Insulin Concentrations and Time-Action Profiles of Three Different Intermediate-acting Insulin Preparations in Nondiabetic Volunteers Under Glucose-controlled Glucose Infusion Technique

P. BOTTERMANN, H. GYARAM, K. WAHL, R. ERMLER, AND A. LEBENDER

This study describes the pharmacokinetics of three intermediate-acting insulin preparations, NPH porcine insulin, NPH human insulin (recombinant DNA), and "Depot-A" insulin, a mixture of 20% regular and 80% NPH human insulin from Eli Lilly and Company. Metabolic healthy normal weight volunteers were selected for the study. After overnight fasting, each test person received 0.4 U of each insulin per kg body weight injected subcutaneously in the triceps area of the arm. To prevent severe hypoglycemia, the test persons were connected to a "GCIIS Biostator" with blood glucose clamp at the 60 mg/dl level. Peripheral blood was sampled at regular intervals for glucose, insulin, and C-peptide determination. More elevated insulin levels were measured after application of both NPH human insulin and "Depot-A" insulin than after NPH porcine insulin. A more rapid decrease in the blood glucose concentration was observed after injection of both human insulin preparations than after porcine insulin. The dextrose output of the "GCIIS Biostator" was more pronounced in both human insulins than after the porcine preparation. After the injection of NPH human and NPH porcine insulin, significant differences were calculated between the concentrations of these two insulins in the blood, from the 2nd to the 10th hour ($P < 0.05$–$P < 0.005$) and between the dextrose output of the "GCIIS Biostator" from the 3rd to the 8.5th hour ($P < 0.05$). The fall of the C-peptide concentration to the lower detection limit of the assay reflects suppression of the endogenous B-cell secretion and confirms the measure of peripheral insulin concentrations as a result of the exogenously applied insulin. Although all investigations were performed under identical experimental conditions and equal dosages of each insulin were injected, higher insulin concentrations and a stronger biologic effect, shown by larger amount of dextrose delivered, were observed in both human insulins than in porcine insulin. Why this phenomenon occurs is as yet unclear. The clamp technique used with the "GCIIS Biostator" enables establishment of the biologic profile of any insulin, and thus represents a valuable tool in comparative studies. DIABETES CARE 5 (SUPPL. 2): 43–52, 1982.

For successful treatment of patients with insulin-dependent diabetes mellitus (IDDM) with subcutaneous insulin injections, it is necessary to know exactly the pharmacokinetics of the applied insulins. One has to give to each patient "the right insulin in the right dose at the right time." Therefore, the aim of this investigation was to study the behavior of the insulin concentration in blood after subcutaneous injection of three different insulin preparations. One of them was a well-established intermediate-acting porcine insulin, the two others were newly developed preparations of human insulin (recombinant DNA).

MATERIALS AND METHODS

The three different insulins that were tested are: (1) NPH porcine insulin (Eli Lilly and Company, Indianapolis, Indiana); (2) NPH human insulin (Eli Lilly and Company); and (3) "Depot-A" insulin, a mixture of 20% regular and 80% NPH human insulin (Eli Lilly and Company). All the insulin preparations are neutral solutions with a pH value of 7.4; all contained small amounts of metacresol as a preservative against bacterial destruction. The insulin concentration of all three preparations was 40 U/ml.

The insulins were tested not in diabetic patients, but in metabolic healthy normal weight volunteers. The age of the

test persons ranged from 22 to 30 yr. Prior to selection for the study, all volunteers underwent an oral glucose tolerance test (OGTT) with "Dextro-OGT" (Boehringer Mannheim Corp.),[1] containing 100 g oligosaccharides per 400 ml water, to exclude an impaired glucose tolerance[2] (fasting blood glucose < 100 mg/dl, after 1 h < 160 mg/dl, after 2 h < 120 mg/dl, after 3 h < 100 mg/dl).

Additionally, glycohemoglobin was determined as HbA_1 fraction by an own laboratory microcolumn method.[3] All values lay within the normal range (upper limit 7.7%).

After overnight fasting, 0.4 U per kg body weight of each insulin preparation was given in the triceps area of one arm subcutaneously. Prior to injection and at hourly intervals thereafter for 24 h, venous blood samples were collected from a vein of the other arm for determination of glucose, insulin, and C-peptide. The test persons fasted throughout the experiment.

As could be expected (that such a high dose of insulin like 0.4 U/kg body weight—a total amount of about 20–32 U)—would provoke severe hypoglycemia in nondiabetics) all test persons were connected to the glucose-controlled insulin infusion system "GCIIS Biostator" (Miles/Life Science Instruments) to prevent dangerous falls of blood glucose concentration. This apparatus,[4] commonly known as artificial B-cell, is normally used for treatment of diabetic patients.[5–7] It enables the continuous determination of blood glucose concentration from peripheral vein. From the actual blood glucose level and the rate of change in the blood glucose level, the momentary insulin need is established by an incorporated computer and subsequently infused intravenously. To prevent hypoglycemia after a possible overdose of insulin, the "GCIIS Biostator" possesses an additional feature to deliver dextrose.

In our experiments the "GCIIS Biostator" was programmed to infuse 20% dextrose solution in a counterregulatory manner when the blood glucose concentration falls below 60 mg/dl.

The control algorithms[6,8] for the rate of dextrose infusion were:

$$DR = RD\left(\frac{BD - G}{QD} + 1\right)^4;$$

$$\text{if } \frac{BD - G}{QD} + 1 \leq 0, \text{ then } DR = 0,$$

where RD = rate of dextrose infusion at BD; BD = the preprogrammed "desired" glucose level, where DR = RD; QD = the reciprocal of the static gain; G = the last minute's average glucose reading; and DR = dextrose infusion rate. By these algorithms, the rate of dextrose infusion is regulated in a "static" manner. Minute by minute, the difference between the actual and the desired value of blood glucose concentration is calculated and, according to the preselected constants, dextrose is infused above the desired glucose concentration, first in very small amounts, then rapidly increasing with falling blood glucose level. In a pilot study before it was seen, that with constants such as: RD = 50 (mg/min), BD = 60 (mg/dl), and QD = 25, the best counterregulatory effect was reached.[9] With these constants, it was possible to avoid oscillations of the actual blood glucose concentration around the desired value of 60 mg/dl, caused by the lag time of 90–120 s in measurement due to the void volume of the tubing system between the forearm vein and the glucose-measuring enzyme electrode in the apparatus.

Strictly speaking, the "GCIIS Biostator" was transformed in its function into a "glucose-controlled dextrose infusion system." Therefore, onset, intensity, and duration of biologic effect of each insulin applied can be estimated according to the dextrose infusion rate. Independent of the continuous blood glucose measurements of the "GCIIS Biostator," the blood glucose concentrations were determined in the venous blood samples by the glucose dehydrogenase method.[10] Insulin and C-peptide concentrations were measured radioimmunologically.[11,12] The lower limit of detection of C-peptide assay is 0.29 ± 0.02 ng/ml ($\bar{x}$ ± SEM.; N = 36).

Additionally, the average dextrose infusion rate for 30-min periods was calculated and drawn graphically reflecting the time-action profiles of the blood glucose-lowering effect of the applied insulins.

In a first series of experiments NPH porcine insulin and NPH human insulin (rDNA) were compared in a randomized fashion on the same seven volunteers. In a second series of experiments the NPH/regular mixture of human insulin (rDNA) was tested in another group of seven persons.

For the statistic analysis of the results the Student's *t* test was used.

RESULTS

The data (mean values and SEM) of the concentrations of glucose, insulin, and C-peptide in the venous blood samples and the calculated average dextrose infusion rates can be read from Tables 1–3 for each insulin tested. For better presentation and comparison, the mean value curves of insulin, glucose, dextrose-infusion rate, and C-peptide are shown for all three preparations in Figures 1–4.

Insulin concentration (*Figure 1*). After subcutaneous injection of all three insulin preparations, the insulin concentration in blood increases in the following 3–4 h. NPH porcine insulin gives a clearcut maximum of the insulin concentration after 3 h. Until the 4th hour, a sharp decline of the insulin concentration follows. Later on, until the end of the investigation, the insulin concentration falls in a nearly uniform manner.

After subcutaneous administration of both human insulin preparations, the maxima of the insulin concentrations are seen at the 4th hour. The increase of the insulin concentration after the "Depot-A" mixture is faster in comparison with the pure NPH preparation. Also the fall of the insulin concentration from the maximum at the 4th hour up to the 10th–12th hour is pronounced in the "Depot-A" preparation

TABLE 1
Behavior of blood glucose concentration, "GCIIS Biostator" dextrose output, and serum insulin and C-peptide concentration in seven metabolically healthy persons after s.c. injection of 0.4 U/kg body wt NPH porcine insulin under conditional dextrose delivery

Time (h)	Blood glucose (mg/dl)		DR (mg/min)		Insulin (pmol/L)		C-peptide (ng/ml)	
	x̄	SEM	x̄	SEM	x̄	SEM	x̄	SEM
−0.25	81.17	1.09			113.46	14.70	1.10	0.12
0	78.34	1.63			97.43	11.96	1.10	1.10
0.5			4.39	1.69				
1.0	74.58	2.32	9.50	4.74	172.62	25.51	0.84	0.08
1.5			12.73	6.36				
2.0	69.06	2.79	15.11	6.18	287.48	106.90	0.63	0.10
2.5			38.09	11.91				
3.0	64.06	4.37	72.32	18.57	366.75	109.51	0.49	0.09
3.5			73.75	19.72				
4.0	61.56	2.97	71.08	22.02	260.03	45.73	<0.29	—
4.5			86.09	21.65				
5.0	59.94	2.98	88.31	20.49	242.96	26.30	<0.29	—
5.5			89.50	19.13				
6.0	58.56	3.60	95.63	15.75	259.97	30.97	<0.29	—
6.5			128.23	21.69				
7.0	58.11	4.04	108.95	14.07	245.21	20.53	<0.29	—
7.5			111.25	14.77				
8.0	60.57	3.67	108.80	12.98	226.44	19.62	<0.29	—
8.5			105.87	13.13				
9.0	61.41	2.41	108.48	22.91	215.08	16.03	<0.29	—
9.5			108.39	16.52				
10.0	59.57	2.39	101.93	13.40	219.15	13.73	<0.29	—
10.5			103.17	13.48				
11.0	60.94	2.14	98.72	18.62	206.82	18.28	<0.29	—
11.5			89.14	17.03				
12.0	61.73	2.85	83.83	12.05	212.47	18.34	<0.29	—
12.5			87.58	8.63				
13.0	62.70	3.58	83.87	10.37	208.09	20.71	0.40	0.04
13.5			71.83	10.56				
14.0	66.36	3.38	76.46	15.56	171.35	19.31	0.36	0.02
14.5			88.40	14.28				
15.0	66.58	1.77	59.72	9.66	142.98	10.81	0.34	0.02
15.5			50.95	8.39				
16.0	66.04	2.24	47.81	7.21	145.65	16.94	0.34	0.02
16.5			51.47	7.09				
17.0	66.94	2.63	56.09	10.42	143.77	13.91	0.35	0.02
17.5			52.13	8.35				
18.0	66.68	2.98	51.95	3.09	144.20	14.39	0.34	0.02
18.5			82.70	26.26				
19.0	68.50	4.34	86.82	15.29	141.28	22.59	0.37	0.02
19.5			47.96	10.32				
20.0	67.48	4.02	45.81	9.69	125.79	11.60	0.46	0.10
20.5			38.80	9.81				
21.0	69.70	7.76	35.77	5.02	109.94	12.63	0.42	0.04
21.5			34.19	3.57				
22.0	67.90	2.55	31.07	3.66	116.92	13.73	0.40	0.04
22.5			32.06	5.99				
23.0	66.63	3.82	27.80	4.73	115.53	12.69	0.40	0.04
23.5								
24.0	65.25	2.16			109.21	16.09	0.38	0.03

x̄ = mean value; SEM = standard error of the mean; DR = dextrose infusion rate of the "GCIIS Biostator" as mean rate during the previous 30-min period.

TABLE 2
Behavior of blood glucose concentration, "GCIIS Biostator" dextrose output, and serum insulin and C-peptide concentration in seven metabolic healthy persons after s.c. injection of 0.4 U/kg body wt NPH human insulin under conditional dextrose delivery

Time (h)	Blood glucose (mg/dl)		DR (mg/min)		Insulin (pmol/L)		C-peptide (ng/ml)	
	x̄	SEM	x̄	SEM	x̄	SEM	x̄	SEM
−0.25	79.28	2.39			132.10	9.60	1.16	0.21
0	78.34	2.54			124.41	7.89	0.94	0.13
0.5			5.51	2.77				
1.0	73.74	3.09	6.66	1.97	205.77	15.12	0.72	0.12
1.5			14.79	3.11				
2.0	65.30	3.29	32.98	5.65	354.89	51.92	0.51	0.07
2.5			69.95	19.90				
3.0	54.65	2.03	113.44	30.96	353.44	17.35	<0.29	—
3.5			130.23	17.42				
4.0	53.14	2.09	135.68	15.37	453.93	40.03	<0.29	—
4.5			146.02	15.64				
5.0	57.18	2.51	172.20	17.80	401.68	44.30	<0.29	—
5.5			181.88	19.45				
6.0	55.51	2.97	176.70	20.23	417.72	62.31	<0.29	—
6.5			174.57	29.02				
7.0	55.93	3.28	195.06	29.32	385.78	37.79	<0.29	—
7.5			183.70	27.64				
8.0	57.40	1.68	171.55	29.32	401.09	37.53	<0.29	—
8.5			176.91	30.48				
9.0	58.18	2.19	160.58	33.73	333.07	40.81	<0.29	—
9.5			148.69	28.35				
10.0	51.41	2.85	141.60	25.36	315.19	52.05	<0.29	—
10.5			139.25	28.13				
11.0	57.84	2.40	138.80	28.86	279.51	20.90	<0.29	—
11.5			128.85	18.42				
12.0	60.67	2.59	124.14	17.65	259.40	21.29	<0.29	—
12.5			99.81	13.85				
13.0	62.94	3.00	97.40	9.71	228.57	19.72	0.34	0.02
13.5			91.29	13.23				
14.0	63.81	4.29	89.97	21.53	236.20	27.60	0.35	0.02
14.5			74.84	12.10				
15.0	66.71	4.73	69.90	13.66	201.10	25.37	0.46	0.11
15.5			71.03	9.85				
16.0	68.33	2.56	62.44	12.91	174.95	18.26	0.46	0.11
16.5			54.50	8.78				
17.0	71.27	3.59	59.03	18.18	182.96	27.08	0.53	0.17
17.5			44.77	5.85				
18.0	69.56	2.99	42.50	11.24	182.96	27.08	0.49	0.13
18.5			56.28	11.01				
19.0	69.34	2.72	45.78	10.45	156.87	18.93	0.51	0.10
19.5			44.57	10.78				
20.0	69.98	2.45	48.75	11.43	154.84	17.45	0.52	0.13
20.5			36.29	9.95				
21.0	71.33	3.33	38.59	6.04	148.33	16.50	0.47	0.09
21.5			38.55	7.67				
22.0	71.6	2.32	30.37	5.82	151.68	15.97	0.48	0.09
22.5			18.78	5.72				
23.0	71.88	1.33	18.01	5.52	150.04	15.25	0.45	0.07
23.5			13.29	4.50				
24.0	68.18	1.86			153.65	15.25	0.40	0.04

x̄ = mean value; SEM = standard error of the mean; DR = dextrose infusion rate of the "GCIIS Biostator" as mean rate during the previous 30-min period.

TABLE 3
Behavior of blood glucose concentration, "GCIIS Biostator" dextrose output, and serum insulin and C-peptide concentration in seven metabolic healthy persons after s.c. injection of 0.4 U/kg body wt "Depot-A" insulin (20% regular and 80% NPH human insulin) under conditional dextrose delivery

Time (h)	Blood glucose (mg/dl)		DR (mg/min)		Insulin (pmol/L)		C-peptide (ng/ml)	
	x̄	SEM	x̄	SEM	x̄	SEM	x̄	SEM
−0.25	84.03	1.14			122.5	12.94	1.17	0.19
0	80.57	2.26			142.6	23.48	1.05	1.13
0.5			9.47	9.34				
1.0	73.54	3.96	29.41	23.17	287.8	75.97	0.92	0.17
1.5			11.67	5.00				
2.0	66.27	4.10	46.23	18.73	440.8	114.46	0.48	0.08
2.5			65.56	25.05				
3.0	57.87	3.66	86.14	27.70	458.1	130.65	<0.29	—
3.5			124.57	26.24				
4.0	55.83	3.10	135.13	19.65	485.9	103.98	<0.29	—
4.5			153.71	24.72				
5.0	55.60	2.66	149.01	25.43	382.2	56.41	<0.29	—
5.5			135.89	25.72				
6.0	57.24	2.17	131.27	24.62	356.7	38.85	<0.29	—
6.5			123.45	23.93				
7.0	59.27	2.97	115.76	25.72	330.4	20.14	<0.29	—
7.5			114.05	29.40				
8.0	60.66	2.52	100.67	27.86	306.5	22.10	<0.29	—
8.5			93.98	27.03				
9.0	62.64	3.19	89.41	30.40	283.5	24.62	<0.29	—
9.5			80.45	24.33				
10.0	60.53	2.27	77.19	21.05	285.6	43.79	<0.29	—
10.5			81.84	21.73				
11.0	63.66	3.76	86.79	27.08	270.1	44.93	0.32	0.02
11.5			73.82	21.14				
12.0	67.04	3.17	84.96	20.92	252.2	41.16	0.32	0.02
12.5			60.56	15.30				
13.0	65.13	2.67	54.68	13.67	284.4	41.95	0.35	0.03
13.5			58.73	13.95				
14.0	66.60	2.53	80.49	21.52	223.7	36.30	0.38	0.04
14.5			40.56	9.19				
15.0	67.17	3.99	30.49	8.11	200.7	28.98	0.36	0.04
15.5			43.15	13.76				
16.0	68.33	3.88	28.10	5.46	186.5	27.11	0.39	0.04
16.5			41.19	16.94				
17.0	67.29	2.25	46.38	22.19	179.8	29.45	0.49	0.08
17.5			44.14	23.99				
18.0	70.54	3.03	33.84	15.69	171.5	29.04	0.52	0.11
18.5			47.25	34.12				
19.0	70.74	2.60	45.53	25.38	155.6	23.54	0.47	0.08
19.5			41.44	18.29				
20.0	71.60	1.80	32.41	13.69	143.9	20.26	0.44	0.04
20.5			21.18	8.54				
21.0	72.14	2.41	14.41	6.31	128.4	11.33	0.46	0.04
21.5			8.97	3.29				
22.0	74.63	4.03	10.24	4.99	133.8	15.43	0.42	0.04
22.5			12.21	3.91				
23.0	71.89	1.38	13.67	5.33	125.0	15.10	0.46	0.04
23.5			8.32	2.71				
24.0	71.10	1.95	10.19	3.19	122.9	17.04	0.47	—

x̄ = mean value; SEM = standard error of the mean; DR = dextrose infusion rate of the "GCIIS Biostator" as mean rate during the previous 30-min period.

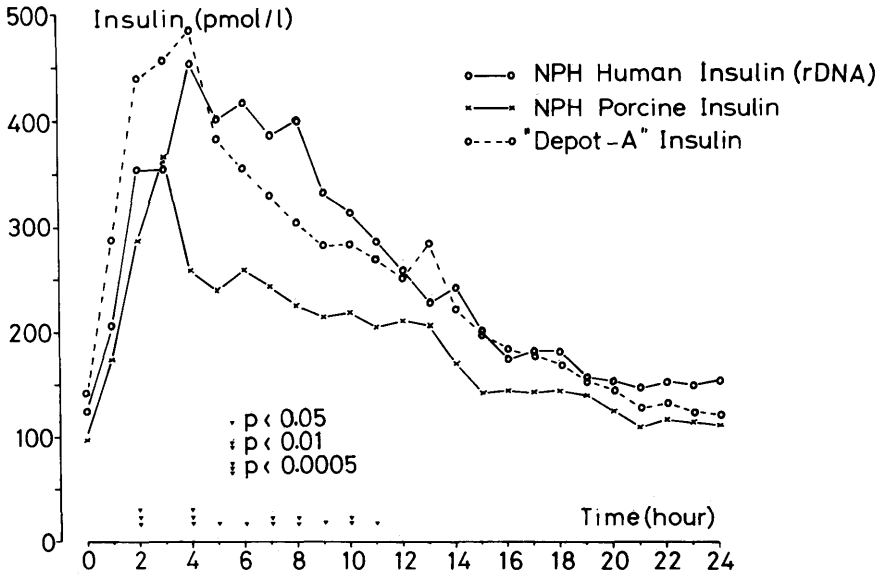

FIG. 1. Behavior of the serum insulin concentration in seven metabolic healthy persons after s.c. injection of 0.4 U/kg body wt NPH porcine insulin, NPH human insulin, and "Depot-A" insulin, a mixture of 20% regular and 80% NPH human insulin. The black triangles denote the time where significant differences exist between NPH human insulin and NPH porcine insulin.

in comparison with the pure NPH human insulin.

Comparing the porcine NPH insulin with the two human insulin preparations, one can see that at all times the insulin levels following administration of the human insulin preparations are higher than after application of the porcine preparation. Statistical analysis which was performed between the two NPH preparations shows that from the second to the 10th hour after subcutaneous injection the differences are significant ($P < 0.05$–$P < 0.0005$).

Glucose concentration (*Figure 2*). As can be expected, initially a quick drop of the blood glucose concentration is seen after subcutaneous injection of the above-mentioned insulins. According to the faster increase of the insulin concentration after application of the both human insulin preparations, the initial decrease of the blood glucose level is pronounced in these experiments. Between the 2nd and the 3rd hour after insulin injection, the blood glucose concentration falls slightly below the 60-mg/dl level for the following 5–10 h. After administration of the NPH porcine insulin preparation the blood glucose decrease is more protracted;

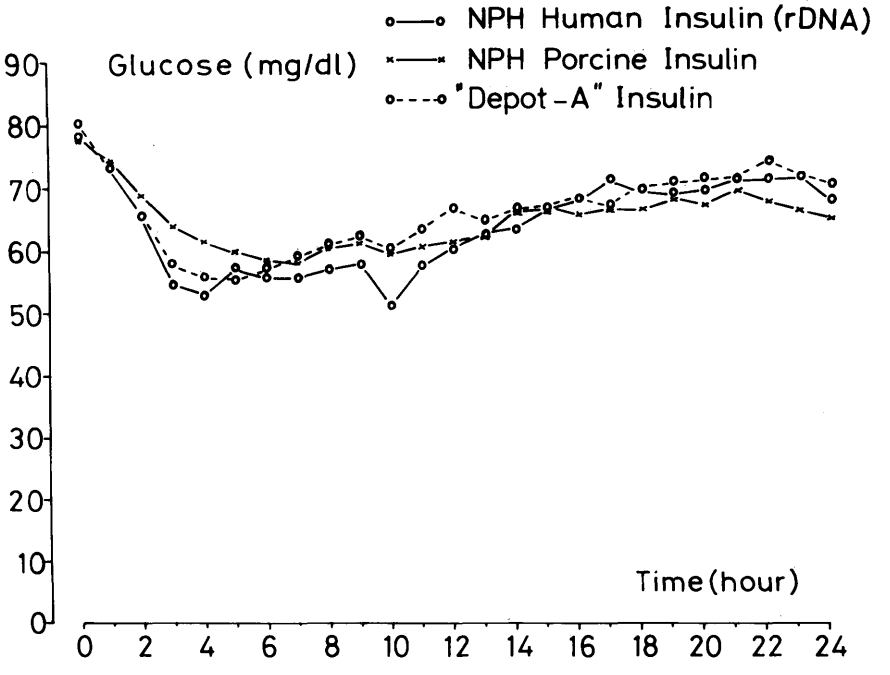

FIG. 2. Behavior of the blood glucose concentration in seven metabolic healthy persons after s.c. injection of 0.4 U/kg body wt NPH porcine insulin, NPH human insulin, and "Depot-A" insulin, a mixture of 20% regular and 80% NPH human insulin.

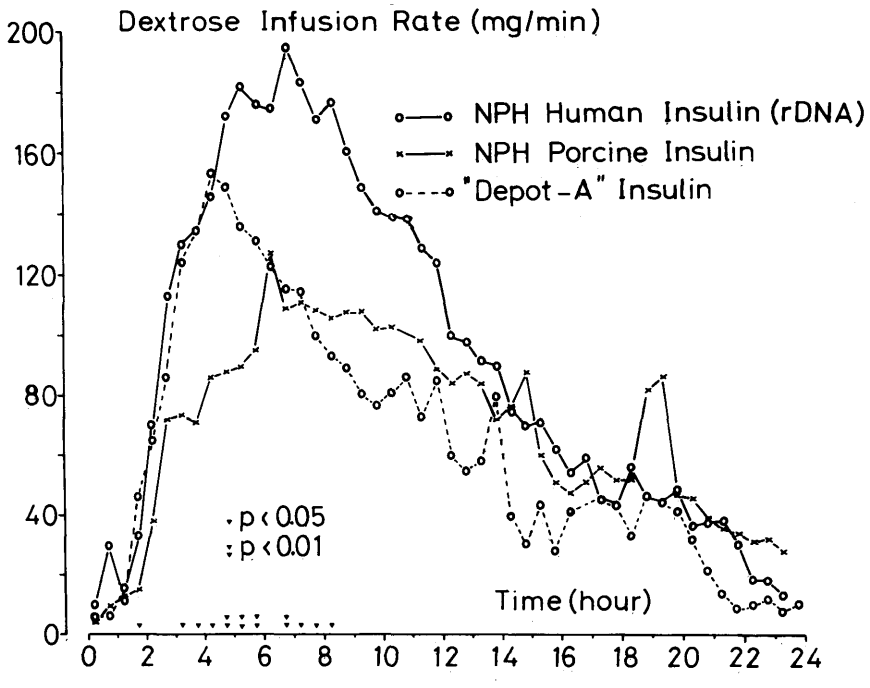

FIG. 3. Behavior of the dextrose infusion rate of the "GCIIS Biostator" in seven metabolic healthy persons after s.c. injection of 0.4 U/kg body wt NPH porcine insulin, NPH human insulin, and "Depot-A" insulin, a mixture of 20% regular and 80% NPH human insulin. The black triangles denote the time where significant differences exist between NPH human insulin and NPH porcine insulin.

therefore, the 60 mg/dl level is reached later around the 5th hour after insulin injection. But all these differences were statistically not significant.

After the several nadirs, the increase of the blood glucose concentration proceeds in the same amount in all three insulin preparations. But until the end of the test period the initial blood glucose level is not reached.

Dextrose infusion rate (*Figure 3*). The dextrose infusion rate (DR) shows how much dextrose is necessary to prevent the blood glucose concentration from falling below 60 mg/dl level. Reflecting the onset and intensity of the blood glucose lowering effect of the applied insulins the "GCIIS Biostator" delivers glucose in increasing amount.

In the first 2 h, the increase in the dextrose infusion rate is nearly identical in all three insulins. Then the curve of the dextrose infusion rate is flattened in NPH porcine insulin, reaching its maximum between the 6th and 6.5th hour after insulin injection. In comparison, after NPH human insulin the rapid increase of the dextrose infusion rate lasted longer according to the higher increase of the insulin concentration and gives higher values, but reaches the maximum at nearly the same time as NPH porcine insulin.

After administration of the "Depot-A" preparation the maximum of the dextrose infusion rate is seen about the 4th hour after injection.

In all three insulins, a continuous fall of the dextrose infusion rate follows the maxima. But at the end of the test periods, the infusion rate is slightly elevated according to the blood glucose levels, which are not normalized completely, as seen before (Figure 2). The oscillations of the dextrose infusion rate in the last hours of the test period, seen in the "Depot-A" and the NPH porcine insulin preparations, can be attributed more to technical problems in the continuous blood sampling by the venous double lumen catheter in some test persons than to any biologic effect of the insulins.

As dextrose infusion rate curves represent the actual profiles of the biologic effect of the insulins, one can see that NPH human insulin has a higher hypoglycemic potency than NPH porcine insulin. The differences between the dextrose infusion rates are statistically significant from the 3rd to the 8.5th h. The dextrose infusion rate of "Depot-A" insulin shows a significant difference to NPH human insulin at 7, 8.5, 9.5, 10, 11.5, and 12.5 h after injection ($P < 0.05$).

C-peptide concentration (*Figure 4*). The C-peptide concentration reflects the endogenous B-cell secretion. After application of all three types of insulin, the C-peptide levels fall in the first hours of the test period to the lower detection limit of the assay. The small C-peptide increase in the second part of the test period correlates in time with the rise of the blood glucose level over 60 mg/dl, as well as with the fall of the dextrose infusion rate and the fall in the insulin concentration.

DISCUSSION

The method described in this study enables the establishment of insulin time-action profiles in nondiabetic persons, and the profiles point out clearly the onset, intensity, and duration of the biologic effect of different insulin preparations. The occurence of severe hypoglycemia, which is expected after application of 0.4 U insulin per kg body weight, can be prevented by the counterregulatory dextrose delivery from the "GCIIS Biostator." Below an adjustable blood glucose level, the ap-

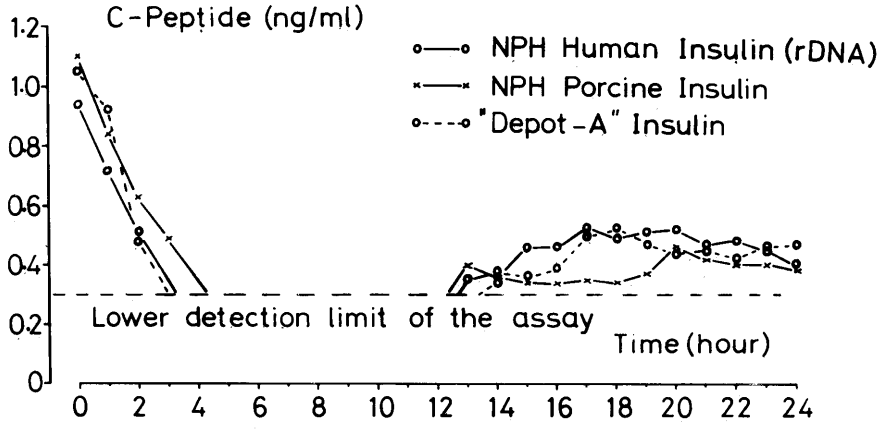

FIG. 4. Behavior of the serum C-peptide concentration in seven metabolic healthy persons after s.c. injection of 0.4 U/kg body wt NPH porcine insulin, NPH human insulin, and "Depot-A" insulin, a mixture of 20% regular and 80% NPH human insulin.

paratus hinders a further fall in the blood glucose concentration through compensatory dextrose output until the hypoglycemic effect of the insulin applied, fades away. From the time and intensity of the dextrose delivery from the "GCIIS Biostator" the biologic activity of the insulins can be estimated. The serum insulin curves and the corresponding insulin biologic, time-action profile curves show similar configuration.

The measured insulin must be of exogenous origin; this means injected insulin, because of the endogenous B-cell secretion is suppressed as one can see by the C-peptide levels.

At the beginning of our experiments with estimating time-action profiles of insulins with the help of the counterregulatory dextrose delivery of the "GCIIS Biostator," we tried to clamp the blood glucose level at the fasting blood glucose values of the test persons. But pilot studies showed unsatisfactory results. Firstly, it was seen that it was not possible to clamp exactly at the fasting value. The "static" algorithm of the "GCIIS Biostator" for dextrose infusion needs a difference between the actual blood glucose value and the preprogrammed value in order that the computer of the apparatus is able to calculate the amount of dextrose that has to be infused. Depending on the constants given to the apparatus' computer, therefore, either the blood glucose concentration is allowed to fall slightly below the preprogrammed value, in which case the apparatus infuses dextrose in amounts to hinder a further continuous decrease of glucose concentration, or the blood glucose concentration is not allowed to fall below the preprogrammed level. In this case, the apparatus will infuse large amounts of glucose in short time intervals over short periods. This provokes oscillations of the blood glucose concentration around or above the preprogrammed fasting value. Furthermore, the bursts of glucose from the apparatus with rapid blood glucose increases stimulate the endogenous B-cell secretion. Thereafter, it is difficult to decide how much of the insulin concentration measured is of exogenous or endogenous origin. Secondly, at the beginning of the studies, we believed that the dextrose infusion rate would give a clearcut time-related parameter of the insulin biologic action. We hoped that the dextrose delivery from the apparatus would stop when the blood-glucose-lowering effect of the administered insulin had finished. Instead of this, the apparatus infused continuously small amounts of dextrose, presumably substituting the endogenous glucose production of the fasting state. To a certain degree, this change of endogenous glucose production to exogenous glucose delivery by the apparatus is also observed under the present experimental conditions.

Therefore, the desired blood glucose level is preprogrammed at 60 mg/dl to avoid overshooting dextrose infusion with stimulation of endogenous insulin secretion. As is well-known, at this level the B-cell secretion is suppressed. This can be seen in our experiments also by the C-peptide levels, which fall to the lower detection limit of the assay. Only in the last part of the test period together with the slow increase of the blood glucose level, there is a slow increase of the C-peptide concentration.

Since during the time of C-peptide suppression, the measured insulin must be of exogenous origin, the initial increase and the following fall of the insulin concentration reflect the pharmacokinetics of the injected insulin. The dextrose infusion rate roughly reflects the biologic activity of the administered insulin, giving time-action profiles. As one can see, there is a good correlation between the curves of the insulin concentration and the dextrose delivery rates.

To a certain amount endogenous glucose production by counterregulatory secretion of glucagon may influence the time-action profiles and may lower the absolute amount of the dextrose infusion rates. This could have been circumvented by another experimental design with continuous somatostatin infusion. Indeed, together with glucagon, the endogenous insulin secretion would have been suppressed. But this would have complicated the experimental procedure in the healthy volunteers. Furthermore, the primary interest in these studies was not to look at the absolute amount of glucose, which is necessary to compensate a certain amount of injected insulin in a normal, nondiabetic person, but was to look at the behavior of the insulin concentration as a result of subcutaneous application and absorption to compare

several insulin preparations with another. The dextrose infusion rate gives additional information which allows the estimation of the biologic potency of the insulin preparations.

Considering the results in detail, this investigation shows that every insulin preparation manifests a characteristic kinetic behavior with corresponding time-action biologic profiles.

The most interesting point is the astonishing difference between the NPH porcine insulin and NPH human insulin. Both preparations were given in the same amount to the same subcutaneous region of the same test persons under the same experimental conditions. But the insulin level as well as the dextrose infusion rate shows a clearcut higher increase after the human than after the porcine preparation.

The difference between the insulin concentrations cannot be explained by insufficient radioimmunologic measurements. Normally, human insulin in blood is determined against a human insulin standard. Really, the insulin antibody in our assay reacts to a lesser degree with porcine than with human insulin. But this fact was compensated for by measuring against a porcine standard. Additionally, the time-action profile of the dextrose infusion rate shows significantly lower values after NPH porcine than NPH human insulin. The differences may be explained by a better absorption of the NPH human than the NPH porcine insulin from the injection site or a smaller extent of degradation of human than porcine insulin by subcutaneous tissue.

Human insulin is more hydrophilic than porcine insulin due to threonin instead of alanin in the B-30 position of the molecule. Whether the discrepancy noted in our study is a result of this property or of small galenic differences between the preparations cannot be decided.

Between the two human preparations the differences exist because the "Depot-A" preparation consists of only 80% NPH and 20% regular human insulin in comparison with the pure NPH human insulin preparation. Because of the regular part the increase of the insulin concentration after "Depot-A" injection occurs faster. Also, the fall of the insulin concentration from the maximum is faster in the first hours, as the NPH part is smaller. But all differences are small and statistically not significant. The prolonged effect of the NPH human preparation may be shown by the dextrose infusion rate, which is more pronounced.

The aim of this investigation was to show that every insulin preparation manifests its own characteristics. This is useful in meeting satisfactorily the individual need of an insulin-dependent diabetic patient. To avoid misunderstandings, it must be emphasized that these studies have been established after subcutaneous injection, which is the conventional application route. Naturally, application of insulin by other routes like i.v., i.m., or i.p. under identical experimental conditions would give different profiles and kinetics because of the different bioavailability of the insulin preparations.[13–17]

The pharmacokinetics of the insulins applied depend on many factors. In patients, muscle exercise, injection site, local skin temperature, and other factors have a great influence on the insulin bioavailability. However, in group studies such as those described here, these factors can be neglected because the test persons stayed in bed for the experiment. The injection site was the same. Therefore, this method justifies its reliability for investigating the hypoglycemic potence of any insulin preparation as general orientation in the insulin pharmacokinetics.

From the II. Medizinische Klinik und Poliklinik der Technischen Universität München, Klinikum rechts der Isar, München, West Germany.

Address reprint requests to P. Bottermann, II. Medizinische Klinik und Poliklinik der Technischen Universität München, Klinikum rechts der Isar, Ismaninger Straße 22, D-8000 München 80, West Germany.

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