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# Proinsulin

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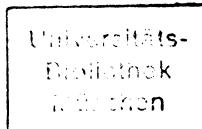
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## Comparative Study of Hormonal Counterregulation During GCIIS-Guided Hypoglycemia Tests Using Human Proinsulin and Human Insulin (Recombinant DNA)

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### Summary

Counterregulatory effects following administration of biosynthetic human proinsulin (BHPI) and human insulin (BHI) were compared during hypoglycemia standardized by means of a glucose controlled insulin infusion system (GCIIS).

A total of  $0.148 \pm 0.010$  U/kg of BHPI had to be given by the GCIIS in order to obtain a minimal blood glucose (BG) of  $26 \pm 2$  mg/dl ( $\bar{x} \pm \text{SEM}$ ) at  $43 \pm 2$  min. In contrast,  $0.083 \pm 0.004$  U/kg of BHI were sufficient to produce a minimal BG of  $21 \pm 1$  mg/dl (n.s.) at  $35 \pm 1$  min. ( $P < 0.005$ ). Moreover, BHPI infusion resulted in prolonged hypoglycemia and delayed blood glucose recovery. On a molar basis, the acute BG lowering effect of BHPI was about 13% that of BHI (BHPI  $3.94 \pm 0.27$  vs. BHI  $0.51 \pm 0.03$  nmol/kg). Serum proinsulin after BHPI reached its

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maximum of  $19.4 \pm 2$  pmol/ml at 20 min, and still exceeded basal values markedly at the end of the test period at 240 min. Serum insulin peaked at 10 min. ( $162 \pm 48$   $\mu$ U/ml) and had already returned to basal values ( $7.5 \pm 1$   $\mu$ U/ml) after 45 min. No severe side effects were observed and there was no need for glucose administration, but clinical symptoms of hypoglycemia were more pronounced after BHPI. Compared to BHI, BHPI produced a higher cortisol peak ( $252 \pm 16$  vs.  $168 \pm 10$  ng/ml), a more pronounced secretion of ACTH and GH as well as a stronger decline of serum potassium ( $3.20 \pm 0.06$  vs.  $3.58 \pm 0.08$  mmol/l). Counterregulatory prolactin secretion did not differ significantly. Urinary epinephrine secretion following hypoglycemia after BHPI exceeded that after BHI ( $10.3 \pm 4.8$  vs.  $3.0 \pm 0.5$  ng/120 min.). Serum lactate increase after BHPI was more prolonged ( $1.68 \pm 0.24$  vs.  $0.37 \pm 0.14$  mmol/l at 120 min.). BHPI-induced inhibition of lipolysis, as determined by free fatty acid patterns, was delayed and less pronounced.

Our results indicate that the observed more distinct glucose counterregulation is due to prolonged hypoglycemia rather than to any specific BHPI action on the hypothalamic-pituitary axis. We regard this as a consequence of the prolonged circulating and biological half-life. A preferential proinsulin action on the liver may play an additional role. Whether this "depot effect" may be beneficial in the treatment of diabetes mellitus remains to be established.

**Key-Words:** *Human Proinsulin, Recombinant DNA Technology, Glucose Counterregulation, Glucose Controlled Insulin Infusion System*

## Introduction

Recently, large amounts of human proinsulin have become available by recombinant DNA technology (Frank, Pettee, Zimmerman and Burck 1981), thereby facilitating extensive metabolic in vitro and in vivo studies.

Investigations carried out in healthy volunteers up to now, have shown distinct differences in hormone effects between human proinsulin and insulin.

Apart from its relatively weak biological activity, human proinsulin is more potent in suppressing hepatic glucose production than in stimulating peripheral glucose disposal (Revers, Henry, Schmeiser, Kolterman, Cohen, Bergenstal, Polonsky, Jaspan, Rubenstein, Frank, Galloway and Olefsky 1984). In addition, the deactivation of the in vivo actions of proinsulin is markedly prolonged and, as a striking feature, the recovery of hepatic glucose production following cessation of proinsulin infusion is disproportionately delayed (Glauber, Revers, Henry, Schmeiser, Wallace, Kolterman, Cohen, Rubenstein, Galloway,

Frank and Olefsky 1986). These findings may be partially explained by pharmacokinetic factors such as circulating half-life (Glauber et al. 1986) metabolic clearance rate (Revers et al. 1984), distribution volume and compartmentalization (Bottermann, Gray, Zilker, Heinzel, Ermler, Wahl and Lebender 1985). Furthermore, differences in the cellular actions of proinsulin and insulin may play a role.

The prolonged blood glucose-lowering effect of proinsulin as well as its preferential sustained action on the liver could be of therapeutic benefit in the treatment of diabetes mellitus (Bergenstal, Cohen, Lever, Polonsky, Jaspan, Blix, Revers, Olefsky, Kolterman, Steiner, Charrington, Frank, Galloway and Rubenstein 1984).

Keeping this potential therapeutic use in mind, we decided to compare the counterregulatory effects following the administration of either biosynthetic human proinsulin (BHPI) or human insulin (BHI) during hypoglycemia tests guided by a glucose controlled insulin infusion system (GCIIS). In addition, myocardial contractility was determined during hypoglycemia noninvasively.

## Materials and Methods

### Subjects

8 healthy volunteers (3 female, 5 male;  $25.3 \pm 1.0$  years;  $63.4 \pm 3.0$  kg (mean  $\pm$  SEM), taking no medications, were studied. Each subject underwent 2 hypoglycemia tests: one with human proinsulin and one with human insulin. The study was approved by the ethic committee of the Medical University Lübeck. All subjects gave written informed consent.

### Materials

Biosynthetic human proinsulin (BHPI) was prepared and characterized as previously described (Frank et al. 1981) and was supplied by Eli Lilly and Company, Indianapolis, Indiana/USA (CT-5765-2C). Biosynthetic human insulin (Biohumaninsulin normal) was provided by Eli Lilly and Company, Bad Homberg/FRG.

### Glucose Controlled Insulin Infusion System (GCIIS)

The details of the GCIIS (Biostator<sup>®</sup>, Life Science Instruments, Miles Laboratories, Elkhart, Indiana/USA) have been described elsewhere (Pfeiffer, Thum and Clemens 1974; Fogt, Dodd, Jenning and Clemens 1978; Clemens, Chang and Myers 1978). The Biostator GCIIS was used on static control. The following constants were chosen: BI 35, QI 10, RI 20, FI 300 (Müller-Esch, Ball, Heidbüchel, Wood and Scriba 1984).

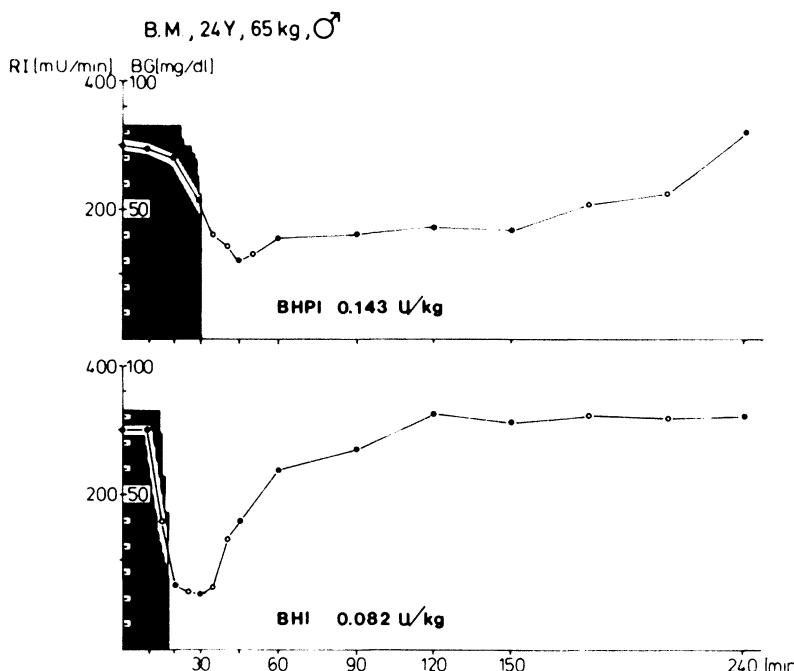


Fig. 1 Blood glucose (BG) and infusion rates (RI) of bio-synthetic human proinsulin (BHPI) and biosynthetic human insulin (BHI) during GCIS-guided hypoglycemia tests in a healthy volunteer. The total dosage administered is 0.143 U/kg for BHPI and 0.082 U/kg for BHI.

### Experimental Protocol

After an overnight fast and bed rest, the subjects were connected to the GCIS between 8 a.m. and 9 a.m. Feedback controlled infusion of BHPI or BHI was discontinued and the device was only used for blood glucose monitoring when blood glucose had fallen by 40% of baseline values and initial clinical symptoms of hypoglycemia occurred (Fig. 1). Venous blood samples were drawn at  $-10, \pm 10, +10, +20, +30, +45, +60, +90, +120, +150$  and  $+240$  min., respectively, from an indwelling catheter placed in an antecubital vein. In addition, urine was collected during four 120 min. periods before, during and after hypoglycemia tests.

### Analytical Methods

Serum ACTH (INC; Stillwater, Minnesota/USA), cortisol (Clinical Assays; München/Germany), GH (Sorin, Hamburg/Germany), prolactin (Becton-Dickinson; Heidelberg/Germany), insulin (Sorin; Hamburg/Germany) and C-peptide (Mallinkrodt; Dietzenbach/Germany) were determined by commercially available radioimmunoassays.

Serum proinsulin measurements were carried out by Eli Lilly and Company, using a recently developed specific radioimmunoassay (Cohen, Nakabayashi, Blix, Rue, Shoelson, Root, Frank, Revers and Rubenstein 1985).

Urinary epinephrine was determined by HPLC with electrochemical detection (Kraas, Schütt and Knuppen 1982).

Free fatty acids (FFA) were measured by HPLC (Ikeda, Shimada and Sakaguchi 1983).

Serum potassium was determined by flame photometry, serum lactate enzymatically.

### Systolic Time Intervals

In 6 volunteers, systolic time intervals (PEP = pre-ejection period; LVET = left ventricular ejection time; QS2 = electromechanical systole and Q = PEP divided by LVET (Weissler, Harris and Schoenfeld 1969) were continuously monitored before the hypoglycemia tests, immediately after stopping feedback controlled proinsulin or insulin infusion and at 150 min. (AVL Myocard Check 970; Schaffhausen/Switzerland).

### Statistical Methods

Results are expressed as mean  $\pm$  SEM. Wilcoxon's test for paired differences and analysis of variance for repeated measures over time (Winer 1971) were used.

### Results

Figures 2–6 and Tables 1–4 show the results obtained with BHPI and BHI. The curves were achieved by calculating the mean  $\pm$  SEM at identical points of time, whereas Table 1 gives the mean  $\pm$  SEM of the individual peak or nadir levels, which differed slightly in time from the overall means.

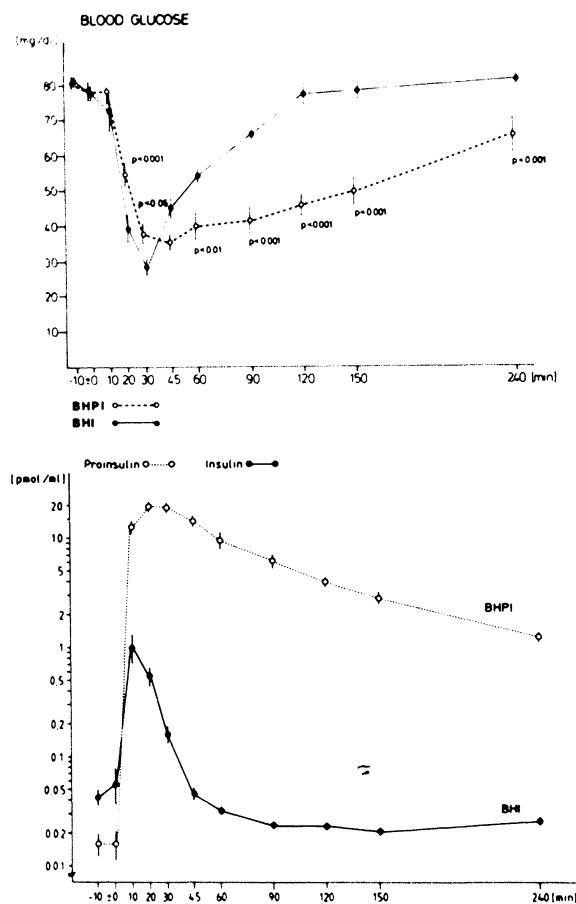


Fig. 2 Blood glucose levels during GCIIS-guided hypoglycemia tests with BHPI (○-----○) and BHI (●—●) in 8 healthy volunteers ( $\bar{x} \pm \text{SEM}$ ). The corresponding proinsulin serum levels (○-----○) during BHPI-infusion and serum insulin levels (●—●) during BHI-infusion on a logarithmic scale are also shown.

A total of  $0.148 \pm 0.010$  U/kg of BHPI had to be given by the GCIIS in order to obtain a minimal blood glucose of  $26 \pm 2$  mg/dl at  $43 \pm 2$  min. (Tables 2-3). In contrast,  $0.083 \pm 0.004$  U/kg of BHI ( $P < 0.001$ ) were sufficient to produce a minimal blood glucose of  $21 \pm 1$  mg/dl (n.s.) at  $35 \pm 1$  min. ( $P < 0.005$ ). Feedback controlled insulin infusion was stopped after  $18 \pm 4$  min., whereas proinsulin infusion was discontinued after  $29 \pm 5$  min. ( $P < 0.001$ ).

BHI administration resulted in prolonged hypoglycemia and delayed blood glucose recovery as compared to BHI (Fig. 2). Accordingly, the calculated area under the blood glucose curve (AUC) was significantly lower for BHPI than for BHI (BHPI:  $12.209 \pm 776$  vs. BHI:  $16.350 \pm 326$  mg/dl·min.;  $P < 0.01$ ).

On a molar basis, the blood glucose lowering effect of BHPI was about 13% that of BHI (BHPI  $3.94 \pm 0.27$  vs. BHI  $0.51 \pm 0.03$  nmol/kg).

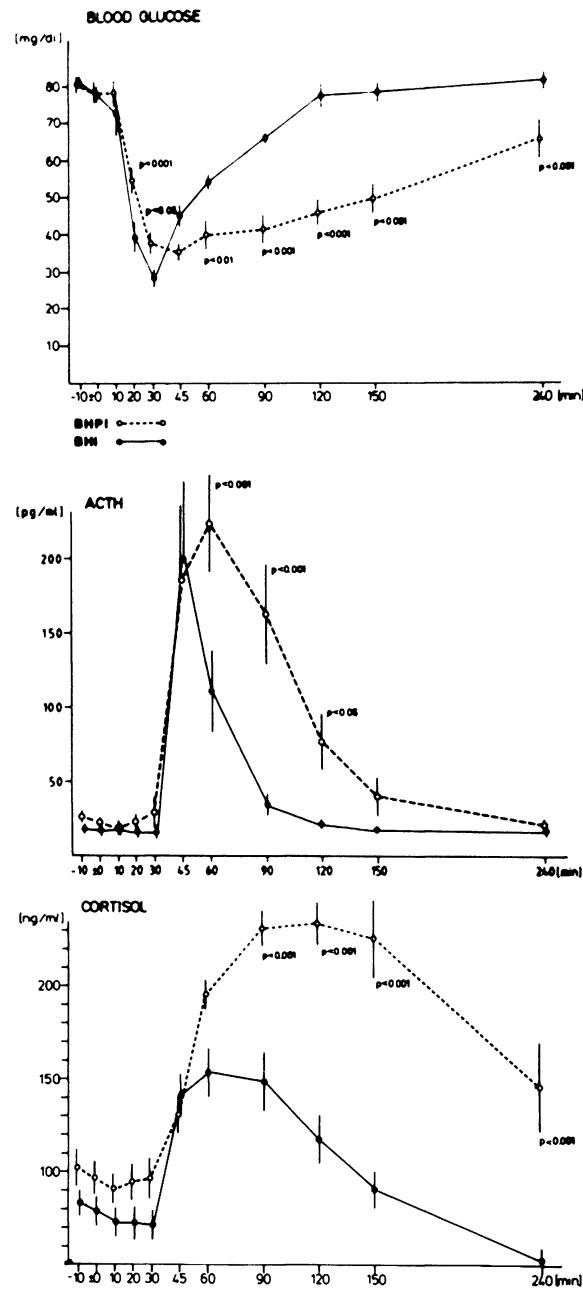


Fig. 3 Serum levels for ACTH and cortisol during GCIIS-guided hypoglycemia tests with BHPI (○-----○) and BHI (●—●) in 8 healthy volunteers ( $\bar{x} \pm \text{SEM}$ ).

No severe side effects were observed and there was no need for glucose administration, but clinical symptoms of hypoglycemia were more pronounced after BHPI infusion.

The basal serum proinsulin level was  $0.02 \pm 0.01$  pmol/ml; during BHPI-infusion, a maximum of  $19.4 \pm 2$  pmol/ml was reached after 20 min. (Fig. 2). At the end of the test period (240 min.) serum proinsulin levels still exceeded basal values markedly. In contrast, serum insulin peaked with  $162 \pm 48$   $\mu\text{U}/\text{ml}$  at

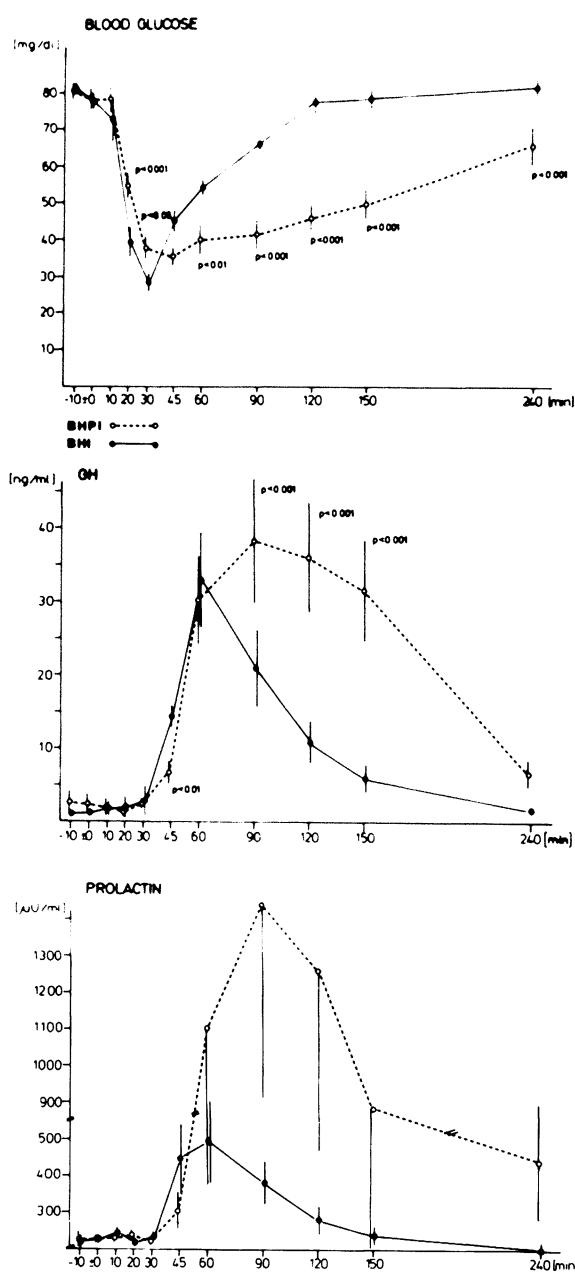


Fig. 4 Serum levels for GH and prolactin during GCIIS-guided hypoglycemia tests with BHPI (○-----○) and BHI (●—●) in 8 healthy volunteers ( $\bar{x} \pm \text{SEM}$ ).

10 min. and had already returned to basal values ( $7.5 \pm 1 \mu\text{U}/\text{ml}$ ) after 45 min.

When comparing serum proinsulin and insulin levels on a molar basis, serum proinsulin concentration required for the induction of hypoglycemia exceeded that of insulin 20-fold.

Mean basal C-peptide levels before proinsulin and insulin infusion were identical ( $1.1 \pm 0.1 \mu\text{g}/\text{l}$ ). BHI-induced C-peptide inhibition reached its maximum between 45 and 90 min. ( $0.41 \pm 0.07 \mu\text{g}/\text{l}$ ).

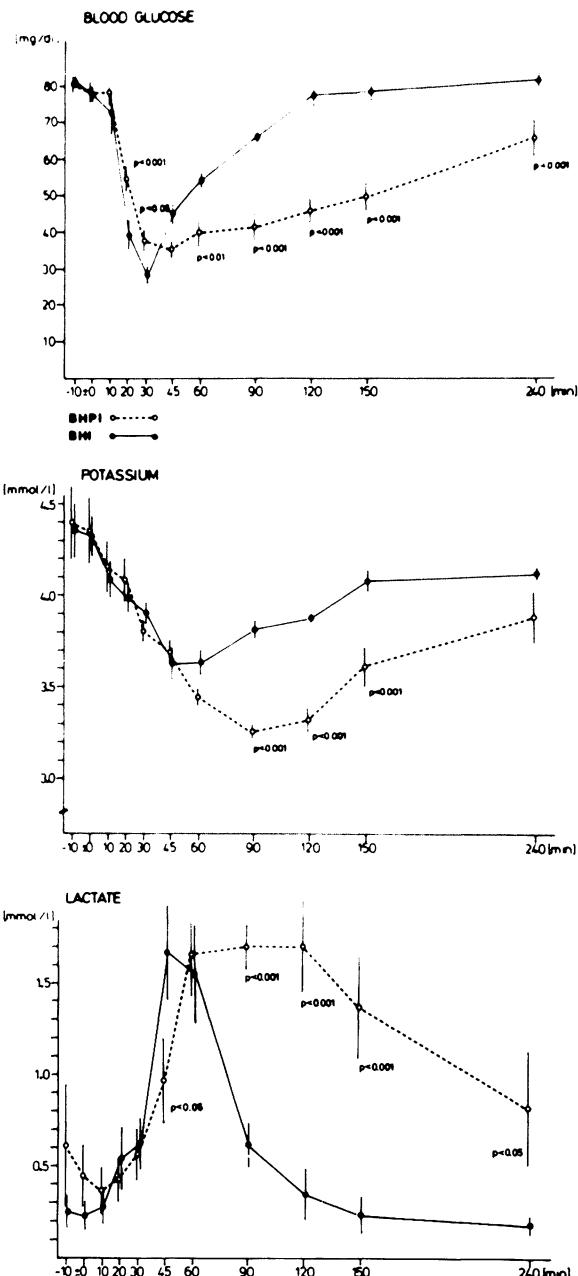


Fig. 5 Serum potassium and lactate during GCIIS-guided hypoglycemia tests with BHPI (○-----○) and BHI (●—●) in 8 healthy volunteers ( $\bar{x} \pm \text{SEM}$ ).

Due to the known cross-reactivity between C-peptide and proinsulin in the C-peptide assay used, C-peptide serum levels during proinsulin infusion could not be determined. This problem would have been overcome by a preceding proinsulin extraction (Revers et al. 1984) or by using a specific C-peptide assay (Hampton, Beyzavi and Marks 1985); however, both methods were not available.

Compared to BHI, hormonal counterregulation after BHPI was characterized by delayed and partially more

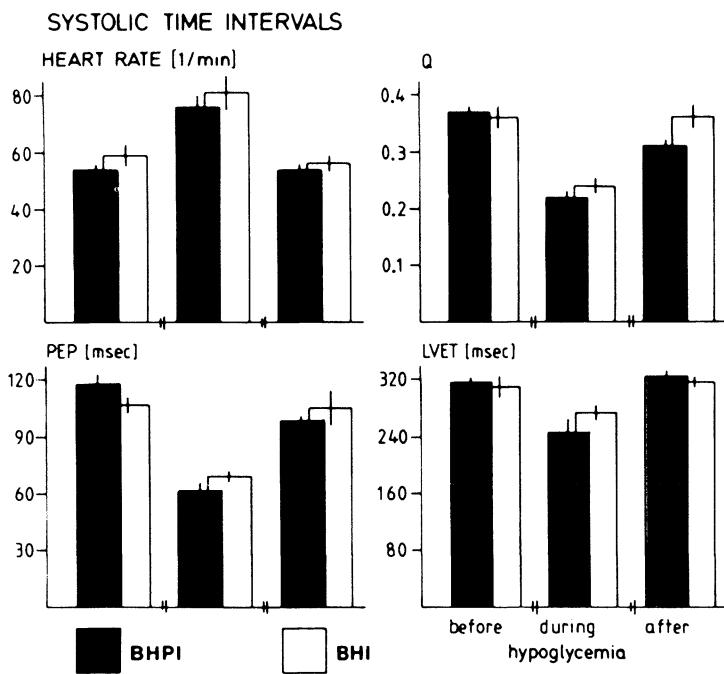


Fig. 6 Systolic time intervals during GCIIS-guided hypoglycemia tests with BHPI (■) and BHI (□) in 6 healthy volunteers ( $\bar{x} \pm \text{SEM}$ ). PEP = preejection period, LVET = left ventricular ejection time, Q = PEP/LVET.

Table 1 Feedback-controlled infused BHPI and BHI (U/kg) in 8 healthy volunteers.

|                          | BHPI              | BHI               |
|--------------------------|-------------------|-------------------|
| 1                        | 0.207             | 0.085             |
| 2                        | 0.150             | 0.101             |
| 3                        | 0.151             | 0.090             |
| 4                        | 0.163             | 0.090             |
| 5                        | 0.134             | 0.070             |
| 6                        | 0.143             | 0.082             |
| 7                        | 0.108             | 0.071             |
| 8                        | 0.129             | 0.078             |
| $\bar{x} \pm \text{SEM}$ | $0.148 \pm 0.010$ | $0.083 \pm 0.004$ |
|                          | $P < 0.001$       |                   |

distinct maximum hormone responses as well as by prolonged recovery.

As can be seen from Figures 3–4, prolonged secretion of ACTH, cortisol and BG corresponded well with prolonged hypoglycemia; the higher cortisol maximum is statistically significant (Table 2).

Due to the large standard deviation in a limited number of subjects, statistically significant differences in prolactin secretion could not be detected, although there was a tendency towards higher prolactin levels after BHPI infusion (Table 3).

Urinary epinephrine excretion after BHPI during the third collection period (120–240 min.) exceeded that after BHI 3-fold (BHPI  $10.3 \pm 4.8$  vs. BHI  $3.0 \pm 0.5$   $\mu\text{g}/120 \text{ min.}$ ;  $P < 0.05$ ).

BHPI infusion resulted in a more distinct delayed hypokalemia with prolonged recovery (Fig. 5).

Lactate maxima after BHPI and BHI were comparable, but the peak after BHPI occurred later (BHPI  $113 \pm 21$  vs. BHI  $48 \pm 5$  min.) and lactate levels did not reach basal values at the end of the test period (Fig. 5).

Table 4 shows the free fatty acid patterns during the hypoglycemia tests with BHPI and BHI. Proinsulin-induced inhibition of lipolysis was delayed and clearly less pronounced.

BHPI and BHI led to a comparable reversible decrease of the systolic time intervals PEP, LVET, QS2 and Q during hypoglycemia; the transient heart rate increase was identical (Fig. 6).

## Discussion

The aim of this study was to compare the counterregulatory effects following administration of BHPI and BHI during GCIIS-guided hypoglycemia tests. Using the GCIIS with appropriate constants on static control (Müller-Esch et al. 1984), blood glucose nadirs obtained by feedback controlled BHPI and BHI infusion did not differ significantly. The total BHPI dosage administered exceeded that of BHI approximately 1.8-fold (0.148 vs. 0.083 U/kg).

On a molar basis, a nearly 8-fold higher dosage of BHPI had to be given (3.94 vs. 0.51 nmol/kg). This calculation is based on the assumption, that the biological potency, determined by rabbit hypoglycemia test, of BHPI is 4 U/mg (Clinical Investigation Manual 1984) and that of BHI 28 U/mg.

If one defines biological potency as the quotient of blood glucose decrease (mg/dl) and applied hormone

Table 2 Mean basal values (bas.) and maximum (max.) or minimum (min.) levels obtained during GCIIS-guided hypoglycemia tests with BHPI and BHI for blood glucose (BG), GH, ACTH, cortisol, prolactin, potassium, lactate and free fatty acids (FFA) in 8 healthy volunteers ( $\bar{x} \pm \text{SEM}$ ). \*P < 0.05; \*\*P < 0.01.

|      | BG (mg/dl) |            | GH (ng/ml)    |            | ACTH (pg/ml)   |              | Cortisol (ng/ml) |               | Prolactin ( $\mu\text{U}/\text{ml}$ ) |                | Potassium (mmol/l) |                   | Lactate (mmol/l) |               | FFA ( $\mu\text{mol}/\text{l}$ ) |               |
|------|------------|------------|---------------|------------|----------------|--------------|------------------|---------------|---------------------------------------|----------------|--------------------|-------------------|------------------|---------------|----------------------------------|---------------|
|      | bas.       | min.       | bas.          | max.       | bas.           | max.         | bas.             | max.          | bas.                                  | max.           | bas.               | min.              | bas.             | min.          | bas.                             | min.          |
| BHPI | 79 $\pm$ 8 | 26 $\pm$ 2 | 1.9 $\pm$ 1.2 | 39 $\pm$ 8 | 23.5 $\pm$ 3.0 | 250 $\pm$ 42 | 100 $\pm$ 10     | 241 $\pm$ 14* | 213 $\pm$ 14                          | 1212 $\pm$ 470 | 4.35 $\pm$ 0.18    | 3.20 $\pm$ 0.06** | 0.45 $\pm$ 0.17  | 2.1 $\pm$ 0.2 | 664 $\pm$ 110                    | 326 $\pm$ 41* |
| BHI  | 78 $\pm$ 2 | 21 $\pm$ 1 | 0.9 $\pm$ 0.3 | 32 $\pm$ 5 | 18.5 $\pm$ 3.5 | 198 $\pm$ 45 | 88 $\pm$ 9       | 168 $\pm$ 10  | 223 $\pm$ 15                          | 482 $\pm$ 98   | 4.32 $\pm$ 0.11    | 3.58 $\pm$ 0.08   | 0.24 $\pm$ 0.08  | 1.7 $\pm$ 0.3 | 552 $\pm$ 104                    | 216 $\pm$ 32  |

Table 3 Individual serum prolactin levels ( $\mu\text{U}/\text{ml}$ ) during GCIIS-guided hypoglycemia tests with BHPI and BHI in 8 healthy volunteers.  $\bar{x} \pm \text{SEM}$  is also shown.

|                          | BHPI         |              |              |              |              |              |               |                |                |               |               |  |
|--------------------------|--------------|--------------|--------------|--------------|--------------|--------------|---------------|----------------|----------------|---------------|---------------|--|
|                          | -10          | $\pm$ 0      | 10           | 20           | 30           | 45           | 60            | 90             | 120            | 150           | 240 min       |  |
| 1                        | 199          | 214          | 225          | 264          | 241          | 285          | 3500          | 3500           | 3500           | 2960          | 1300          |  |
| 2                        | 301          | 292          | 281          | 293          | 281          | 273          | 314           | 352            | 377            | 361           | 274           |  |
| 3                        | 275          | 225          | 195          | 237          | 180          | 176          | 1090          | 3202           | 2464           | 1104          | 503           |  |
| 4                        | 193          | 219          | 217          | 216          | 190          | 600          | 805           | 761            | 624            | 369           | 277           |  |
| 5                        | 192          | 190          | 183          | 198          | 244          | 192          | 312           | 354            | 303            | 250           | 186           |  |
| 6                        | 208          | 217          | 254          | 229          | 202          | 209          | 599           | 470            | 300            | 230           | 173           |  |
| 7                        | 166          | 195          | 186          | 254          | 228          | 227          | 246           | 429            | 320            | 340           | 227           |  |
| 8                        | 198          | 152          | 162          | 181          | 198          | 222          | 429           | 341            | 253            | 230           | 100           |  |
| $\bar{x} \pm \text{SEM}$ | 217 $\pm$ 16 | 213 $\pm$ 14 | 213 $\pm$ 14 | 234 $\pm$ 13 | 221 $\pm$ 12 | 273 $\pm$ 48 | 912 $\pm$ 383 | 1180 $\pm$ 477 | 1018 $\pm$ 442 | 731 $\pm$ 334 | 374 $\pm$ 139 |  |
| BHI                      |              |              |              |              |              |              |               |                |                |               |               |  |
| 1                        | 258          | 248          | 197          | 202          | 176          | 233          | 238           | 198            | 176            | 174           | 224           |  |
| 2                        | 280          | 268          | 322          | 290          | 280          | 276          | 351           | 432            | 340            | 355           | 282           |  |
| 3                        | 185          | 195          | 149          | 215          | 233          | 360          | 302           | 246            | 203            | 176           | 117           |  |
| 4                        | 198          | 204          | 260          | 149          | 297          | 685          | 897           | 600            | 417            | 252           | 220           |  |
| 5                        | 178          | 198          | 208          | 231          | 224          | 274          | 275           | 258            | 216            | 196           | 162           |  |
| 6                        | 217          | 241          | 240          | 226          | 215          | 857          | 918           | 574            | 336            | 277           | 185           |  |
| 7                        | 296          | 279          | 290          | 275          | 236          | 495          | 437           | 394            | 338            | 215           | 140           |  |
| 8                        | 191          | 151          | 146          | 182          | 185          | 212          | 237           | 220            | 168            | 166           | 153           |  |
| $\bar{x} \pm \text{SEM}$ | 225 $\pm$ 16 | 223 $\pm$ 15 | 227 $\pm$ 22 | 221 $\pm$ 16 | 231 $\pm$ 15 | 424 $\pm$ 83 | 457 $\pm$ 101 | 365 $\pm$ 57   | 274 $\pm$ 33   | 226 $\pm$ 30  | 185 $\pm$ 19  |  |

Table 4 Total free fatty acids (FFA) and free fatty acid pattern (C14:0, C16:0, C16:1, C18:0, C18:1, C18:2) (μmol/l) during GCIS-guided hypoglycemia tests with BHPI and BHI in 8 healthy volunteers (x±SEM). \*P &lt; 0.05; \*\*P &lt; 0.01.

|            | -10    | ±0     | 10      | 20       | 30      | 45       | 60     | 90      | 120    | 150    | 240 min |
|------------|--------|--------|---------|----------|---------|----------|--------|---------|--------|--------|---------|
| C14:0 BHPI | 48±7   | 50±10  | 55±7    | 55±9     | 43±10   | 50±8     | 54±9   | 59±13   | 44±10  | 56±8   | 50±9    |
| C14:0 BHI  | 50±11  | 37±7   | 28±3**  | 25±5**   | 28±7    | 29±5     | 30±5   | 33±8    | 40±9   | 39±5   | 54±7    |
| C16:0 BHPI | 195±23 | 210±35 | 207±31  | 164±31   | 137±27  | 172±35   | 180±35 | 152±23  | 133±23 | 142±27 | 191±20  |
| C16:0 BHI  | 176±23 | 160±23 | 121±23* | 94±20*   | 125±16* | 102±16   | 98±16  | 117±23  | 164±23 | 180±16 | 238±20  |
| C16:1 BHPI | 20±4   | 19±4   | 22±5    | 15±4     | 11±3    | 12±3     | 11±3   | 13±3    | 13±4   | 13±3   | 18±3    |
| C16:1 BHI  | 20±4   | 17±2   | 15±3    | 11±3     | 11±2    | 11±2     | 13±4   | 11±2    | 17±4   | 19±2   | 28±4    |
| C18:0 BHPI | 42±3   | 45±3   | 40±2    | 33±2     | 29±4    | 38±8     | 53±13  | 44±8    | 40±6   | 36±7   | 45±5    |
| C18:0 BHI  | 43±6   | 40±3   | 29±4**  | 26±3**   | 29±5    | 32±4     | 34±3   | 29±3    | 33±3   | 41±3   | 50±4    |
| C18:1 BHPI | 232±33 | 237±53 | 252±47  | 176±39   | 139±30  | 177±38   | 176±45 | 154±29  | 145±24 | 157±26 | 225±21  |
| C18:1 BHI  | 190±35 | 170±28 | 127±27* | 92±29*   | 62±10*  | 112±28   | 91±23  | 120±25  | 184±28 | 214±27 | 282±17  |
| C18:2 BHPI | 98±12  | 98±20  | 94±18   | 74±17    | 61±12   | 77±12    | 72±15  | 73±13   | 69±14  | 74±14  | 89±10   |
| C18:2 BHI  | 104±14 | 95±9   | 65±11*  | 51±13*   | 30±5**  | 60±15    | 56±12  | 64±13   | 81±11  | 92±9   | 120±14  |
| FFA        | BHPI   | 637±73 | 664±110 | 669±96   | 516±96  | 430±61   | 528±92 | 552±104 | 492±71 | 442±73 | 478±81  |
| FFA        | BHI    | 580±98 | 520±65  | 380±63** | 298±69* | 250±35** | 346±64 | 334±54  | 372±64 | 520±69 | 586±57  |

dosage (nmol/kg) (Jones, Dron, Ellis, Sönksen and Brandenburg 1986), BHPI has only a potency of 13% compared to BHI.

If an identical dosage (U/kg) of BHPI and BHI, as applied during hypoglycemia tests by bolus injection, would result in identical blood glucose nadirs, the total molar BHPI dosage would exceed that of BHI 4-fold. In this case, the biological potency of BHPI would be 23% that of BHI.

It is known, that the biological potency of insulin preparations determined by insulin hypoglycemia tests may vary up to 50% (Galloway, Spradlin, Root and Fineberg 1981; Home, Shepherd, Noy, Massi-Benedetti, Hanning, Burrin and Alberti 1983). Most recent preliminary data have reported on identical blood glucose nadirs after injection of 0.15 U/kg BHPI and BHI (Wright, Hampton, Stout, Morgan and Marks 1985) as well as on a smaller blood glucose decrease following administration of 0.1 U/kg BHPI (Williams, Berelowitz and Frohman 1985). The distinct interindividual variation of the dosage required for the induction of feedback-controlled hypoglycemia (Table 1) confirms the assumption that the degree of blood glucose decrease achieved by bolus injection of insulin or proinsulin is a rather insensitive indicator of biological potency and underlines the importance of feedback-controlled hormone infusion according to the individual sensitivity by means of a GCIS.

In comparison to BHI, BHPI infusion resulted in delayed blood glucose decrease reaching a comparable nadir, prolonged hypoglycemia and retarded recovery. Certainly, this is due to differences in the pharmacokinetics of both hormones including different factors such as a higher distribution volume (Bottermann et al. 1985), compartmentalization (Glauber et al. 1986) and a decreased metabolic clearance rate (Revers et al. 1984). The prolonged circulating half-life of human proinsulin, perhaps the most important factor (Glauber et al. 1986), is demonstrated in Figure 2.

On the other hand, one can speculate that a preferential disproportionately long-lasting effect on the liver (suppression of hepatic glucose production) relative to the periphery (stimulation of glucose disposal) must play an additional role (Revers et al. 1984; Probst, Hartmann, Jungermann and Creutzfeldt 1985; Hartmann, Kramer and Creutzfeldt 1985).

Counterregulatory responses of ACTH, cortisol, GH and prolactin were markedly delayed and prolonged after BHPI infusion. Maximum hormone responses after either BHPI or BHI were clearly related in time to blood glucose nadirs; the greater AUC for ACTH, cortisol and GH corresponded well with prolonged hypoglycemia and delayed blood glucose recovery.

Our results indicate that the course of time of hormonal counterregulation depends on the rate of glucose decline during hypoglycemia and that the degree of counterregulatory hormone secretion is determined not only by the absolute glucose concentration, the absolute glucose decrement or the rate of glucose decline (Cryer 1981) but also by the duration of induced hypoglycemia.

A direct specific pituitary action of proinsulin on ACTH and GH secretion could not be detected. With the assumption that epinephrine secretion is reflected with sufficient reliability by urinary epinephrine excretion during the collection periods, the more distinct urinary epinephrine excretion after BHPI is a result of the prolonged hypoglycemia, too.

Studies on the regulation of serum potassium during insulin hypoglycemia tests have shown that the initial potassium decrease is due to an insulin-induced cell-influx, whereas the second phase during blood glucose recovery is attributed to epinephrine secretion in response to hypoglycemia (Petersen, Schlüter and Kerp 1982; Brown, M.J., Brown, D.C., and Murphy 1983). Therefore, prolonged hypoglycemia and increased epinephrine secretion following BHPI must result in prolonged and more distinct hypokalemia as well as in delayed serum potassium recovery (Fig. 5).

Intracellular neuroglucopenia is an effective stimulus for prolactin secretion (Woolf, Lee, Leebaw, Thompson, Lilalivathana, Brodows and Campbell 1977); a direct blood glucose-independent insulin effect on prolactin secretion has not been proven. The prolactin response is not mediated by catecholamines (Woolf et al. 1977) and – in comparison with other counterregulatory hormones – is characterized by a physiological interindividual variation which can be demonstrated in Table 3.

Thus, in studies with an only limited number of subjects, differences due to the large standard deviation may be misinterpreted, hypothesizing e.g. a different "hypothalamic handling" of homologous and heterologous insulins (Rosak, Althoff, Enzmann and Schöffling 1982). Our data do not support a specific proinsulin effect on the hypothalamic-pituitary axis, which could have been postulated because of the partial structural homology with IGF I (Rinderknecht and Humbel 1978).

The delayed and prolonged lactate increase is a consequence of a more distinct epinephrine-induced lactate production in muscle (Rosak, Vogel, Althoff, Neubauer, Brecht and Schöffling 1982).

Proinsulin induced suppression of lipolysis with its delayed onset was clearly less pronounced in comparison to insulin. With regard to the known sensitivity of the antilipolytic action of insulin (Zierler and Rabinowitz 1964) this finding is rather unexpected and points to further differences in metabolic actions of proinsulin and insulin in different organs.

Reversible heart rate increase and shortening of the systolic time intervals during hypoglycemia were identical for BHPI and BHI. They reflect an increase in myocardial contractility as a result of 1. counterregulatory epinephrine secretion and 2. a direct inotropic proinsulin or insulin effect (Lee and Downing 1976; Page, Smith and Watkins 1976; Hilsted, Bondes-Petersen, Nørgaard, Greniman, Christensen, Parving and Suzuki 1984).

In conclusion, biosynthetic human proinsulin, when infused during GCIIS-guided hypoglycemia test, results in delayed and prolonged hypoglycemia with consecutively more distinct hormonal counterregulation in comparison to human insulin. We regard this as a consequence of a prolonged circulating and biological half-life. In addition, a preferential effect on the liver (prolonged suppression of hepatic glucose production) may be of importance. A specific blood glucose independent action of human proinsulin on the hypothalamic-pituitary axis cannot be demonstrated. Whether this "depot effect" may be beneficial in the treatment of diabetes mellitus remains to be established.

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