and after previous subcutaneous administration of purified porcine insulin (PPI) or human insulin (BHI) (x: $P < 0.01$; xx: $P < 0.005$ or 0.001).

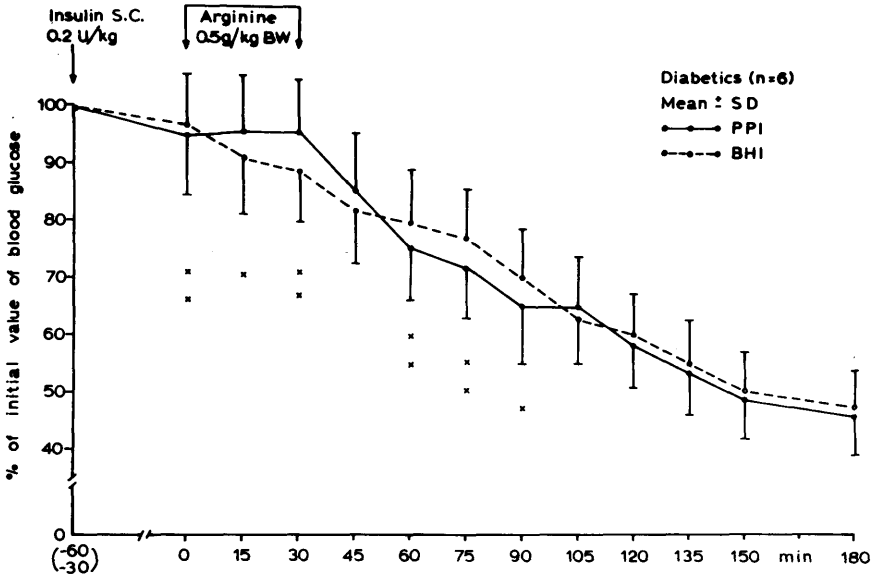

FIG. 3. *Percentage fall of blood glucose in six maturity-onset diabetic subjects after the intravenous infusion of arginine and after previous subcutaneous administration of purified porcine insulin (PPI) or human insulin (BHI) (x: P < 0.01; xx: P < 0.005 or 0.001).*

In normal subjects (Figure 1) the fall in blood glucose is the same after PPI and human insulin up to the 90th min. Then there is a statistically significant greater fall in blood glucose with human insulin to the 120th min than with PPI. The hypoglycemic symptoms at comparable blood sugar levels were less pronounced in patients on human insulin than on PPI, as we had observed in a previous study.[1]

In diabetic patients (Figure 2) the course of blood glucose fall appears to be the same with both types of administered insulin. However since in this case the initial values of blood glucose were higher when the patients were receiving the human insulin, we estimated the glucose fall on a percentage basis compared to the initial values.

Thus, it is clearly demonstrated (Figure 3) that during the first 30 min, the hypoglycemic action of human insulin is greater than that of PPI, while between 60 and 90 min, the opposite holds true. After the 150th min there is no difference in the lowering effect of either insulin.

At this point it has to be emphasized that after administration of porcine insulin as well as of human insulin, the known moderate increase of blood sugar that occurs as a rule in human beings during the first 30 min of arginine infusion, was not observed.

Radioimmunologically measurable insulin in serum of healthy volunteers (Figure 4) is slightly but significantly higher in the PPI group. In the overweight diabetic patients (Figure 5) the concentration of insulin was much higher than in normal subjects. The course of the curve is generally the same after the administration of either PPI or human insulin, despite the fact that in certain points either insulin may predominate significantly.

The arginine-stimulated C-peptide secretion in healthy volunteers (Figure 6) is inhibited statistically more after BHI administration than after PPI. This inhibition is clear and quite obvious up to the 150th min of observation. Between the 150th and 180th min there is no longer any difference

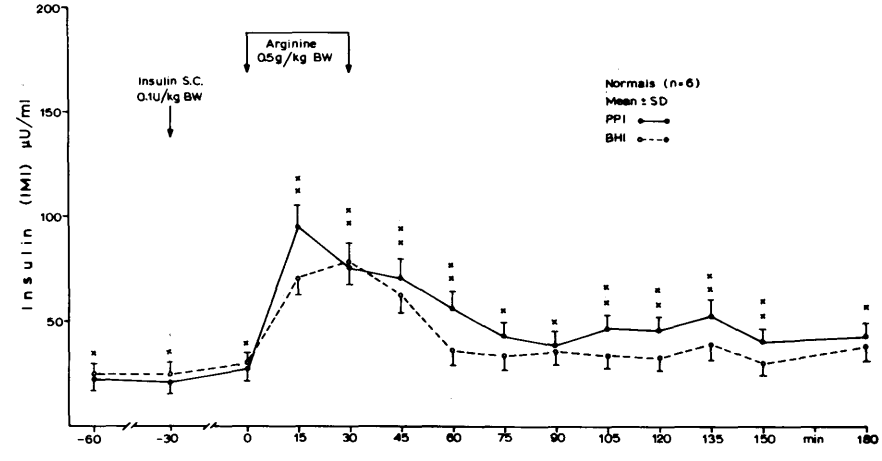

FIG. 4. *Radioimmunologically measurable insulin in six healthy volunteers after the intravenous infusion of arginine and after previous subcutaneous administration of purified porcine insulin (PPI) or human insulin (BHI) (x: P < 0.001; xx: P < 0.005 or 0.001).*

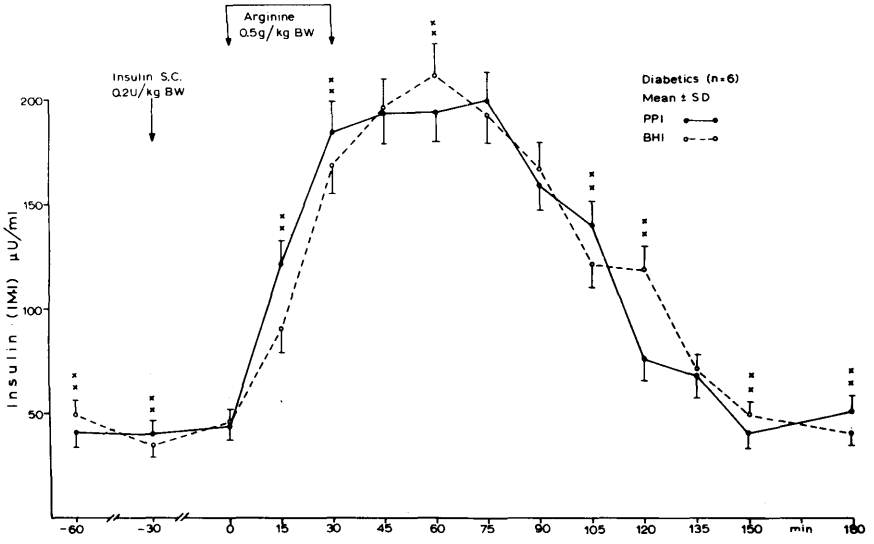

FIG. 5. Radioimmunologically measurable insulin in six maturity-onset diabetic subjects after the intravenous infusion of arginine and after previous subcutaneous administration of purified porcine insulin (PPI) or human insulin (BHI) (x: $P < 0.01$; xx: $P < 0.005$ or 0.001).

between the two insulins. In contrast, in type II diabetics (Figure 7) besides C-peptide concentration after arginine administration being higher compared to that of the healthy volunteers, its inhibition is not entirely distinct between the two types of insulins studied. However while between the 30th and 60th min C-peptide inhibition is greater with human insulin, in the second portion of the curve the inhibition is greater with porcine insulin. Before the 30th min, the comparison is not feasible since basal values are different. For this reason we estimated the surface area of C-peptide in each case (Figure 8). So the latter was only 210 ng · min/ml with human insulin, while with PPI 320 ng · min/ml. The difference was statistically significant. Regarding pancreatic glucagon determination the situation appears more clear. Arginine-stimulated glucagon secretion in healthy volunteers (Figure 9) is obviously inhibited during the whole experiment period, statistically significantly more after human insulin administration than after PPI. The difference between the two groups in the first 60 min of the experiment is more characteristic; the inhibition of glucagon by human insulin is greater and occurs much earlier than by PPI. The curve has been shifted to the right. Maximal stimulation of glucagon by arginine is obtained in 30 min after PPI administration, in contrast to 60 min after human insulin. After that time and until the end of the experiment the inhibition of glucagon after human insulin administration remains statistically significantly greater. The same changes are observed in the group of maturity-onset diabetics as well (Figure 10). The basal and the reactive glucagon values in this group are higher than in the group of healthy volunteers. The fall of

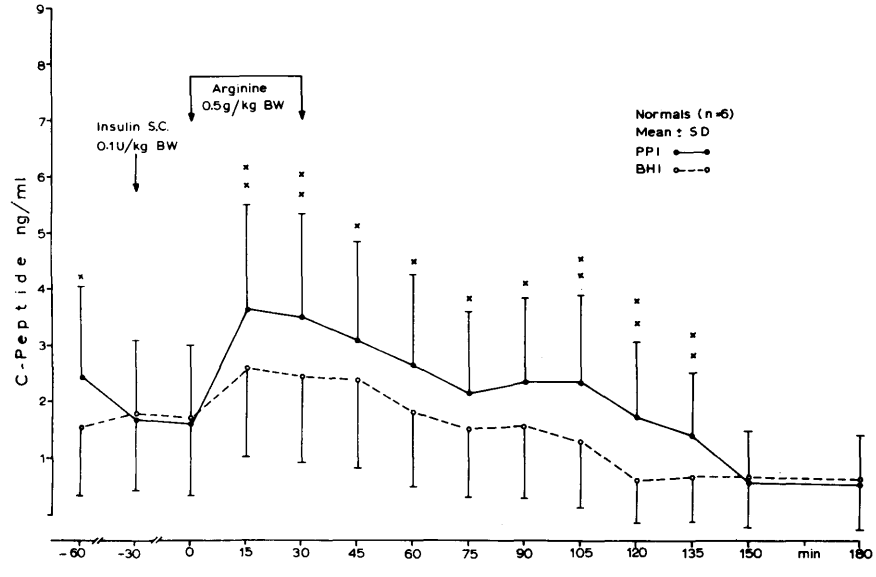

FIG. 6. Radioimmunologically measurable C-peptide in six healthy volunteers after the intravenous infusion of arginine and after previous subcutaneous administration of purified porcine insulin (PPI) or human insulin (BHI) (x: $P < 0.001$; xx: $P < 0.005$ or 0.001).

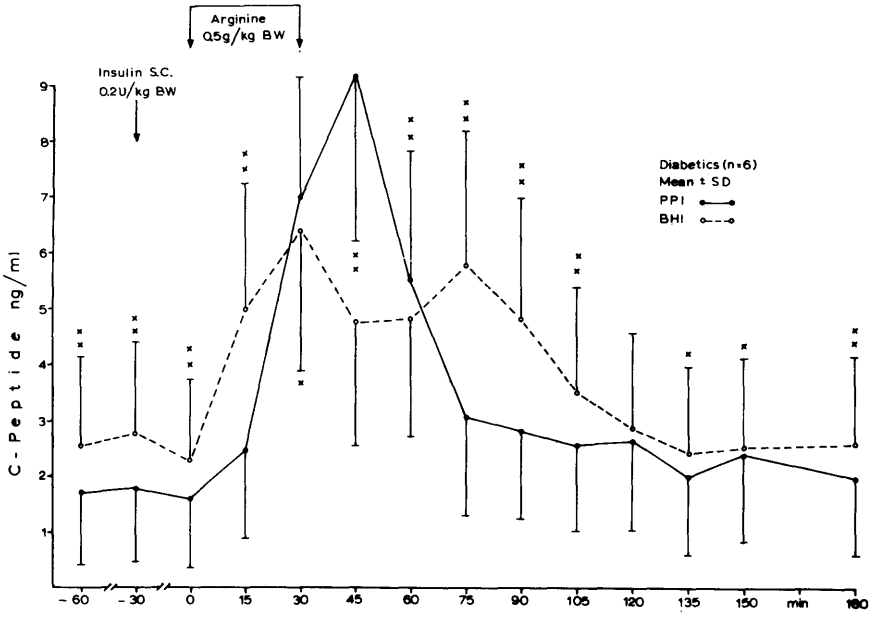

FIG. 7. Radioimmunologically measurable C-peptide in six maturity-onset diabetic subjects after the intravenous infusion of arginine and after previous subcutaneous administration of purified porcine insulin (PPI) or human insulin (BHI) (x: $P < 0.01$; xx: $P < 0.005$ or 0.001).

free fatty acids in healthy volunteers (Figure 11) is significantly greater after human insulin than after PPI. The stronger antilipolytic action of human insulin in comparison to PPI is quite obvious after the 90th min. The differences were also characteristic in the first part of the curve. More specifically, while 30 min after the insulin injection there is a drastic fall of free fatty acids in the human insulin group and the infusion of arginine cannot bring about an increase of their circulating concentration in the blood, in the PPI group arginine is able to break through this inhibition and lead to a statistically significant increase of FFA. The same occurs in the group of diabetic patients (Figure 12).

Human insulin, in contrast to PPI, is also in a position to inhibit the arginine-induced increase of free fatty acids. The further course and antilipolytic action of human insulin and PPI do not differ in such an obvious manner in diabetic as occurs in normal subjects. At this point it has to be stressed that basal values in this group of diabetic patients are statistically significantly higher than in healthy volunteers. If the surface area of FFA in diabetic patients is calculated during the whole 3-h follow-up (Figure 13), then the value with human insulin is found to be -49.296 ± 28.854 μeq · min/ml, while with PPI only -31.638 ± 31.038 μeq · min/ml. Despite that, the mean values of both groups are greatly different which points in an obvious way to the greater antilipolytic action of human insulin in comparison to PPI. This same difference is not, however, statistically significant, because the fluctuations between patients were large, with the consequence of a large standard deviation.

Adverse effects. Except for the classical mild symptoms of hypoglycemia no adverse effect was observed after arginine administration, nor was there any local skin reaction after subcutaneous injection of either human insulin or PPI.

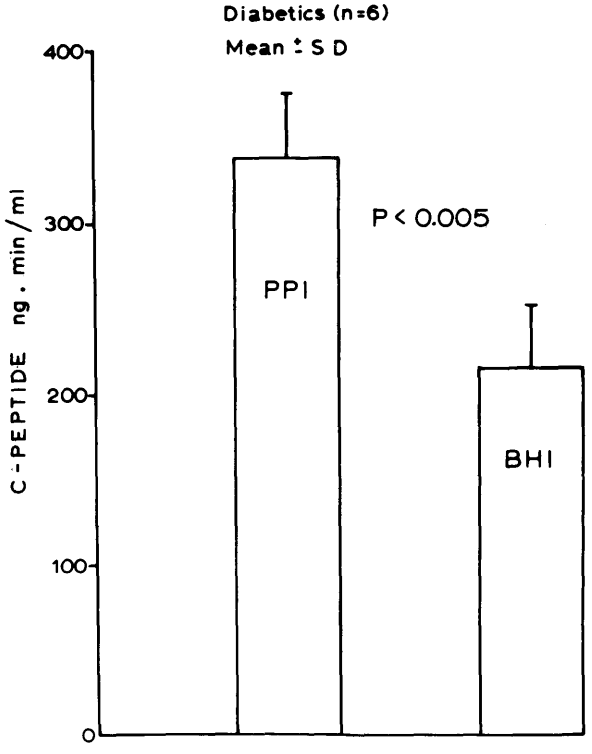

FIG. 8. Surface C-peptide areas calculated during the whole observation period of 180 min in six maturity-onset diabetic subjects after the intravenous infusion of arginine and after previous subcutaneous administration of purified porcine insulin (PPI) or human insulin (BHI).

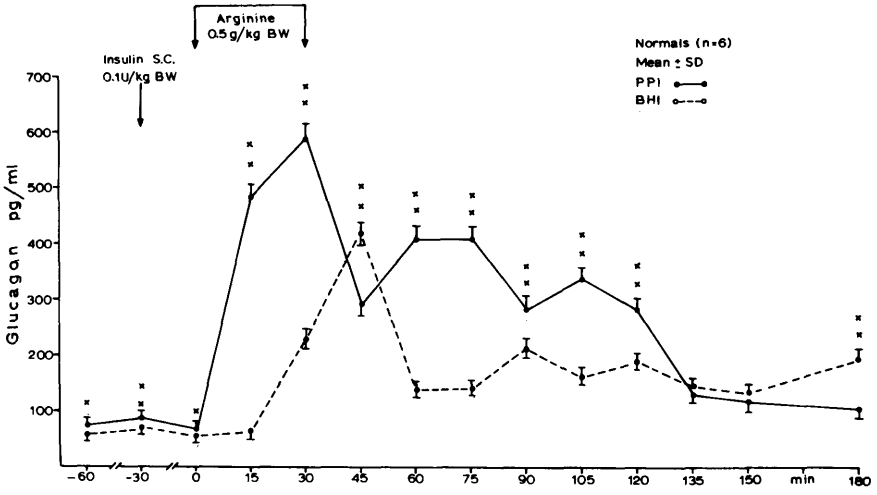

FIG. 9. Radioimmunologically measurable pancreatic glucagon in six healthy volunteers after the intravenous infusion of arginine and after previous subcutaneous administration of purified porcine insulin (PPI) or human insulin (BHI) (x: $P < 0.01$; xx: $P < 0.005$ or 0.001).

DISCUSSION

The above findings demonstrate that human insulin may possess certain significant differences in biologic action in comparison to PPI. The difference in fall of blood glucose in normal subjects after subcutaneous administration of insulin after PPI and human insulin occurs only at 105 and 120 min of observation, as was mentioned. The difference is, however, characteristic in the diabetic patients. The fall is faster after human insulin than after PPI, which speaks in favor of the faster absorption of human insulin from the subcutaneous tissue. (The phenomenon of the feeling of hypoglycemia between human insulin and PPI is characteristically different.) As we were the first to describe it previously[1] during a comparable fall of blood glucose, the group that received human insulin did not experience the feeling of hypoglycemia so markedly as did the PPI group. The reason is probably the different stimulation of growth hormone and cortisol after hypoglycemia created by human insulin.[1]

The concentration of radioimmunologically measurable insulin in the blood after PPI administration was found to be higher compared to the concentration after human insulin. This of course cannot be explained with certainty by the fact that PPI's absorption from the site of injection is more complete and faster, because other factors take part as well. Initially, the administration of arginine infusion during the first 30 min led to the secretion of endogenous insulin from the pancreas too. Besides this, antibodies against insulin employed in our radioimmunological method were raised with porcine insulin in guinea pigs. These recognize, in all likelihood, porcine insulin better than human insulin. The observed increased concentration of insulin in type II diabetic patients in comparison to healthy volunteers is due to the double amount of injected insulin on one hand and the exceeding weight of patients and their existent hyperinsulinism on the other.

It is characteristic that although after PPI administration insulin concentration in the blood is higher, the greater fall in blood sugar occurs after human insulin administration.

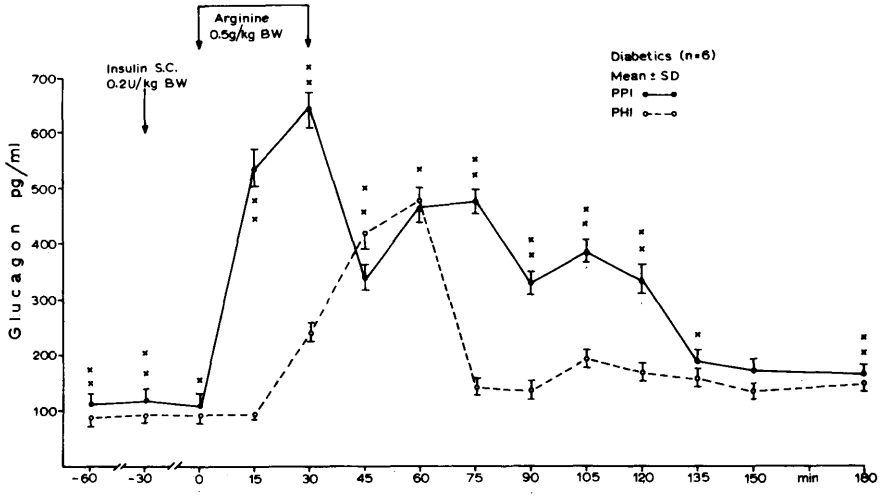

FIG. 10. Radioimmunologically measurable pancreatic glucagon in six maturity-onset diabetic subjects after the intravenous infusion of arginine and after previous subcutaneous administration of purified insulin (PPI) or human insulin (BHI) (x: $P < 0.01$; xx: $P < 0.005$ or 0.001).

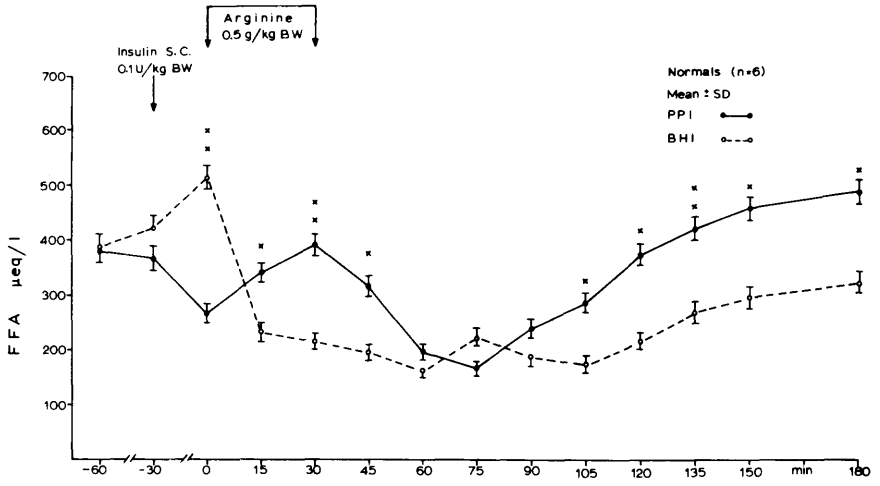

FIG. 11. Free fatty acids concentration in six healthy volunteers after the intravenous infusion of arginine and after previous subcutaneous administration of purified porcine insulin (PPI) or human insulin (BHI) (x: $P < 0.01$; xx: $P < 0.005$ or 0.001).

It seems therefore that either the peripheral tissues or the different sites of insulin action recognize human insulin as more homologous, with the result that its biologic action is greater. Not only peripheral tissues, but β-cells of pancreas as well seem to recognize human insulin more than PPI, as is apparent from the greater inhibition of C-peptide after human insulin administration. We had noted greater inhibition of C-peptide by BHI in a previous study of our own.[1]

Considering the importance of glucagon secretion in diabetes mellitus, its greater and faster inhibition after human insulin administration acquires a characteristic and probably crucial role. This signifies the more rapid absorption of injected human insulin on one hand and its greater biologic action on the other. Therefore it seems that α-cells of pancreas recognize human insulin better, resulting in more drastic inhibition of glucagon secretion. If one thinks of the increased concentrations of glucagon in diabetes mellitus[2] as well as of its insulin-opposing action,[4] one can easily realize the importance of this property of human insulin in terms of clinical significance—the prompt and of comparatively greater inhibition of pancreatic glucagon secretion.

This greater inhibition of glucagon as well as the stronger biologic activity of human insulin, and possibly its direct action on the fat cell as well, lead to its higher antilipolytic action in comparison to PPI. This diminution of free fatty acids leads not only to a better action of insulin at the peripheral tissues[13,14] but to a better performance of cardiac muscle[15] as well.

As is known, the more frequent complications of diabetes mellitus originate in the cardiovascular system. Therefore a greater inhibition of lipolysis by human insulin is probably beneficial. It is known from recent observations[16,17] on myocardial metabolism that there is an interaction between FFA concentration and oxygen need in man. Depression of myocardial FFA utilization leads to enhanced glucose utilization and consequently to a decrease in oxygen consumption.[18] Furthermore the inhibition of lipolysis alone leads to a profound reduction in the size of myocardial infarcts, for ex-

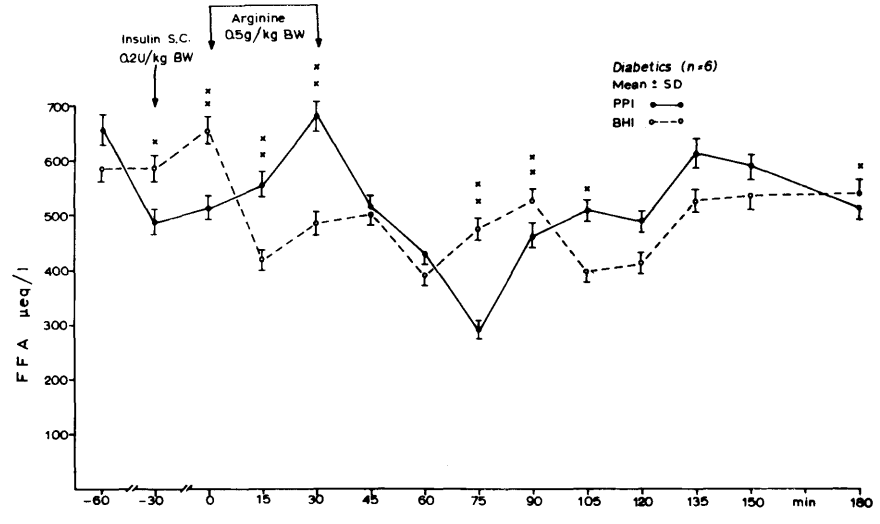

FIG. 12. Free fatty acids concentration in six maturity-onset diabetic subjects after the intravenous infusion of arginine and after previous subcutaneous administration of purified porcine insulin (PPI) or human insulin (BHI) (x: $P < 0.01$; xx: $P < 0.005$ or 0.001).

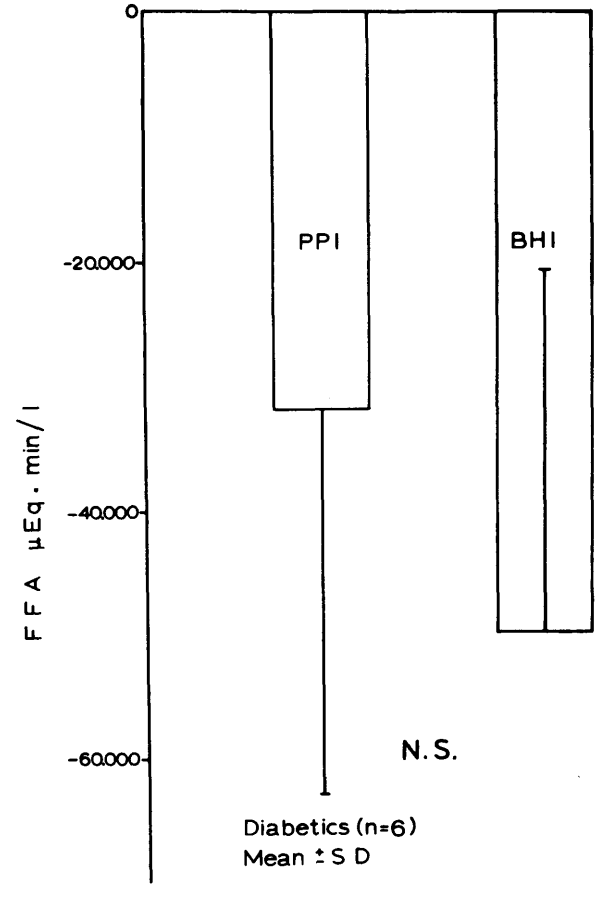

FIG. 13. Surface free fatty acids areas calculated during the whole observation period of 180 min in six maturity-onset diabetic subjects after the intravenous infusion of arginine and after previous subcutaneous administration of purified porcine insulin (PPI) or human insulin (BHI).

ample, and to a lower incidence of dysrhythmias in experimental coronary occlusion.[19,20]

More profound antilipolytic action of human insulin in comparison to PPI has been documented also by Viberti et al.[21] and Rosak and co-workers[22] (and Rosak et al., pp. 82–89). Massi-Benedetti et al.[23] found that not only lipolysis but also ketogenesis were suppressed more by human insulin.

We concluded that quite probably the above-described differences between human insulin and PPI have clinical relevance and may result in improved diabetic control and ultimately in the diminished occurrence of late complications in patients treated with human insulin.

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