# Differential Effects of Human and Pork Insulin–Induced Hypoglycemia on Neuronal Functions in Humans

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**Insulin has been found to cross the blood-brain barrier, and insulin receptors have been detected in different structures of the brain. However, the biological significance of insulin acting in the brain remains unclear. Reports of differential awareness of hypoglycemic symptoms during human insulin (HI)- and pork insulin (PI)-induced hypoglycemia hint at a modulatory influence of insulin on sensory processing. In a double-blind study, we recorded auditory-evoked potentials (AEPs), indexing neuronal transmission along sensory pathways, in 30 healthy male subjects during a baseline condition and HI- and PI-induced mild hypoglycemia of 2.65 mM. Fifteen subjects were tested after 20 min and another 15 after 50 min of constant hypoglycemia. During hypoglycemia, subjects had to indicate the severity of hypoglycemic symptoms and their current mood. Hypoglycemia increased latencies of the P3 component and reduced amplitudes of the N1, P2, and P3 components. Despite identical blood glucose and serum insulin levels in both sessions, effects of PI-induced hypoglycemia on AEP components were significantly stronger than those of HI-induced hypoglycemia ($P < 0.05$). Differences between the effects of the insulins were consistently apparent after 20 min of hypoglycemia, indicating a short-term action of these hormones on central nervous system functions. Also, after 20 min, but not after 50 min, of steady-state hypoglycemia, subjects felt more excited during PI than HI infusion ($P < 0.05$). The results indicate different influences of HI and PI on sensory function during hypoglycemia. These differences, occurring during early hypoglycemia, could contribute to the differential awareness of hypoglycemic warning symptoms during HI- and PI-induced hypoglycemia in diabetic patients.** *Diabetes* **39:1091–98, 1990**

The central nervous system has been traditionally considered independent of the influence of insulin. However, in the last few years, evidence has accumulated that insulin and its receptors are present in the brain, and physiological, behavioral, and developmental influences of insulin on the brain have been documented (for review, see ref. 1). Insulin has rapid access to the brain via the circumventricular organs lacking the blood-brain barrier (BBB), where it is concentrated on neuron endings and transported to deeper sites within the brain (2,3). It has also been suggested that insulin crosses the BBB via a receptor-mediated transport system in endothelial cells of brain microvessels (3–5). In several species, including humans, parallel changes in plasma and cerebrospinal fluid insulin concentrations are well documented (6,7). Whether insulin is also synthesized in the brain is still a matter of discussion. Insulin receptors are widely spread in the brain, with the highest concentrations in the olfactory bulb, in the hypothalamus, and throughout the limbic system of the rat brain (8–11). Thus, insulin may exert widespread modulatory influences on various neuronal pathways. Insulin, for example, inhibits firing of neurons in the hippocampus (12) and hypothalamus (13) and alters the response of single units in the olfactory bulb and amygdala to olfactory stimuli (14). However, the significance of insulin acting on the brain for the entire organism remains unclear. This study was done to demonstrate afferent influences of insulin on human brain functions. Human insulin (HI) and pork insulin (PI) were compared. Because both of these insulins have similar potency with regard to inducing hypoglycemia (15), the demonstration of differential effects of these insulins on human brain functions would substantially support the hypothesis of an afferent action of insulin on human brain functions. Reports of a different awareness of hypoglycemic symptoms during HI- and PI-induced hypoglycemia can be considered first

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Received for publication 16 October 1989 and accepted in revised form 27 April 1990.

This article is dedicated to Prof. Dr. P. Pauschinger on the occasion of his 65th birthday.

hints at a differential central nervous system efficacy of both insulins (16–20). Moreover, because PI is more lipophilic than HI (21), it may cross the BBB faster than HI.

In this study, stimulus-evoked brain potentials were used to assess the effects of these insulins on brain functions. Stimulus-evoked brain potentials representing physiological indicators of sensory processing were expected to reflect differential influences of insulin-induced hypoglycemia on sensory processing more precisely than subjective reports of hypoglycemia awareness.

## RESEARCH DESIGN AND METHODS

Subjects were 30 healthy male volunteers (aged 18–34 yr) with normal hearing and body weight (±10%) and without personal or family history of diabetes. All subjects were nonsmokers, and none were taking medication. Twelve hours before testing, subjects had to fast and abstain from coffee and alcoholic beverages. Subjects with cross-sleep disturbances in the nights before the experimental sessions were excluded. The study was approved by the Committee on Research Involving Human Subjects of the University of Ulm, and written informed consent was obtained from all subjects.

The experiments were double blind and designed according to a within-subject crossover comparison. Each subject was tested twice, with an interval of 1 wk between both sessions. On one occasion, subjects received biosynthetic HI (Velasulin H, Nordisk, Bethesda, MD); on the other, they received purified PI (Velasulin, Nordisk). The order of administration was counterbalanced across subjects. Experiments took place in a sound-attenuated and darkened room between 0900 and 1300 with the subjects sitting in bed. One hour before testing, two catheters were inserted into veins of the dorsal hand, which was warmed up to 55°C. With this procedure, arterialized blood could be sampled for continuous blood glucose monitoring with a glucose analyzer (Biostator Glucose Controller, Life Science; 22) and for determination of serum insulin and plasma cortisol. A third intravenous catheter in the opposite arm was used to infuse insulin and glucose. Blood samples were taken every 20 min for determination of serum insulin, plasma cortisol, and blood glucose (by a Beckman Glucose Analyzer II) to calibrate the Biostator. Cortisol was assessed as an indicator of counterregulatory mechanisms, which is only a crude measure, because glucagon and epinephrine responses begin somewhat earlier in the course of hypoglycemia.

After a 40-min baseline period, an initial bolus of insulin (30 mU/kg i.v.) was administered, and thereafter, insulin was infused at a constant rate of 1.5 mU · $kg^{-1}$ · $min^{-1}$. At a plasma glucose concentration of ~2.6 mM, hypoglycemia was maintained by an additional manually controlled glucose infusion (20% solution) for ~1.5 h, after which blood glucose was increased to baseline level.

Auditory-evoked potentials (AEPs) indexing cortical sensory processing were recorded while the subjects performed a vigilance task (oddball paradigm). This task required the subjects to discriminate and to covertly count target pips (840 or 1200 Hz, 60-ms duration), which were randomly interspersed among frequent standard pips of lower pitch (800 Hz). The task consisted of three sequences, each containing ~340 pips. Tone pips were presented binaurally through headphones, with a mean interstimulus interval of 1.5 s. Brain potentials evoked by both standard and target pips contain a prominent component complex ~100 ms poststimulus, which is termed the *vertex response* and is made up of first vertex negative deflection (N1) and a subsequent positive deflection (P2). The vertex potential reflects a nonspecific cortical arousal induced by the stimulus presentation (23). The task-relevant target pips, in addition, evoke a large positive component (P3) peaking ~350–450 ms poststimulus over parietal cortical regions. The P3 is an indicator of the target processing within short-term memory (24). Its latency is known to depend on the discriminability of the target; its amplitude depends on the probability of the target (25). Accordingly, probability and pitch of the target tones were varied in each of the three sequences to differentiate direct effects of insulin on P3. The following conditions were run: *1*) pitch of target tones = 1200 Hz, $P = 0.1$; *2*) pitch = 840 Hz, $P = 0.1$; *3*) pitch = 1200 Hz, $P = 0.5$. The order of presentation of the three sequences was counterbalanced across subjects. AEPs were recorded during the baseline phase and during constant hypoglycemia (2.65 mM) after either 20 min ($n = 15$) or 50 min ($n = 15$) of steady-state conditions.

To determine subjective awareness of hypoglycemic symptoms, at the end of each AEP-recording phase, subjects rated from 1 (none) to 7 (severe) the following symptoms: hunger, sweating, palpitations, tremor, tiredness, nervousness, dizziness, faintness, irritability, embarrassment, blurred vision, and difficulty in thinking. Furthermore, a checklist of adjectives was presented to the subjects, in which 65 items were used to describe the subject's mood according to the dimensions of activation, deactivation, fatigue, numbness, arousal, and anxiety (26). Subjects had to indicate whether each adjective reflected aspects of his current mood. Thereafter, heart rate and blood pressure were measured.

Recordings were obtained of EEGs (5-s time constant, 70 Hz/12 decibels [dB] high-frequency, 0.045 Hz/6 dB low-frequency roll off) from Fz, Cz, and Pz electrode locations referenced to linked electrodes attached to the earlobes. An electrode attached at Fpz served as a ground. For artifact recognition, the vertical electrooculogram (EOG) was monitored. Nonpolarizable silver–silver chloride electrodes of 16 mm diam were used for all recordings. EEG and EOG signals were amplified by a Nicolet 1A97 polygraph and digitized (sampling rate 385 Hz) for on-line averaging of AEPs.

Serum insulin was determined by radioimmunoassay (Pharmacia Insulin RIA 100) with interassay error measured as coefficient of variation (C.V.) <5.4%. Intraassay variation was <4.5% in all cases. Plasma cortisol was also measured by radioimmunoassay (Hermann Biermann) with a sensitivity of 5.5 nM, interassay C.V. of 5%, and intra-assay C.V. of <3% in the concentration range between 27 and 1380 nM. The same kit was used for all samples of an individual subject.

AEPs were averaged separately according to experimental conditions: glucose level (baseline versus hypoglycemia), type of insulin (HI versus PI), topography (Fz, Cz, Pz), tone pip (standard versus target), and type of sequence (with pitches and probabilities, respectively, 1200 Hz, $P = 0.1$; 840 Hz, $P = 0.1$; and 1200 Hz, $P = 0.5$). The averaging epoch covered a 40-ms baseline and a 740-ms poststimulus

interval. EEG epochs were excluded from analysis if they contained gross eye movements or other artifact potentials >50 μV. Measures derived from AEP waveforms were as follows. *1*) Latencies and baseline-to-peak amplitudes of the N1 and P2 components: the latency bin accounting for N1 was 70–140 ms poststimulus. For P2 determination, the maximum positive voltage between 130 and 230 ms poststimulus was used. Also, N1-P2 difference amplitude was calculated. *2*) The latency and baseline-to-peak amplitude of the P3 component after the task-relevant target stimuli: P3 was defined with regard to the maximum positive amplitude within 280 to 740 ms poststimulus.

Effects on AEP measures were assessed by repeated-measures analyses of variance (ANOVAs) containing the factors glucose level (baseline versus hypoglycemia), type of insulin (HI versus PI), topography (Fz, Cz, Pz), tone pip (standard versus target), and type of sequence (with pitches and probabilities, respectively, 1200 Hz, $P = 0.1$; 840 Hz, $P = 0.1$; and 1200 Hz, $P = 0.5$). Supplementary ANOVAs were performed with an additional group factor to assess whether effects of hypoglycemia depended on the time since onset of the steady-state hypoglycemia (20 vs. 50 min). Analyses of covariances (ANCOVAs) to further evaluate effects of the different insulins were performed, inducting the baseline measurements as covariates.

Cardiovascular parameters and measures of blood glucose, serum insulin, and plasma cortisol levels of both sessions were similarly assessed by ANOVAs and ANCOVAs. Analyses of self-report measures included nonparametric statistical tests (Friedman's and Wilcoxon's).

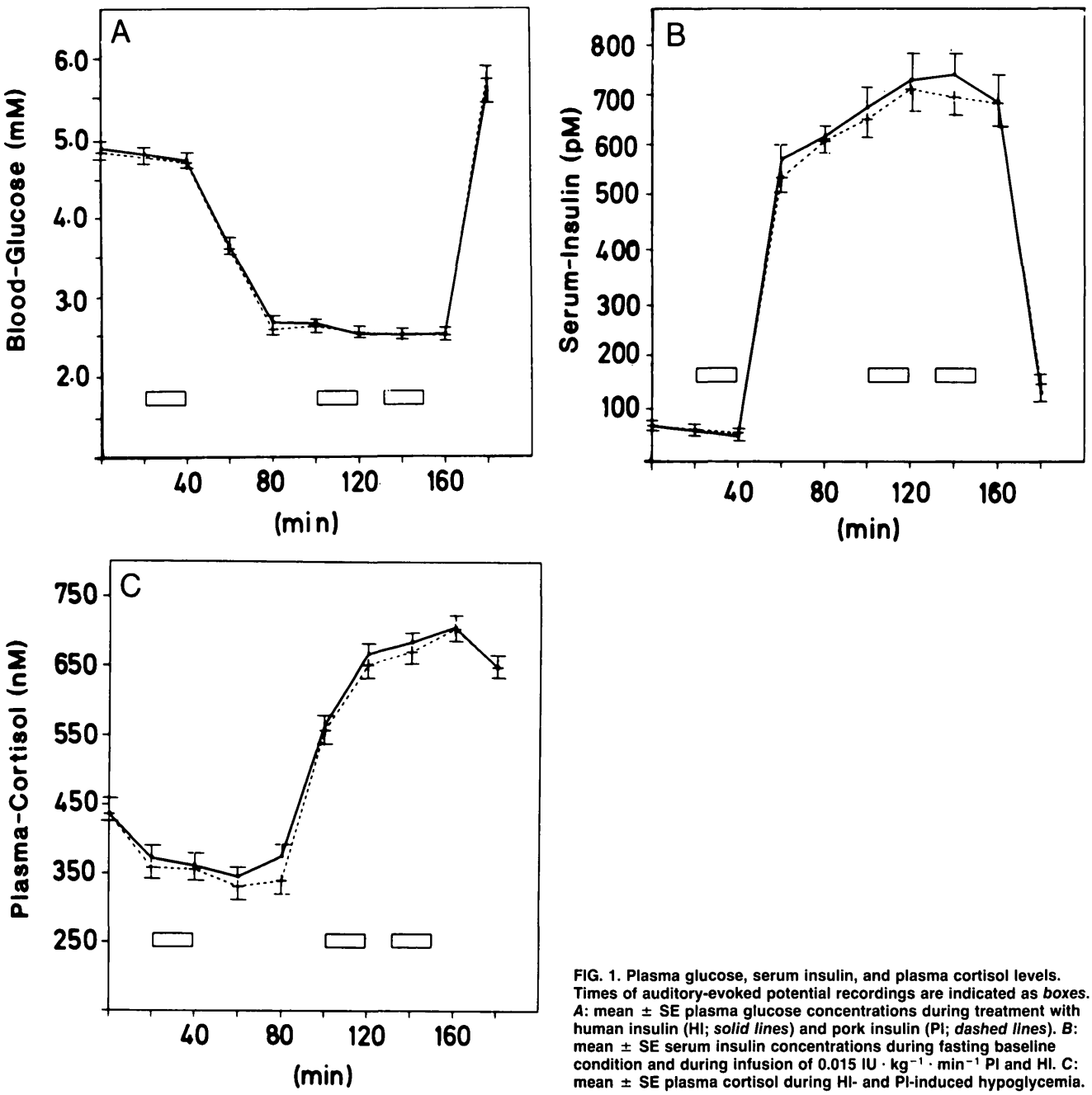


**FIG. 1. Plasma glucose, serum insulin, and plasma cortisol levels. Times of auditory-evoked potential recordings are indicated as *boxes*. *A*: mean ± SE plasma glucose concentrations during treatment with human insulin (HI; *solid lines*) and pork insulin (PI; *dashed lines*). *B*: mean ± SE serum insulin concentrations during fasting baseline condition and during infusion of 0.015 IU · $kg^{-1}$ · $min^{-1}$ PI and HI. *C*: mean ± SE plasma cortisol during HI- and PI-induced hypoglycemia.**

TABLE 1
Baseline-to-peak amplitudes and latencies of auditory-evoked potential components N1, P2, and P3 and N1-P2 difference amplitude during human insulin (HI) and pork insulin (PI) session

| | HI | | PI | | *P* | |
|---|---|---|---|---|---|---|
| | Baseline | Hypoglycemia | Baseline | Hypoglycemia | Hypoglycemia | H × I |
| N1 | | | | | | |
| Amplitude (μV) | −5.1 ± 0.4 | −4.2 ± 0.4 | −5.4 ± 0.4 | −3.9 ± 0.4 | <0.0001 | <0.05 |
| Latency (ms) | 97.3 ± 2.1 | 96.0 ± 2.0 | 97.4 ± 2.1 | 99.7 ± 2.3 | NS | <0.05 |
| P2 | | | | | | |
| Amplitude (μV) | 5.7 ± 0.5 | 5.4 ± 0.4 | 5.1 ± 0.4 | 4.8 ± 0.4 | <0.1 | NS |
| Latency (ms) | 202.7 ± 4.1 | 197.6 ± 4.0 | 204.3 ± 3.7 | 203.8 ± 4.0 | <0.1 | <0.01 |
| N1-P2 amplitude (μV) | 10.8 ± 0.6 | 9.6 ± 0.5 | 10.6 ± 0.5 | 8.7 ± 0.4 | <0.0001 | <0.03 |
| P3 | | | | | | |
| Amplitude (μV) | 13.4 ± 1.3 | 9.5 ± 1.0 | 14.4 ± 1.2 | 9.5 ± 1.2 | <0.0001 | NS |
| Latency (ms) | 356.7 ± 9.2 | 392.0 ± 17.3 | 356.7 ± 7.9 | 417.7 ± 21.3 | <0.002 | NS |

Values are means ± SE averaged across all subjects. H × I, differences between HI- and PI-induced hypoglycemia.

## RESULTS

Mean plasma glucose and serum insulin levels during the HI and PI sessions are shown in Fig 1. In both groups, mean ± SE blood glucose concentrations (HI, 4.89 ± 0.08 mM; PI, 4.86 ± 0.06 mM) and serum insulin values (HI, 56.7 ± 9.3 pM; PI, 60.3 ± 7.2 pM) during baseline were not significantly different. After continuous infusion of 1.5 mU · $kg^{-1}$ · $min^{-1}$ HI and PI, nearly the same serum insulin levels were obtained (HI, 686.4 ± 46.5 pM; PI, 672.0 ± 37.3 pM), and during this phase, blood glucose concentrations were reduced to nearly the same extent by HI and PI (HI, 2.68 ± 0.08 mM; PI, 2.66 ± 0.05 mM).

As expected, N1 and P2 showed maximum baseline-to-peak amplitude recordings from Cz, whereas the P3 component after target pips dominated at parietal recording sites (topography $P < 0.0001$). P3 amplitude was larger for targets occurring with a probability of $P = 0.1$ than for targets with a probability of $P = 0.5$ ($P < 0.06$). P3 latency was increased when targets were hard to discriminate (840 Hz) compared with the sequences in which the targets strongly deviated in pitch from the standard pips ($P < 0.01$).

Table 1 summarizes the effects of HI- and PI-induced hypoglycemia on AEP components. During hypoglycemia, N1 amplitude was significantly reduced ($P < 0.001$); a similar trend was found for P2 amplitude ($P < 0.1$). The N1 amplitude reduction after PI-induced hypoglycemia (mean ± SE −1.5 ± 0.2 μV) was more distinct than after HI-induced hypoglycemia (differences between PI- and HI-induced hypoglycemia [H × I], −0.9 ± 0.2 μV, $P < 0.05$). PI- and HI-induced hypoglycemia also differentially influenced latencies of the vertex potential components. Although N1 and P2 latencies tended to be shorter during HI-induced hypoglycemia than during baseline recordings, during PI-induced hypoglycemia, N1 latency tended to increase (H × I, $P < 0.05$); the reductions in P2 latency during PI-induced hypoglycemia were only marginal compared with the effect during HI-induced hypoglycemia (H × I, $P < 0.01$). Similarly, N1-P2 difference amplitude was distinctly reduced during hypo-

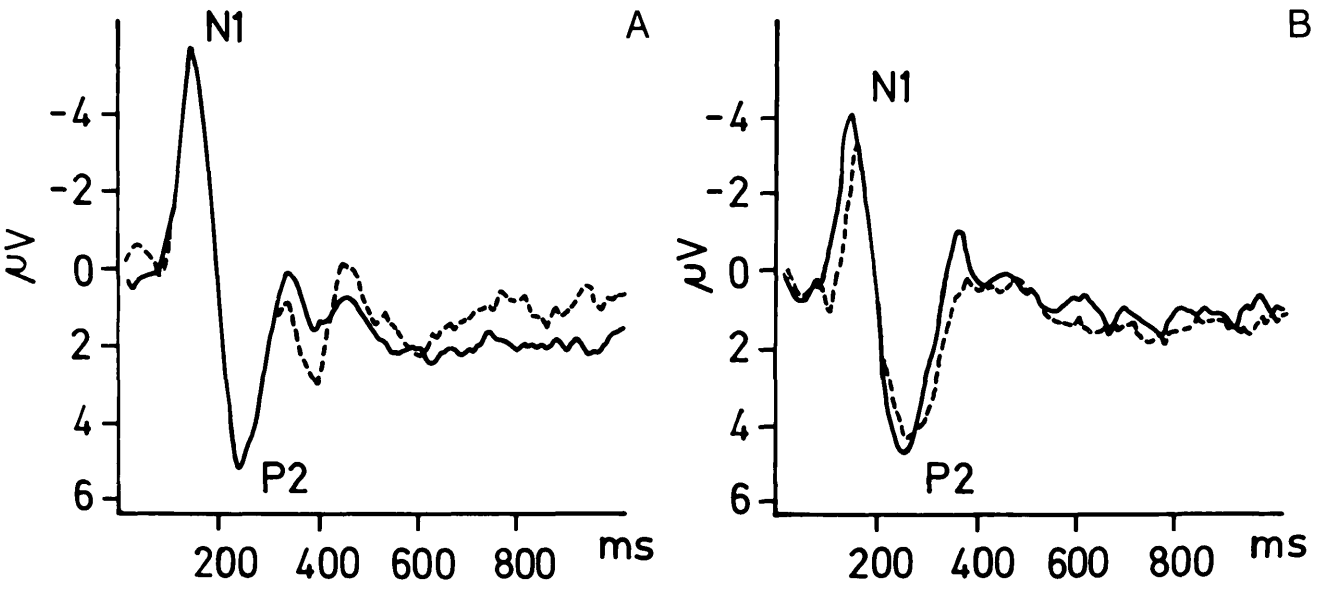


**FIG. 2. Grand average auditory-evoked potential responses to standard tones from 15 subjects under baseline condition (*A*) and after 50 min of steady-state hypoglycemia (*B*) during human insulin (HI; *solid lines*) and pork insulin (PI; *dashed lines*) sessions. N1-P2 difference amplitude distinctly decreased during hypoglycemia. Reductions of N1-P2 difference amplitude during PI infusion were significantly stronger than during HI infusion.**

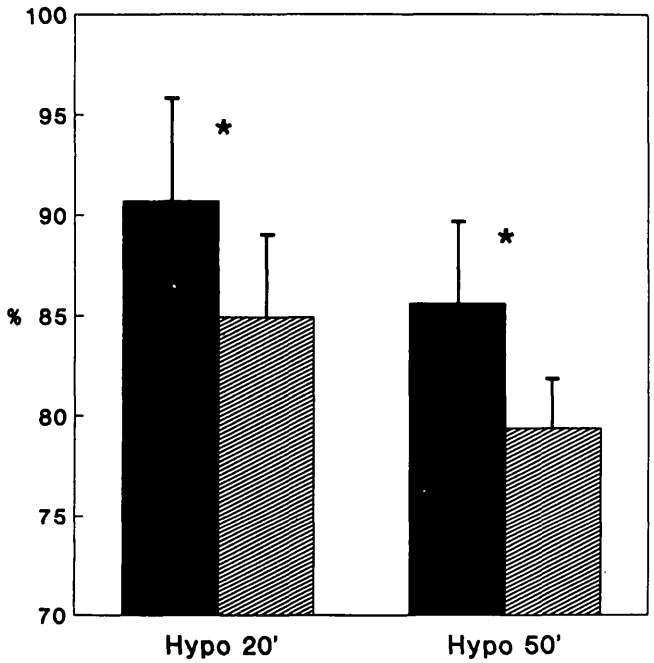


**FIG 3. Percentage reductions of N1-P2 difference amplitude for auditory-evoked potential recordings obtained after 20 min (Hypo 20') and 50 min (Hypo 50') of steady-state hypoglycemia. Significant differences between human (*solid bars*) and pork (*hatched bars*) insulin were apparent on both occasions with respect to baseline values (*$P < 0.05$).**

glycemia ($P < 0.0001$), and this effect was significantly stronger during PI-induced ($-1.9 \pm 0.3$ μV) than HI-induced ($-1.2 \pm 0.4$ μV) hypoglycemia (H × I, $P < 0.03$) (Fig. 2). The stronger effect of PI-induced hypoglycemia compared with HI-induced hypoglycemia on the N1-P2 difference amplitude was equally apparent in recordings obtained after 20 and 50 min of steady-state hypoglycemia (Fig. 3).

Target tones elicited a distinct P3 component. Lowering blood glucose markedly reduced P3 amplitude (hypoglycemia, $-4.4 \pm 0.9$ μV, $P < 0.0001$) and also prolonged its latency (hypoglycemia, $+48.2 \pm 15.4$ ms, $P < 0.002$). These effects did not depend on target probability or discriminability and were not modulated by the type of insulin. However, comparing effects after 20 and 50 min of hypoglycemia revealed that, during the earlier recordings, the increase in P3 latency during PI-induced hypoglycemia ($+97.4 \pm 27.6$ ms) was much stronger compared with the HI condition ($+30.2 \pm 19.8$ ms, H × I, $P < 0.05$) (Fig. 4); this difference disappeared with time spent in hypoglycemia (Fig. 5).

The mean ± SE deviation of the counts from the correct number of target pips presented was 3.13 ± 0.70 under baseline conditions. Counts tended to be less accurate during hypoglycemic phases than at baseline. However, counting accuracy varied considerably among subjects (Table 2).

Amplitudes of blood pressure increased during hypoglycemia. There were no significant differences concerning blood pressure between HI- and PI-induced hypoglycemia.

Plasma cortisol, as one indicator of mechanisms counterregulating hypoglycemia, began to increase when blood glucose levels were at ~2.8 mM (Fig. 1). At the time of the first AEP recording (i.e., after 20 min of steady-state hypoglycemia), plasma cortisol levels reached ~60% of maximum value. Maximum concentrations were observed during the later recording phase after 50 min of hypoglycemia. There were no significant differences in cortisol concentrations between the HI and PI conditions.

Ratings concerning mood, feelings of hunger, activation, etc., were distinctly affected by hypoglycemia. With low plasma glucose concentrations, subjects felt more inactive, more tired, more numb, more anxious, more excited, and less active than during euglycemic baseline conditions ($P < 0.05$). The effects on fatigue and deactivation were stronger after 50 than 20 min of hypoglycemia ($P < 0.05$). The effects on excitement—particularly after 20 min of hypoglycemia—appeared to be stronger during PI- than HI-induced hypoglycemia (H × I, $P < 0.02$) (Table 3).

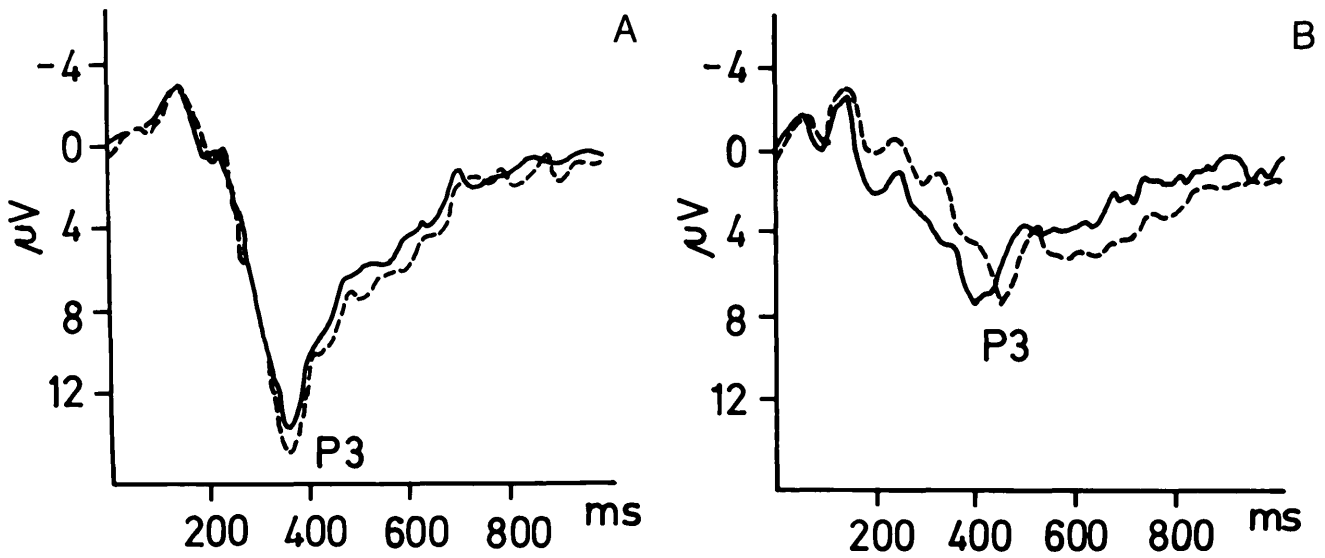


**FIG. 4. Auditory-evoked potential waveforms averaged across all target tones from 15 subjects under baseline condition (*A*) and after 20 min of steady-state hypoglycemia (*B*) during human insulin (HI; *solid lines*) and pork insulin (PI; *dashed lines*) sessions. Hypoglycemia caused decreased amplitudes and lengthened latencies of P3 component. Increases of P3 latency during PI infusion were significantly stronger than those of HI.**

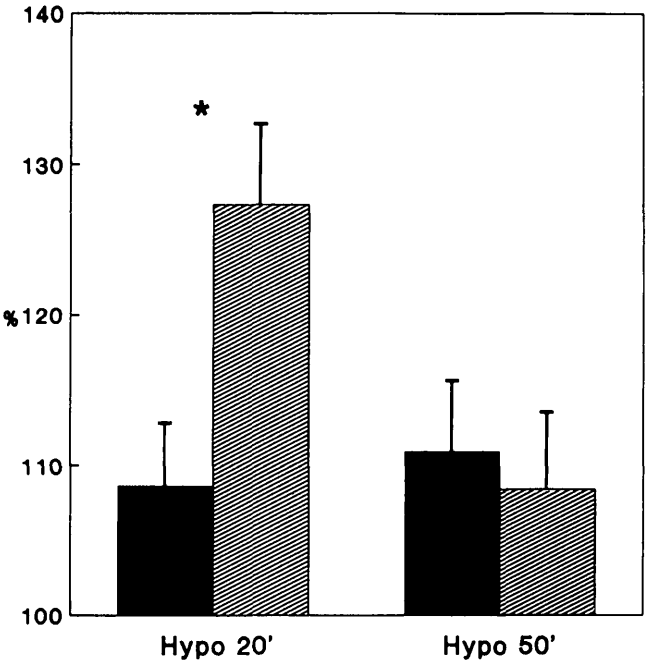


**FIG. 5. Percentage increase of P3 latency with respect to baseline values after 20 min (Hypo 20') and 50 min (Hypo 50') of hypoglycemia. Effects during pork insulin (*hatched bars*) infusion were significantly stronger than those of human insulin (*solid bars*). These differences were restricted to earlier hypoglycemic phase. *$P < 0.05$.**

## DISCUSSION

In this study, AEPs proved to be sensitive to influences of hypoglycemia. Lowering blood glucose to 2.65 mM decreased N1, P2, and P3 amplitudes and increased P3 latency. These findings agree with previous reports of decreased amplitudes of visually evoked potentials (27) and prolonged latencies of AEP components generated at the cortical (28) and brainstem (29,30) levels.

In this study, we also compared the effects of HI- and PI-induced hypoglycemia on sensory function within the auditory pathways. Both types of insulin appeared to modulate differentially the effect of hypoglycemia on each of the AEP components evaluated; i.e., changes during HI- and PI-induced hypoglycemia were generally in the same direction, but the changes during PI-induced hypoglycemia were significantly stronger than those during HI treatment, indicating greater decay of sensory functions during PI-induced hypoglycemia. Thus, the vertex response, indicating a stimulus-induced cortical arousal mediated by mesencephalic reticular structures, was distinctly reduced during hypoglycemia. This reduction was more pronounced during PI than HI treatment on both recording occasions (20 and 50 min after steady-state hypoglycemia had been achieved). Likewise, P3 latency, which indicates the time needed for controlled stimulus categorization, was prolonged during hypoglycemia. The latency increase during PI treatment exceeded that during HI treatment. However, these latter differences only emerged during the early hypoglycemic phase (i.e., after 20 min of hypoglycemia). At this time, subjects also felt significantly more excited after administration of PI than HI.

Because blood glucose and serum insulin levels were almost identical in both sessions, the differential effects on AEPs and mood suggest that both types of insulin exert direct but different influences on neuronal functions. The structures of HI and PI differ only in one amino acid. However, PI has been reported to be more lipophilic than HI (21). Thus, PI probably crosses the BBB more easily than HI, resulting in higher concentrations of PI in the brain during hypoglycemia. These findings complement previous reports of differential effects of HI- and PI-induced hypoglycemia on human AEP components (i.e., wave V) generated in the brainstem (30). The fact that HI effects appeared to be stronger than PI-induced hypoglycemia effects at the brainstem level contrasts with our observations of more pronounced changes in AEPs after PI treatment at the cortical level. However, given that changes in AEPs reflect a direct effect of insulin on neuronal processing, a unitary influence of the hormone at each level of stimulus processing cannot be expected.

It may be argued that changes in AEPs are a consequence of alterations of the peripheral auditory system. For example, some early reports showed effects of hypoglycemia on microphonic potentials of the cochlea, although others did not (31–33). In a previous study, we did not find any differences of PI- and HI-induced hypoglycemia on wave I of the auditory brainstem response (30), which reflects the summation ac-

TABLE 2
Systolic and diastolic blood pressure, heart rate, and counting accuracy (deviation from correct value) during baseline conditions and after 20 and 50 min of human insulin (HI)- and pork insulin (PI)-induced steady-state hypoglycemia

| | | Hypoglycemia | | |
|---|---|---|---|---|
| | Baseline | 20 min | 50 min | Hypoglycemia *P* |
| Systolic blood pressure (mmHg) | | | | |
| HI | 122.0 ± 1.7 | 133.0 ± 2.6 | 133.0 ± 2.4 | <0.05 |
| PI | 122.0 ± 1.6 | 133.0 ± 2.5 | 132.0 ± 2.8 | <0.05 |
| Diastolic blood pressure (mmHg) | | | | |
| HI | 83.0 ± 1.2 | 67.0 ± 2.2 | 62.0 ± 2.7 | <0.05 |
| PI | 84.0 ± 1.3 | 63.0 ± 2.6 | 59.0 ± 2.1 | <0.05 |
| Heart rate (beats/min) | | | | |
| HI | 65.0 ± 1.7 | 65.0 ± 1.9 | 64.0 ± 2.0 | NS |
| PI | 60.0 ± 1.4 | 63.0 ± 1.7 | 64.0 ± 1.8 | NS |
| Counting accuracy | | | | |
| HI | 3.37 ± 0.79 | 4.94 ± 0.99 | 7.15 ± 1.75 | <0.1 |
| PI | 2.89 ± 0.62 | 4.60 ± 0.89 | 5.84 ± 1.95 | <0.1 |

Values are means ± SE. Baseline values are means of both groups, because they were nearly identical during both baseline conditions.

TABLE 3
Scores on dimensions of adjective checklist during baseline and after 20 and 50 min of steady-state hypoglycemia

| | Baseline | Hypoglycemia | |
|---|---|---|---|
| | | 20 min | 50 min |
| Activation | | | |
| HI | 4.67 ± 0.67 | 3.17 ± 0.58* | 2.27 ± 0.66† |
| PI | 5.03 ± 0.68 | 2.77 ± 0.51* | 2.17 ± 0.70 |
| Deactivation | | | |
| HI | 2.57 ± 0.47 | 5.10 ± 0.74* | 6.73 ± 0.74† |
| PI | 1.80 ± 0.48 | 5.43 ± 0.71* | 7.07 ± 0.77† |
| Fatigue | | | |
| HI | 0.77 ± 0.26 | 2.30 ± 0.41* | 3.93 ± 0.43† |
| PI | 0.53 ± 0.20 | 2.67 ± 0.32* | 3.97 ± 0.40† |
| Numbness | | | |
| HI | 0.17 ± 0.07 | 0.43 ± 0.09* | 0.63 ± 0.09 |
| PI | 0.13 ± 0.06 | 0.43 ± 0.09* | 0.63 ± 0.09 |
| Excitement | | | |
| HI | 1.23 ± 0.26 | 2.07 ± 0.51‡ | 2.87 ± 0.64 |
| PI | 0.73 ± 0.22 | 3.57 ± 0.68*‡ | 3.67 ± 0.74 |
| Anxiety | | | |
| HI | 0.23 ± 0.09 | 0.47 ± 0.15 | 0.70 ± 0.22 |
| PI | 0.23 ± 0.11 | 0.57 ± 0.16* | 0.73 ± 0.23 |

Values are means ± SE. HI, human insulin; PI, pork insulin. Baseline values were averaged across both groups assessed at different times during hypoglycemia.
*$P < 0.05$ vs. baseline.
†$P < 0.05$ vs. 20 min of hypoglycemia.
‡$P < 0.02$ vs. HI- and PI-induced hypoglycemia.

tion potential of the cochlear nerve (34). Moreover, a direct central effect of PI and HI rather than an effect mediated via peripheral changes in the auditory system is also supported by the different time courses of changes in N1-P2 and P3 components, i.e., the stronger effect of PI on the N1-P2 difference amplitude persisted over time, whereas the increase of the P3 latency disappeared with time spent in hypoglycemia.

Thus, influences of insulin may be mediated via changes in glucose metabolism in the brain (35,36) and in the auditory pathways (37). Alternatively, insulin may directly influence neuronal function. For example, a dose-dependent inhibition of insulin on the firing rate of neurons of the hippocampus (12) and the hypothalamus has been reported (13,38,40–45). Evidence that insulin stimulates membrane ion transport and causes hyperpolarization (46) may explain its inhibitory actions on neuronal activity and may be the basis for the reduction in N1-P2 amplitudes and the increase in P3 latency, which was particularly prominent during PI-induced hypoglycemia.

The stronger changes in AEPs during PI-induced hypoglycemia correspond to reports of enhanced awareness of early warning symptoms during PI-induced hypoglycemia compared with HI-induced hypoglycemia (16–20). Also, in this study after 20 min of PI-induced hypoglycemia, subjects felt more excited than during HI treatment. Differential effects of HI- and PI-induced hypoglycemia were already present 20 min after steady-state hypoglycemia had been achieved. Although differential effects on the N1-P2 complex persisted over time, the insulin-dependent changes in P3 latency were not apparent in recordings obtained at 50 min of hypoglycemia. By contrast, cortisol levels gradually increased during hypoglycemia and reached a maximum after 50 min of hypoglycemia. Therefore, the increase in cortisol, being one of several counterregulations emerging during hypoglycemia, cannot account for the different central nervous system actions of the insulins. However, it cannot be excluded that central effects of insulin are mediated via counterregulatory mechanisms other than cortisol. Thus, Heine et al. (47) reported on a greater norepinephrine response after administration of PI than after HI in 8 healthy male volunteers. However, we were unable to confirm these results in a foregoing pilot study with 12 healthy volunteers (48), and several previous studies did not find any differences in counterregulatory hormone responses to HI and PI (49). Given these inconsistencies, it seems unjustified to attribute changes in the AEPs to different responses of norepinephrine to HI and PI.

In this context, note that several studies have reported reduced amplitudes and prolonged latencies of evoked-potential components in insulin-dependent and non-insulin-dependent diabetic patients under euglycemic conditions (50–59). These alterations did not correlate with duration of diabetes, $HbA_1$, and later complications of diabetes (51,52,54,59). Because supraphysiological levels of serum insulin are common in diabetic patients, peripheral insulin receptors appear to be downregulated. By contrast, central nervous system insulin receptors are not downregulated by persisting high insulin concentrations (60,61), thus, it is tempting to speculate that increased insulin concentrations in the brain and an unchanged number of central nervous system receptors may be responsible for evoked-potential changes in these diabetic patients that are similar to those observed in this study in healthy subjects during hypoglycemia.

In summary, our results demonstrate distinct effects of hypoglycemia on amplitudes and latencies of evoked-potential components in humans. Most important, our results indicate that HI- and PI-induced hypoglycemia differ with respect to their pattern of actions, the effects of PI-induced hypoglycemia generally exceeding those of HI-induced hypoglycemia. Further research should clarify to what extent the differences are relevant for the self-awareness of hypoglycemia in diabetic patients.

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