Biologic Activities of Biosynthetic Human Insulin in Healthy Volunteers and Insulin-dependent Diabetic Patients Monitored by the Artificial Endocrine Pancreas

SOTOS RAPTIS, CONSTANTINE KARAISKOS, FRANZ ENZMANN, DIMITRIOS HATZIDAKIS, CHRISTOS ZOUPAS, ATHANASIOS SOUVATZOGLOU, E. DIAMANTOPOULOS, AND SPYRIDON MOULOPOULOS

This study investigates and compares biosynthetic human insulin (BHI) and purified pork insulin, in healthy volunteers and in insulin-dependent diabetic patients, in terms of biologic action, capacity for controlling diabetic patients, and the requirements of the patients on each insulin. The possible importance of this new insulin in the improved long-term control of diabetic patients led to the experimental design of this protocol. DIABETES CARE 4: 155–162, MARCH–APRIL 1981.

The synthesis of the human insulin A- and B-chains through recombinant DNA, using *Escherichia coli* fermentation, was announced in 1979 by Goeddel and co-workers.[1] This general idea and the application of the method were used initially with success for the synthesis of polypeptide hormones, such as somatostatin by Itakura and co-workers in 1977.[2] This remarkable breakthrough started a few years ago, after the rapid progress of basic research in the field of molecular biology, and was hastened by the availability of chemically synthesized codons derived from nucleotide synthesis.[2,3]

Using this same method, with some modifications (see Chance et al., this issue, pp. 147–54), it is possible to synthesize human insulin in significant amounts from *E. coli* with the help of plasmids containing the genes of A- and B-chains of insulin. Recombinant DNA technology promises to contribute to the solution of the problem of treating insulin-dependent diabetic individuals through the production of the appropriate kind of insulin.[4,5] Currently it has been shown[6] that BHI is chemically and physically equivalent to pancreatic human insulin and biologically equivalent to both pancreatic human insulin and a standard pork insulin preparation in various biochemical and animal systems. During the time that the present work was in progress, Keen et al.[7] reported on the hypoglycemic action of BHI in healthy volunteers.

MATERIALS AND METHODS

Healthy volunteers. A total of five metabolically nonobese healthy male volunteers, between 25 and 31 years of age, took part in the study. They were informed according to the rules of the Geneva, Helsinki, and Oslo declaration[8] of the nature of the study and of the symptoms that can develop during administration of insulin, and they gave their written consent to participate.

On the morning of the investigation, the volunteers had been in the supine position for the previous 14 h and were resting in a room with a constant temperature of 20°C. An i.v. catheter was inserted into each arm together with an indwelling mandrin; a 2-h rest period preceded the i.v. injection of insulin. The study was designed to involve a fully randomized intrapatient comparison of the effects of two different dosage regimes of purified pork insulin (PPI) and biosynthetic human insulin (BHI). The experimental procedure consisted of the following:

1. Intravenous administration of insulin in a dose of 0.05 U/kg body wt, diluted in 5 ml saline containing 5% human albumin as bolus.
2. Intravenous administration of insulin in a dose of 0.1 U/kg body wt, diluted in 5 ml saline containing 5% human albumin as bolus, as well.

In all cases an interval of 3 days intervened between each test. All volunteers were placed on a constant diet 3 days before the beginning of the study and remained on the diet, which consisted of 35 kcal/kg body wt (40% carbohydrates, 20% protein, 40% fat), throughout the study.

Before i.v. insulin administration and in constant time intervals after the administration, the following determinations were carried out: blood glucose by the hexokinase method of Schmidt,[9] radioimmunologically measurable insulin according to the method of Melani et al.,[10] and radioimmunologically measurable C-peptide by the method of Beischer et al.[11] In addition, growth hormone levels were determined by the method of Glick et al.,[12] modified by Schröder,[13] and cortisol directly in plasma without extrac-

tion was determined according to the methods of Rolleri et al.[14] and Catt and Tregear.[15]

Gastrin levels were measured according to the method of Raptis et al.,[16] which was completed and improved later by the same investigators.[17] Blood for measurement of gastrin was collected in pre-chilled tubes that contained 100 μl of a solution EDTA-Trasylol (750 mg EDTA, 10 ml 100,000 U Trasylol) in the appropriate proportion for 5 ml blood.

Data were evaluated statistically using the paired *t* test and surface area calculated as described by Thompson et al.[18] Results are given as mean ± SD.

Diabetic patients. The second group consisted of five insulin-dependent diabetic patients (four women and one man), aged 19–64. The requirements of these patients in purified pork insulin (PPI) as well as in BHI were calculated through an artificial endocrine pancreas (AEP), a glucose-controlled insulin infusion system GCIIS-Biostator.[19–21] The patients were connected to the machine in the afternoon, and the next morning calculation of the required amount of insulin was started, after the overnight steady-state period. The requirements were estimated for 24 h, and after the first 24-h period the type of insulin was switched and the required amount for the following 24-h period was measured.

One patient was given pork insulin in the first 24-h period, BHI during the second 24 h, and pork insulin again during the third 24-h period. Another patient was given BHI during the first 24 h, pork insulin during the second period, and BHI again during the third. In another female patient the requirements for BHI via the AEP were estimated before, as well as 20 days after, her possible ideal control via a continuous intravenous infusion through an open-loop system (Promedos–Siemens).[22]

The sequence at the beginning of the experiment with either PPI or BHI was conducted in a randomized fashion. The switch from the one type of insulin to the other took place immediately, without the intervention of any steady-state period. The diet of the patients during the whole period that they were connected to the machine was constant, consisting of 25 kcal/kg body wt (30% protein, 30% carbohydrate, 40% fat) given in six meals.

Before the patients were connected to the apparatus, it was ascertained that they were pure insulinopenic, via the glibenclamide–glucose combined test[23,24] and the C-peptide determination. None of the patients required more than 50 U/day on an ambulatory basis and before their connection to the AEP. Their renal function was normal, as determined by creatinine clearance. In all diabetic patients, as well as in the healthy volunteers, before any insulin administration, intradermal tests with BHI 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.

The BHI applied in the above study had the following characteristics: CT 4648-0B, Lot E-676, July 1980, Lilly Research Centre Ltd. (Windleshom, Surrey, England). The PPI used was the monocomponent Actrapid insulin of Novo (Copenhagen, Lot. No. 35*0,1.5.80). Before any insulin administration and before the intradermal test, 20 cc of blood was obtained from all the patients and from the healthy volunteers for measurement of antibodies against either insulin or *E. coli*, in the future.

RESULTS

As the results show, neither the normal individuals nor the diabetic patients experienced any skin or other reaction in the 24-h period following the intradermal test with BHI as well as PPI. A positive skin reaction of delayed type appeared in only one patient (12 hours after the intradermal injection). However, this reaction should be considered as nonspecific because it occurred not only with the insulins, but with the solvent and the saline as well.

Healthy volunteers. As is well known, insulin administration, besides causing a fall in blood glucose, indirectly induces the release of growth hormone (GH), cortisol, and gastrin through the created hypoglycemia. The height of the secretion of these hormones is directly proportional to the degree of hypoglycemia. Moreover, it seems that insulin exerts some direct action on gastrin release as well as an inhibitory action on the release of C-peptide. For this reason and in order to investigate if there are any differences between BHI and PPI, we measured the above parameters. The

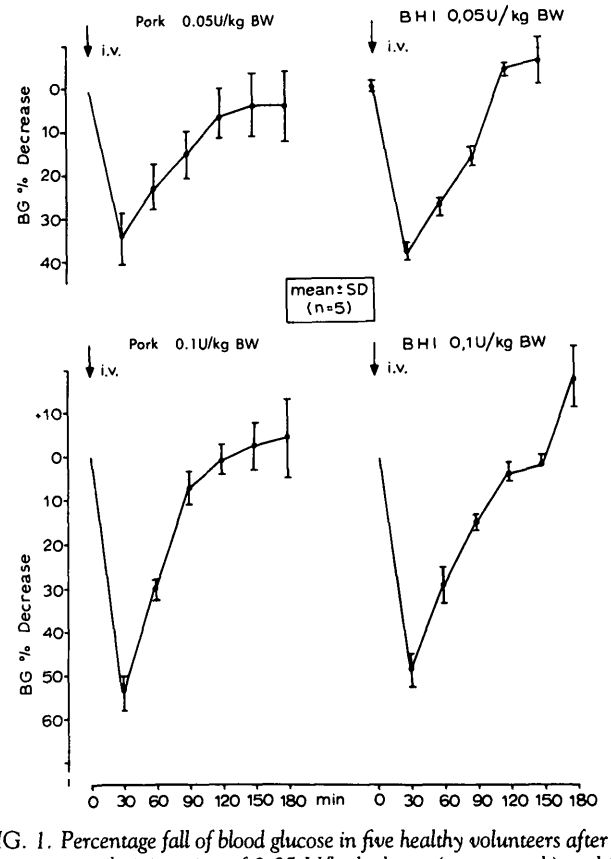

FIG. 1. Percentage fall of blood glucose in five healthy volunteers after the intravenous administration of 0.05 U/kg body wt (upper graph) and 0.1 U/kg body wt (lower graph) of BHI and PPI.

TABLE 1
Surface areas after the i.v. administration of pork insulin and BHI in five healthy volunteers (mean ± SD)

	0.05 U/kg			0.1 U/kg		
	Pork		BHI	Pork		BHI
Blood glucose (mg·min/dl)	−2832 ± 597	P < 0.05	−1702 ± 443	−2268 ± 1281	NS	−2021 ± 263
Insulin (μU·min/ml)	1379 ± 1085	NS	972 ± 1004	2517 ± 922	P < 0.025	5193 ± 919
C-peptide (ng·min/ml)	−29 ± 206	NS	−96 ± 135	−116 ± 65	P < 0.05	−195 ± 35
Growth hormone (ng·min/ml)	870 ± 144	P < 0.025	722 ± 91	1685 ± 262	NS	1483 ± 320
Cortisol (nmol·min/L)	8595 ± 1074	P < 0.0005	21342 ± 1507	14387 ± 4626	P < 0.05	16962 ±2026
Gastrin (pg·min/ml)	114 ± 518	NS	−666 ± 823	1425 ± 493	NS	2206 ± 722

estimated concentration of insulin reflects, in addition to its endogenous secretion, the level of circulating insulin after its i.v. administration.

In healthy volunteers, the intravenous administration of 0.05 U/kg body wt of both BHI and PPI caused a significant fall in blood glucose, which reached its maximum in 30 min after the injection (Figure 1). The percentage of fall in blood glucose in 30 min was greater in the group that received BHI, but the difference is not statistically significant compared with the group that received PPI. In contrast, the level of blood glucose 120 min after the i.v. administration of insulin was statistically higher ($P < 0.0025$) in the group that received BHI.

However, we also estimated the surface area of fall of blood glucose during the period of observation (180 min) (Table 1). The surface area of the fall in blood glucose for the PPI group is greater. This difference has a borderline statistical significance ($P < 0.05$). After the intravenous administration of the greater insulin dose (0.1 U/kg body wt) in the same patients, a statistically significant lower blood glu-

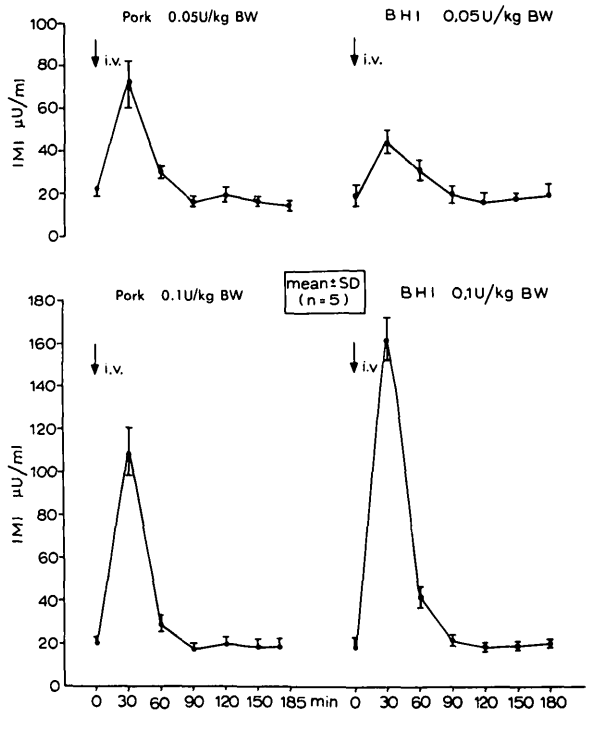

FIG. 2. Radioimmunologically measurable insulin (IMI) in five healthy volunteers after the intravenous administration of 0.05 U/kg body wt (upper graph) and 0.1 U/kg body wt (lower graph) of BHI and PPI.

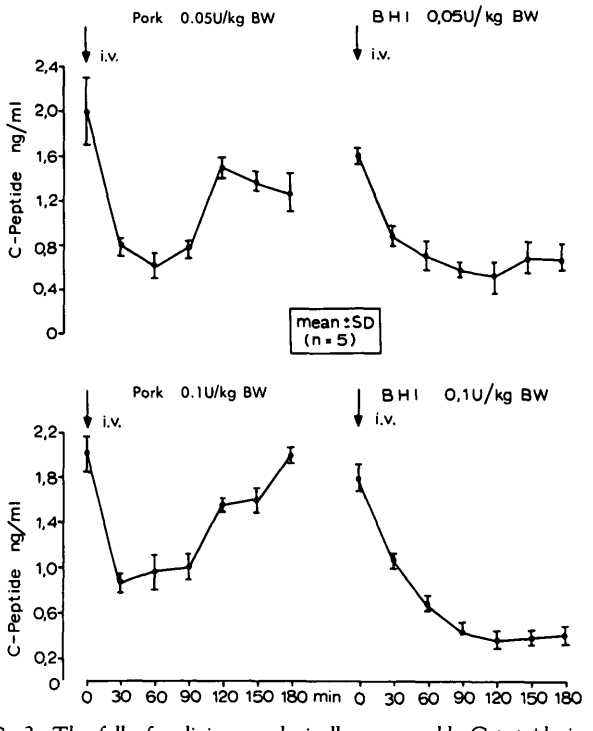

FIG. 3. The fall of radioimmunologically measurable C-peptide in five healthy volunteers after the intravenous administration of 0.05 U/kg body wt (upper graph) and 0.1 U/kg body wt (lower graph) of BHI and PPI.

cose level was obtained in 30 and 60 min ($P < 0.0125$ and $P < 0.05$, respectively) in the PPI group, while significantly lower blood glucose at 90 min ($P < 0.05$) occurred in the BHI group (Figure 1). Calculating the surface area in the fall of absolute values during the 180 min of observation, no statistically significant difference was found between the two groups (Table 1).

At the same time, the radioimmunologically measured insulin in the serum of these five individuals, at a dose of 0.05 U/kg, shows a statistically significant minor increase 30 min after the i.v. injection of BHI ($P < 0.005$) (Figure 2). No statistically significant difference between the PPI and BHI group was found for the surface area of insulin increase or any other value during the 180 min (Table 1).

For the insulin dose of 0.1 U/kg i.v., the values both at 30 min and at 60 and 90 min after the injection are significantly higher in the BHI group ($P < 0.025$; $P < 0.025$; $P < 0.0025$). Also the surface area is statistically greater in the BHI group (Table 1). The fall in C-peptide (Figure 3) in the BHI group after the administration of 0.05 U/kg insulin is statistically greater at the times between 90 and 180 min after the injection ($P < 0.025$; $P < 0.005$; $P < 0.005$), while the surface area of total fall between the two groups is not statistically different (Table 1). There is a high statistical significance ($P < 0.0005$) regarding the major fall at the times between 30 and 180 min after the administration of 0.1 U/kg insulin in the group that received BHI in comparison with the PPI group. The total surface area of the fall in C-peptide has, in this case, a borderline statistical significance ($P < 0.05$) as far as comparison between BHI and PPI groups is concerned.

The i.v. administration of insulin in the dose of 0.1 U/kg body wt causes the release of GH in a higher degree in both groups (BHI and PPI) than with the dose of 0.05 U/kg (Figure 4). The administration of 0.05 U/kg PPI causes exactly the same release of GH at all times up to 180 min as does the BHI. However, by calculation of the total amount of GH released during the whole 3-h period of observation (Table 1), it can be shown that the total GH secreted after the administration of PPI is significantly higher ($P < 0.025$) than in the BHI group. In contrast, after the administration of 0.1 U/kg of insulin, no statistically significant difference is noted in the total amount of GH released between both groups (Table 1). Only a higher level of circulating GH is observed 30 min after the injection in the PPI group (Figure 4).

After the administration of doses of 0.05 U/kg and 0.1 U/kg body wt, a highly significant ($P < 0.0005$) greater value of radioimmunologically measurable cortisol in the BHI group is obtained at all times when blood was drawn for cortisol determination (Figure 5), and also in the total amount of released cortisol during the 3-h period (Table 1).

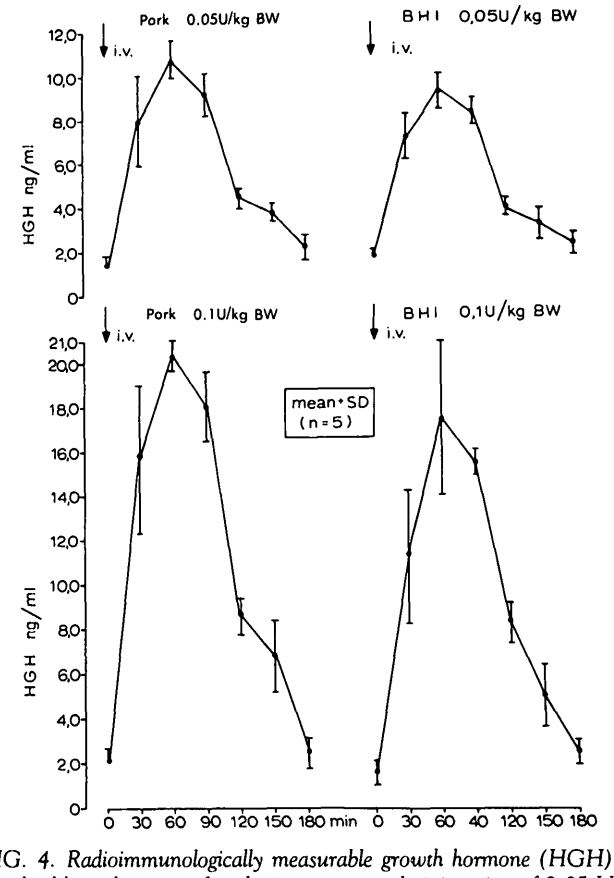

FIG. 4. Radioimmunologically measurable growth hormone (HGH) in five healthy volunteers after the intravenous administration of 0.05 U/kg body wt (upper graph) and 0.1 U/kg body wt (lower graph) of BHI and PPI.

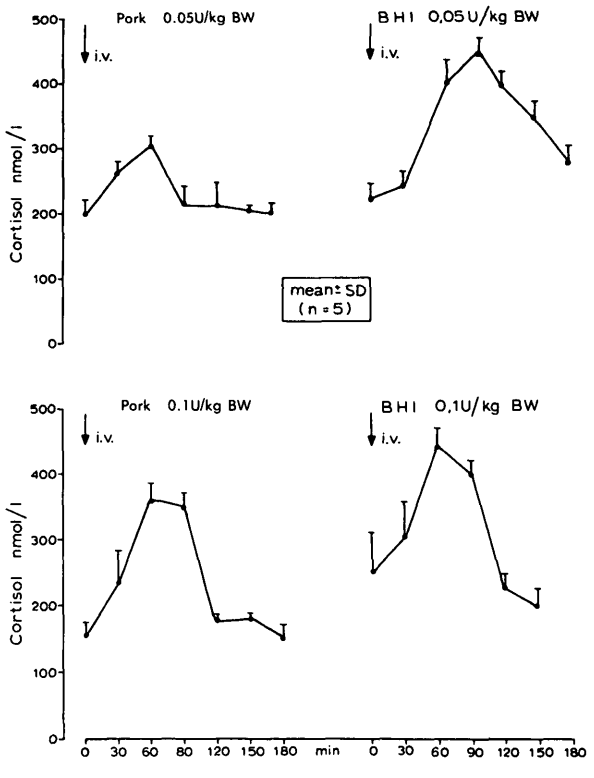

FIG. 5. Radioimmunologically measurable plasma cortisol in five healthy volunteers after the intravenous administration of 0.05 U/kg body wt (upper graph) and 0.1 U/kg body wt (lower graph) of BHI and PPI.

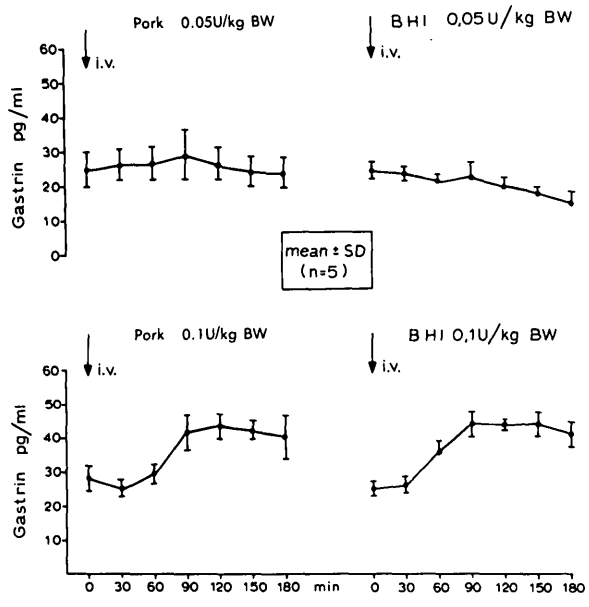

FIG. 6. Radioimmunologically measurable plasma gastrin in five healthy volunteers after the intravenous administration of 0.05 U/kg body wt (upper graph) and 0.1 U/kg body wt (lower graph) of BHI and PPI.

Finally, the radiommunologically measured gastrin in each case did not differ significantly between the groups that received either PPI or BHI. It is worth noting that in neither the BHI group nor the PPI group (Figure 6) is any even minimal increase of plasma gastrin noted after the administration of insulin in a dose of 0.05 U/kg, despite a very significant fall in blood sugar in these patients (Figure 1).

Diabetic patients. The combined glucose–glibenclamide test, applied in all patients, demonstrated (Figure 7) that none of the patients showed even minimal increase of C-peptide in the blood. The first administration of BHI to a diabetic individual took place on July 28, 1980, at 9:23 a.m. in our patient, F.G. The BHI requirements via the AEP were

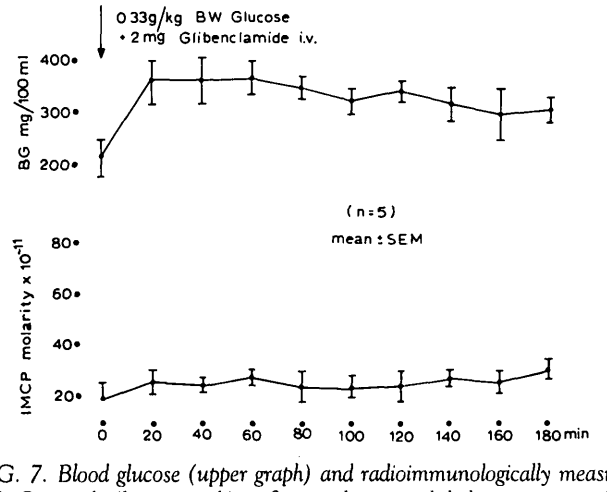

FIG. 7. Blood glucose (upper graph) and radioimmunologically measurable C-peptide (lower graph) in five insulin-treated diabetic patients after the i.v. administration of glibenclamide (2 mg) plus glucose (0.33 g/kg body wt).

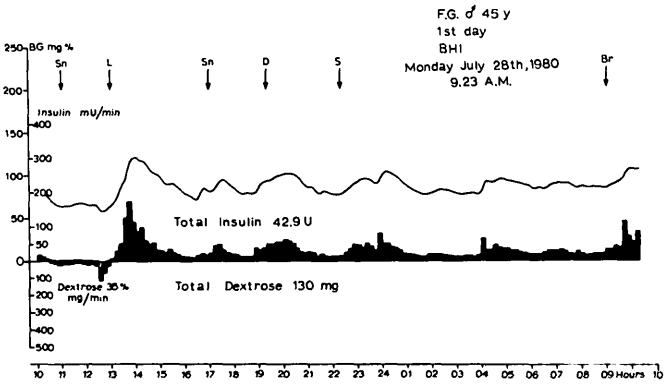

FIG. 8. Blood glucose curve, BHI requirements, and total dextrose amount during the 24-h period as monitored via the artificial endocrine pancreas (GCIIS-Biostator), in the very first diabetic patient who received BHI.

42.9 U for 24 h (Figure 8), and those for dextrose were 130 mg/24 h. During the next 24-h period (Figure 9), BHI was switched to PPI, and at this interval of observation the requirements in insulin were 46.6 U/24 h. Those for dextrose were zero.

By estimating the requirements in insulin of the five patients followed by the AEP (Table 2), we found that there was no statistically significant difference between the days that they received BHI and PPI. In addition, the requirements in dextrose, which was administered through the AEP, were not different in the above patients irrespective of receiving either BHI or PPI, except in the patient F.G. Among the five patients who were given BHI, three required 3–4 U of insulin less than when given PPI. However, such a minimal difference cannot bear any significance, since the difference in insulin requirements from day to day and under the same conditions ranges between 3 and 5 U when the same type of insulin is administered daily via the AEP. The insulin requirements in the two patients in which we changed the sequence of insulin used were the same regardless of the type of alternation, i.e., PPI–BHI–PPI or BHI–PPI–BHI.

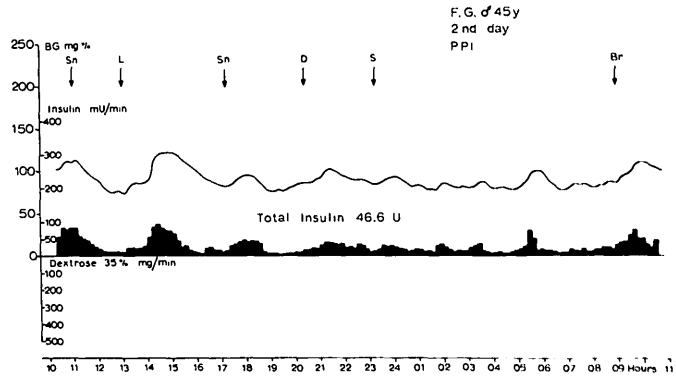

FIG. 9. Blood glucose curve, PPI requirements, and total dextrose amount during the 24-h period of monitoring of the same patient as in Figure 8, via the AEP.

TABLE 2
Insulin requirements (U/24 h) estimated by artificial endocrine pancreas in insulin-dependent diabetic patients

	Pork	BHI
L.P.	65.6	68
T.H.	44.4	45
F.G.	46.6	42.9
C.V.	57.6	54.8
M.K.	49.3	45.5
Mean ± SD	52.70 ± 8.78*	51.40 ± 10.31*

* The difference is not significant.

An open-loop system (Promedos–Siemens) was placed in another female patient (Figure 10), who during her connection with the AEP required 45.5 U/24 h of BHI. This system was administering purified bovine insulin by a special program in an amount of 24 U/24 h intravenously. After 20 days, the patient was connected again with the AEP. The estimation of BHI required for a 24-h period, after a 12-h steady-state period, showed (Figure 11) that at this time only approximately 24 U insulin/24 h were required, i.e., 47% less than during the previous time, before the 20-day ideal blood glucose control, when the insulin pump was used.

DISCUSSION

The above findings demonstrate that BHI has exactly the same biologic activities as PPI. We feel that the differences observed after the statistical analysis of the results may not have any clinical significance as far as the use of these two types of insulin is concerned. Like BHI, fully synthetic human insulin had the same hypoglycemic effects (over a dose range from 20 to 120 μg/kg) as natural, extracted human and pig insulin.[25]

Both in our experiments with healthy volunteers as well as in the studies of Keen and co-workers,[7] it appears that with

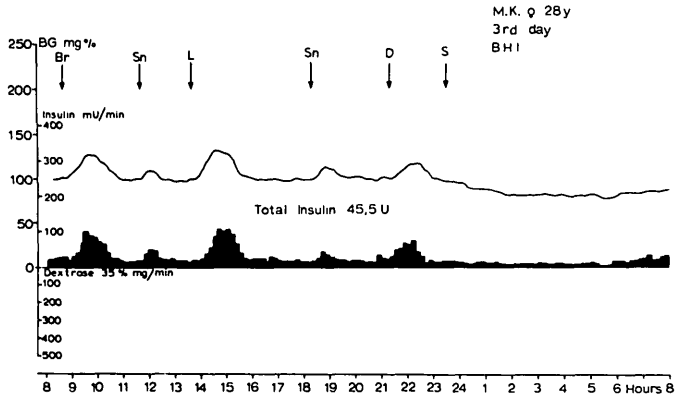

FIG. 10. Blood glucose curve, BHI requirements, and total dextrose amount during the 24-h period of monitoring with the AEP in a diabetic patient.

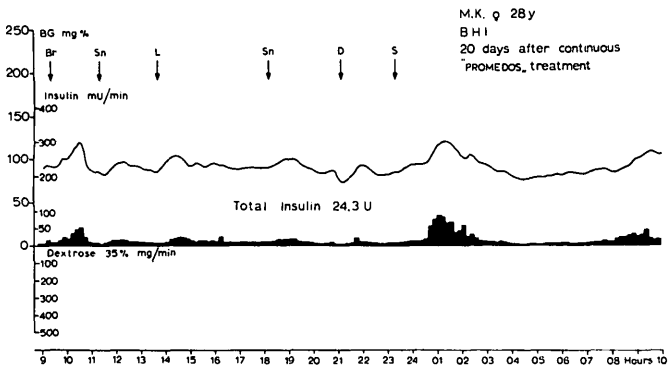

FIG. 11. Blood glucose curve, BHI requirements, and total dextrose amount during the 24-h period monitoring of the same patient as in Figure 10, with AEP after a 20-day ideal glucose control via an open-loop system (Promedos–Siemens).

small doses of i.v. BHI, we obtain a greater hypoglycemic action than with the dose of 0.1 U/kg body wt (see Table 1). It has not been established yet whether some different mechanism of hepatic inhibition and stimulation of peripheral glucose utilization is involved after BHI administration. At any rate, in vitro studies do not seem to show any significant difference between the administration of BHI and that of PPI at this point. Miras and co-workers (personal communication) recently found an increase of phosphodiesterase activity of intact human hepatocytes and membrane preparation[26] following treatment with BHI (12% higher membrane protein phosphorylation) in comparison with PPI.

It is impressive that Keen and co-workers[7] found positive skin reactions. We were not able to trace any positive skin reaction in any of our patients after the intradermal test with BHI, with follow-up for 24 h after the injection. Diem and Teuscher also did not find any skin reaction with fully synthetic insulin.[27,28] At this point, we might have to refer to the findings of Federlin and co-workers (see Federlin et al., this issue, pp. 170–174) who found patients who, despite developing antibodies against *E. coli*, did not react positively in the skin test with BHI. Here it should be emphasized that BHI is produced from *E. coli* strains and the presence of some *E. coli* protein in the solution of insulin cannot be excluded. The results so far indicate that there is no reason to worry about any reaction. However, we must wait for the data of long-term therapy with BHI before we can be sure.

From the findings of biologic actions of BHI and its differences from PPI, the greater release of GH with PPI should be stressed. We know that GH is a diabetogenic hormone,[29,30] and its increase is not a positive sign during the period of control of a diabetic patient. On the other hand, it must be mentioned that our findings were obtained after the i.v. insulin administration and a rapid fall in blood glucose, which is not true for the smooth control of diabetic patients. Also, it is not possible to compare exactly the hypoglycemic action on a unit basis of two different kinds of insulin, especially from different laboratories, without the knowledge of their

nitrogen concentration. Galloway and co-workers (see Galloway et al., this issue, pp. 183–188) made such a comparison with insulins of identical nitrogen content and did not find any statistical difference between BHI and PPI.

The next important difference between BHI and PPI is the significant enhancement of circulating cortisol after BHI administration. This may be the reason why patients who were receiving BHI experienced the feeling of hypoglycemia in a lesser degree than with the same concentration of PPI. This point of greater increase in cortisol after BHI administration may have some remote clinical significance, in terms of protecting the patients in some way during blood glucose fall of equal degree as with PPI in order to avoid hypoglycemia. In contrast, Schülter and co-workers[31] found a lesser increase in plasma cortisol after the administration of fully synthetic human insulin. On the other hand, the same authors[31] found a correlation between the concentration of cortisol and C-peptide.

In our volunteers who received BHI and who had higher cortisol than the PPI group, a greater inhibition of C-peptide was noted. The differences that were observed in the radioimmunologically measurable insulin between the BHI and PPI groups in this study cannot be taken into account entirely since in both groups insulin was measured using pork standard, while it would be more appropriate in the group that took BHI to carry out calculation using human or, better, BHI standard, which, however, was not available. In diabetic patients it appears that the same amount of insulin (either BHI or PPI) is required for blood glucose control, according to what is known through use of the artificial pancreas.

Long-term possible ideal blood glucose control with the assistance of an open-loop system leads to a reduction in BHI requirements, possibly through downregulation of insulin receptors in the periphery. However, as we have previously demonstrated,[32] the same occurs with other insulins as well and this is not characteristic of BHI.

The findings of Klier and co-workers (see Klier et al., this issue, pp. 193–195) are interesting in that they observed a greater delivery of dextrose through the AEP when they were administering BHI to their patients. From Klier's findings it can be concluded that probably BHI creates greater intracellular hypoglycemia, so that the AEP has to compensate by delivering more dextrose.

From our findings so far, as from the in vivo and in vitro studies of other authors, it seems that in the acute experiments BHI does not differ essentially from PPI or the fully synthetic human insulin. Of course, the question as to whether there is any difference during long-term use of this insulin regarding control of patients, possible development of antibodies that bind circulating insulin, and action in other metabolic systems, remains unanswered.

There is no doubt that the production of BHI through the recombinant DNA technique is a significant event, and it becomes more important if we consider that we have it "at hand," freeing us from dependence on animal insulin, which could conceivably run out (for example, if there were a severe epidemic resulting in large-scale death of pigs). Also it is chemically and biologically identical with human insulin and cheaper than fully synthetic human insulin.

In summary, the biologic activity of BHI, as well as its capacity to regulate blood glucose of diabetic patients, in comparison with PPI, was investigated in five healthy volunteers and five insulin-dependent diabetic subjects. The hypoglycemic effect of both insulins is the same; in addition, no statistically significant difference in circulating insulin and gastrin concentration was found for the BHI and PPI groups. BHI is in a position to inhibit C-peptide secretion and to increase plasma cortisol in a statistically significant greater degree than PPI. In contrast, PPI induces a greater increase of growth hormone than BHI. The feeling of hypoglycemia in the group that received BHI was significantly less intense than in the PPI group.

In diabetic patients who were connected to an artificial endocrine pancreas, insulin requirements did not differ between PPI and BHI groups. No skin reactions or other side effects were noted in either healthy volunteers or patients during the application of BHI.

We conclude that, quite probably, in these short experiments there is no clinically significant difference between BHI and PPI. The differences noted would be important for control of diabetic patients if they were observed during long-term treatment with BHI.

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From the Diabetes and Artificial Pancreas Unit, Department of Clinical Therapeutics, Athens University, Greece.

Address reprint requests to S. Raptis, P.O. Box 3127, Athens 606, Greece.

REFERENCES

[1] Goeddel, D. V., Kleid, D. G., Bolivar, F., Heyneker, H. L., Yansura, D. G., Grea, R., Hirose, T., Kraszewski, A., Itakura, K., and Riggs, A. D.: Expression in Escherichia Coli of chemically synthesized genes for human insulin. Proc. Natl. Acad. Sci. USA *76*: 106–10, 1979.

[2] Itakura, K., Hirose, T., Crea, R., Riggs, A. D., Heyneker, H. L., Bolivar, F., and Boyer, H. W.: Expression in Escherichia coli of a chemically synthesized gene for the hormone somatostatin. Science *198*: 1056–63, 1979.

[3] Crea, R., Kraszewski, A., Tadaaki, H., and Itakura, K.: Chemical synthesis of genes for human insulin. Proc. Natl. Acad. Sci. USA *76*: 5765–69, 1978.

[4] Miller, W. L., and Baxter, J. D.: Recombinant DNA—A new source of insulin. Diabetologia *18*: 431–36, 1980.

[5] Recombinant DNA Research. Policy Statements. Diabetes *27*: 877–78, 1978.

[6] Chance, R. E., Kroedd, E. P., and Hoffman, J. A.: Chemical, physical and biological properties of recombinant human insulin. *In*

Insulins, Growth Hormone and Recombinant DNA. Gueriguian, J., Miller, H. I., Schaffeburg, C. A., Gregoire, A. T., and Sobel, S., New York, Raven Press, 1980. In press.

[7] Keen, H., Pickup, J. C., Bilous, R. W., Glynne, A., Viberti, G. C., Jarrett, R. J., and Marsden, R.: Human insulin produced by recombinant DNA technology: safety and hypoglycaemic potency in healthy men. Lancet *2*: 398–401, 1980.

[8] Geneva, Helsinki, Sydney and Oslo Declarations, published by the World Medical Association. Br. Med. J. *1*: 687, 1973.

[9] Schmidt, F.: Methoden der Harn- und Blutzuckerbestimmung, Hexokinase G-6-PDH-Verfahren. *In* Pfeiffer, E. F., Ed. Handbuch des Diabetes Mellitus, vol. 2. München, Germany, Lehmann Verlag, 1971, pp. 936–46.

[10] Melani, F., Ditschuneit, H., Bartlet, K. M., Fridrich, H., and Pfeiffer, E. F.: Über die radioimmunologische Bestimmung von Insulin im Blut. Klin. Wochenschr. *43*: 1000, 1965.

[11] Beischer, W., Keller, L., Maas, M., Schiefer, M., and Pfeiffer, E. F.: Human C-peptide. Part I: radioimmunoassay. Klin. Wochenschr. 54: 709–16, 1976.

[12] Glick, S. M., Roth, J., Yalow, R. S., and Berson, S. A.: The regulation of growth hormone secretion. Rec. Prog. Horm. Res. *21*: 241–83, 1965.

[13] Schröder, K. E.: Radioimmunoassay for human growth hormone: problems and characteristics. Horm. Metab. Res. (Suppl. 3): 36–39, 1971.

[14] Rolleri, E., Zannine, M., Orlandini, S., and Malvano, R.: Direct radioimmunoassay of plasma cortisol. Clin. Chim. Acta 66: 319–27, 1976.

[15] Catt, K., and Tregear, G. W.: Solid phase radioimmunoassay in antibody-coated tubes. Science *158*: 1570–77, 1967.

[16] Raptis, S., Rothenbuchner, G., Schröder, K. E., Straub, K., Birk, J., and Pfeiffer, E. F.: Radioimmunologische Bestimmung von Gastrin im menschlichen Serum. Acta Endocr. Kbh. *152* (Suppl.): 10, 1971.

[17] Raptis, S., Dollinger, H. C., Berger, L. V., Schlegel, W., Schröder, K. E., and Pfeiffer E. F.: Effects of somatostatin on gastric secretion and gastrin release in man. Digestion *13*: 15–27, 1975.

[18] Thompson, J. C., Reeder, D. D., Bunchman, H. H., Becker, H. D., and Brandt, E. N.: Effect of secretion on circulating gastrin. Ann. Surg. *176*: 384–90, 1972.

[19] Pfeiffer, E. F., Thum, Ch., and Clemens, A. H.: The artificial beta-cell—a continuous control of blood sugar by external regulation of insulin infusion (glucose controlled insulin infusion system). Horm. Metab. Res. 6: 339–42, 1974.

[20] Pfeiffer, E. F.: Significance of renormalization of blood glucose concentration in diabetes mellitus. Horm. Metab. Res. 8 (Suppl.): 2–8, 1979.

[21] Pfeiffer, E. F., Thum, Ch., Ziegler, R., and Raptis, S.: Hypoglycemia in diabetics. Horm. Metab. Res. 6 (Suppl.): 112–26, 1976.

[22] Franetzki, M., Prestele, K., and Kresse H.: Technological problems of miniaturized insulin dosing devices and some approaches to clinical trials. Horm. Metab. Res. 8 (Suppl.): 58–65, 1979.

[23] Pfeiffer, E. F., and Raptis, S.: Controlled extension of oral antidiabetic therapy on former insulin dependent diabetics by means of the combined i.v. glibenclamide–glucose-response-test. Diabetologia 8: 41–47, 1972.

[24] Pfeiffer, E. F., Raptis, S., and Schröder, K. E.: Die Bedeutung der einmaligen kombinierten i.v. Glibenclamid-Glukose-Belastung für Auswahl, Indikationsstellung und Prognose der Glibenclamidbehandlung des sogenannten "insulinbedürftigen" Tolbutamid-resistenten Erwachsenendiabetes. Dtsch. Med. Wochenschr. 90: 1281–94, 1974.

[25] Märki, F., and Albrech, W.: Biological activity of synthetic human insulin. Diabetologia *13*: 293–95, 1977.

[26] Marchmont, R. J., and Honslay, M. D.: Insulin triggers cyclic AMP-dependent activation and phosphorylation of a plasma membrane cyclic AMP phosphodiesterase. Nature 286: 904–08, 1980.

[27] Diem, P., and Teuscher, A.: Immunologische Untersuschungen mit vollsythetischem humamem insulin bei Diabetikern. Schweiz. Mediz. Wochenschr. *109*: 1814–16, 1979.

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

[29] Pfeiffer, E. F.: Recognized diabetogenic hormones and diabetes in man. *In* On the Nature and Treatment of Diabetes. Leibel, B. S., and Wrenshall, G. A., Eds. Amsterdam, Excerpta Medica Foundation, 1965, pp. 368–86.

[30] Lundbaek, K.: Growth hormone's role in diabetic microangiopathy. Diabetes *25* (Suppl. 2): 845–49, 1976.

[31] Schülter, K., Petersen, K. G., and Kerp, L.: Unterschiedliche Gegenregulation der Hypoglykämie nach synthetischem Humaninsulin in Vergleich zu Schweinsulin. Akt. Endokrinol. *2*: 201, 1980.

[32] Raptis, S., Karaiskos, K., Hatzidakis, D., Zoupas, Ch., Diamantopoulos, E., and Moulopoulos, S.: Comparison of glycaemic and metabolic control in diabetics achieved by an artificial endocrine pancreas versus an open loop intravenous delivery system. Horm. Metab. Res. (Suppl.). In press.