

Central Nervous System Effects of Intranasally Administered Insulin During Euglycemia in Men

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Insulin receptors have been detected in several structures of the brain, yet the biological significance of insulin acting on the brain remains rather unclear. In humans, direct central nervous effects of insulin are difficult to distinguish from alterations in neuronal functions because of insulin-induced decrease in blood glucose levels. Since several intranasally administered viruses, peptides, and hormones have been shown to penetrate directly from the nose to the brain, we tested whether insulin after intranasal administration likewise has access to the brain. After a 60-min baseline period, insulin (20 IU H-Insulin 100 Hoechst) or vehicle (2.7 mg/ml m-Cresol) was intranasally administered every 15 min to 18 healthy subjects according to a double-blind within-subject crossover design. Auditory-evoked potentials (AEP) indexing cortical sensory processing were recorded while the subjects performed a vigilance task (oddball paradigm) during the baseline phase and after 60 min of intranasal treatment with insulin or placebo. Blood glucose and serum insulin levels were not affected by intranasal insulin. Compared with placebo, intranasal administration of insulin reduced amplitudes of the N1 ($P < 0.005$) and P3 ($P < 0.02$) components of the AEP and increased P3 latency ($P < 0.05$). The reduction in P3 amplitude was most pronounced over the frontal recording site (2.42 ± 1.00 vs. 4.92 ± 0.79 μV , $P < 0.0005$). At this site, after insulin administration, a broad negative shift developed in the AEP between 280 and 500 ms poststimulus (area under the curve -166.0 ± 183.8 vs. 270.8 ± 138.7 $\mu\text{V} \cdot \text{ms}$ after placebo, $P < 0.01$). The results suggest that after intranasal administration, insulin directly enters the brain and exerts distinct influences on central nervous functions in humans. *Diabetes* 48:557–563, 1999

Systemic insulin has rapid access to the brain via the circumventricular organs lacking the blood–brain barrier and via a receptor-mediated transport system located in endothelial cells of brain microvessels (1,2). In several species, including humans, parallel changes in plasma and brain interstitial and cerebrospinal fluid (CSF) insulin concentrations are well documented (3,4).

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AEP, auditory-evoked potential; ANCOVA, analysis of covariance; AUC, area under the curve; CSF, cerebrospinal fluid; EEG, electroencephalogram; EOG, electrooculogram; HI, human insulin; PI, porcine insulin.

In the brain, insulin receptors are widely distributed, with highest densities in the olfactory bulb, hypothalamus, and hippocampus and throughout the limbic system (5).

At the cellular level, insulin exerts a variety of actions on neurons (1,2,5). For example, it inhibits firing of neurons in the hippocampus (6) and hypothalamus (7). Through an influence on membrane sodium transport in choroid plexus epithelial cells (8), insulin could cause widespread neuronal hyperpolarization (9). Moreover, insulin has been found to inhibit reuptake of norepinephrine in dissociated rat brain cells (10), to alter catecholamine turnover in the hypothalamus (11,12), and to stimulate phosphoinositol turnover in the hippocampus via a stimulation of adrenergic activity (13). Insulin has also been shown to differentially influence norepinephrine and dopamine transporter mRNA in neurons (14).

Although multiple insulin effects on various neuronal cell functions are well documented, the consequence of insulin effects on central nervous functions in humans remains unclear. The issue has substantial clinical relevance, considering that patients with type 1 and type 2 diabetes can display hypoinsulinemia or hyperinsulinemia. The potential impact of chronically altered insulin concentrations on brain functions in these patients during daily life (15–18) as well as on the development of hypoglycemia unawareness (19) is hardly understood at present. The lack of information on central nervous insulin effects in humans probably derives from the fact that direct central nervous effects of insulin are difficult to investigate because the concomitant decrease of blood glucose concentrations. Also, with euglycemic hyperinsulinemia, factors linked to the systemic action of the hormone-like increases in cellular potassium uptake, catecholamine and cortisol secretion, and blood pressure (20) can obscure any direct central nervous action of insulin.

Evidence has accumulated that a direct pathway from the nose to the brain exists. Several intranasally administered viruses enter the brain via the olfactory nerve (21–27). Metal ions like cadmium (28,29), aluminum (30), and mercury (31), as well as drugs like cephalixin (32) and proteins like horseradish peroxidase (33,34), have been shown to penetrate directly from the nose to the brain. Functional evidence also exists for a nose–brain pathway for neuropeptides in humans. For example, in the presence of comparable plasma concentrations, arginine vasopressin (35) and cholecystokinin (36) yielded stronger central nervous effects on brain-evoked potential responses when administered intranasally than intravenously. Intranasally administered corticotropin-releasing hormone influenced gastric acid secretion and mood in healthy subjects without a discernible resorption into the bloodstream (37). Whether insulin penetrates via the nose to the brain has not been investigated so far. Since pure insulin is hardly absorbed from the nose into

the blood vessels (38–41), only small changes in blood glucose levels would be expected after intranasal administration. This study aimed at evaluating a possible nose–brain pathway for insulin and the effects of insulin on human brain functions after intranasal administration by assessing auditory-evoked brain potentials (AEP) in healthy humans. Evoked potentials representing physiological correlates of cognitive stimulus processing have been shown to be sensitive even to subclinical changes in brain functions (42–44).

RESEARCH DESIGN AND METHODS

Subjects. Subjects were 18 healthy, normally hearing male volunteers (age 18–34 years) of normal body weight (mean \pm SE BMI, 23.8 ± 1.2 kg/m²) and without personal or family history of diabetes. All subjects were nonsmokers and not under current medication. Fifteen hours before testing, they had to fast and abstain from coffee and alcoholic beverages. Subjects with gross sleep disturbances in the nights preceding the experimental sessions were excluded. The study was approved by the local Ethics Committee on Research Involving Human Subjects, and written informed consent was obtained from all subjects.

Procedure. The experiments were double-blind and designed according to a within-subject crossover comparison. Each subject was tested twice, with an interval of at least 1 week between sessions. At one of the two sessions, the subject received biosynthetic human insulin (H-Insulin 100 Hoechst; Hoechst, Frankfurt, Germany), and at the other session, he received the vehicle solution (2.7 mg/ml m-Cresol; Hoechst) as placebo. The order of administration of placebo and insulin was randomly assigned across subjects, with half of the subjects starting with the placebo condition and the other half with the insulin condition. Experiments took place in a sound-attenuated room between 1300 and 1600 with the subject sitting in supine position in a reclining chair. Thirty minutes before testing, a catheter was inserted into an antecubital vein that was kept patent with a 150 mmol/l NaCl solution. Blood samples were taken every 15 min for determination of blood glucose and serum insulin and every 7.5 min for determination of catecholamines.

After a baseline period of 60 min, insulin (20 IU, corresponding to 10 IU dissolved in 100 μ l vehicle per each nostril) or placebo was intranasally administered every 15 min until the end of the experiment.

AEPs indexing different stages of the brain's stimulus processing were recorded while the subject performed a vigilance task (oddball paradigm). The task required the subject to discriminate and covertly count target pips (1,064 Hz, duration 60 ms, intensity 64 dB SPL, probability 0.1) randomly interspersed among frequent standard pips of lower pitch (1,000 Hz); the task contained ~400 pips. A relatively small difference in pitch between standard and target tones was chosen to prevent the task from being too easy for the subjects—medical students, who usually display above-average discriminative abilities. Increasing the task demands typically increases the sensitivity of the P3 of the AEP to changes in the respective underlying cognitive functions. Tone pips were presented binaurally through headphones, with an interstimulus interval randomly varying between 1,000 and 3,000 ms (mean 2,000 ms). The subject was instructed to press a button with the thumb of the dominant hand immediately when a target pip had been recognized. To avoid electroencephalogram (EEG) artifacts, the subject was asked to fix his eyes on a centrally located dot in front of him and not to blink too often. Brain potentials evoked by both standard and target pips contain a prominent component complex about 100 ms poststimulus, which is termed the vertex response and is made up of a first vertex negative deflection (N1) and a subsequent positive deflection (P2). The vertex potential to a great extent reflects a nonspecific cortical arousal response to the stimulus presentation (45). The task-relevant target pips, in addition, evoke a large positive component (P3, also called P300), peaking ~350–450 ms poststimulus over parietal cortical regions. The P3 is considered an indicator of the target processing within short-term memory (46). AEPs were recorded at the end of the baseline phase and after 60 min of intranasal administration of insulin or placebo.

After each AEP recording, a checklist of adjectives (EWL-N) (47) was presented to the subject, in which a total of 65 items were used to describe the subject's mood on several dimensions such as activation, concentration, deactivation, fatigue, numbness and/or tingling, extraversion, introversion, and anxiety. For each adjective, the subject had to indicate whether or not it reflected aspects of his current state of mood. To determine subjective symptoms, at the end of the experiment subjects rated on a scale from 1 (none) to 7 (severe) the following symptoms: hunger, sweating, palpitations, tremor, tiredness, nervousness, dizziness, faintness, irritability, blurred vision, and difficulty in concentrating. Finally, the subject was asked whether he believed he received placebo or an active agent. Blood pressure and heart rate were measured oscil-

lometrically with a blood pressure monitor (Boso Prestige; Bosch und Sohn GmbH, Jungingen, Germany) every 20 min.

Recordings and apparatus. Recordings were obtained of EEGs (5 s time constant, 70 Hz/12 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 mastoids. 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 diameter were used for all recordings. EEG and EOG signals were amplified by a Nihon Kohden Neurofax 4421 G polygraph (Nihon Kohden, Tokyo, Japan) and digitized (CED 1401; Cambridge Electronic Design, Cambridge, U.K.) with a sampling rate of 385 Hz for offline averaging of AEPs.

Serum insulin concentrations were determined in duplicate by radioimmunoassay (Pharmacia Insulin RIA 100; Pharmacia Diagnostics, Uppsala, Sweden) with a sensitivity of 12 pmol/l and an interassay error, measured as coefficient of variation, of <5.4%. Intra-assay variation was <4.5% in all cases. The same kit was used for all samples of an individual subject. Plasma epinephrine and norepinephrine levels were measured in duplicate by standard high-performance liquid chromatography. Blood glucose levels were measured in duplicate by a Beckman glucose analyzer II (Beckman Instruments, Fullerton, CA) with a coefficient of variation <1.1%.

Data reduction and analysis. AEPs were averaged separately according to experimental conditions: time of recording (baseline versus spray), treatment (insulin versus placebo), topography (Fz, Cz, Pz), and tone pip (standard versus target). The averaging epoch covered a 200-ms baseline and a 800-ms poststimulus interval. EEG epochs were excluded from analysis if they contained gross eye movements or other artifact potentials exceeding ± 50 μ V. Measures derived from AEP waveforms were as follows:

- Latencies and baseline-to-peak amplitudes of the N1 and P2 components after standard pips. 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.
- The latency, baseline-to-peak amplitude, and area under the curve (AUC) between 280 and 700 ms in the AEPs following target pips. P3 was defined with regard to the maximum positive amplitude within 280–700 ms poststimulus. Also, AUC were calculated separately for the 280–500 ms and 500–700 ms latency bins of the AEPs to targets.

Effects on AEP measures were assessed by repeated measures analyses of covariance (ANCOVAs) containing the factors treatment (insulin versus placebo), topography (Fz, Cz, Pz), and tone pip (standard versus target). The baseline measurements served as covariates.

Effects of intranasal insulin (versus placebo) on average measures of blood glucose, serum insulin, catecholamines, systolic and diastolic blood pressure, and heart rate, as well as effects at single points in time on these measures, were statistically assessed by ANCOVA with the average values during the baseline serving as covariates. Additional analyses of variance were run to assess differences between the baseline and treatment phase.

Analyses of self-report measures included nonparametric statistical tests (Friedman, Wilcoxon). A *P* value <0.05 was considered significant. Degrees of freedom were corrected according to the Greenhouse-Geisser procedure.

RESULTS

Blood glucose, serum insulin, and catecholamine concentrations. Mean blood glucose and serum insulin levels during intranasal treatment with placebo and insulin are shown in Fig. 1. Blood glucose concentrations declined during the experiment, with lower levels during the phase of intranasal treatment compared with the pretreatment baseline levels (before and during intranasal treatment, 5.07 ± 0.15 and 4.90 ± 0.10 mmol/l; *P* < 0.01). Blood glucose concentrations during intranasal treatment with insulin did not differ from values during the placebo session (before and during intranasal placebo, 5.20 ± 0.20 and 5.05 ± 0.09 mmol/l; before and during intranasal insulin, 4.92 ± 0.12 and 4.88 ± 0.08 mmol/l, *P* > 0.4). In addition, pairwise comparison at each point of measurement taken every 15 min during treatment did not reveal any significant differences between the effects of insulin and placebo.

There were also no significant differences between the treatment conditions in serum insulin concentrations (before and during intranasal placebo, 64.6 ± 14.2 and 47.1 ± 7.0 pmol/l; before and during intranasal insulin, 62.9 ± 11.1 and 59.5 ± 6.5

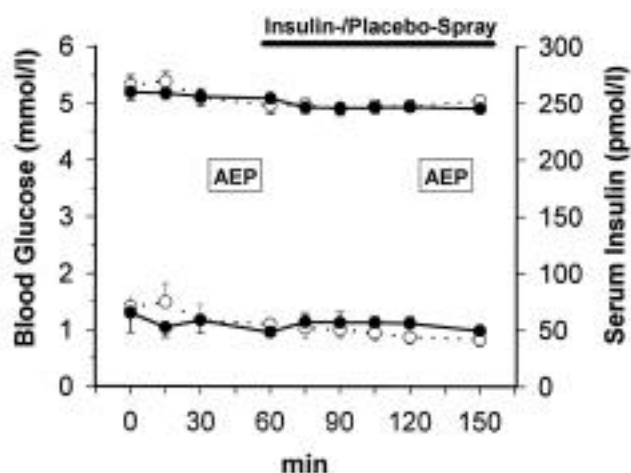


FIG. 1. Mean \pm SE blood glucose and serum insulin concentrations during intranasal administration of placebo (\circ) and insulin (20 IU every 15 min, \bullet). The time of intranasal treatment is indicated by horizontal bar. Blood glucose and serum insulin levels during both conditions were nearly identical and hence can be hardly distinguished in the figure. SEs for most single points in time were smaller than the size of the circles. AEP, time of AEP recording.

pmol/l, $P > 0.3$), plasma norepinephrine levels (before and during intranasal placebo, 1.45 ± 0.10 and 1.33 ± 0.08 nmol/l; before and during intranasal insulin, 1.66 ± 0.15 and 1.56 ± 0.11 nmol/l, $P > 0.5$), and plasma epinephrine levels (before and during intranasal placebo, 255.4 ± 47.5 and 206.3 ± 20.7 pmol/l; before and during intranasal insulin, 228.7 ± 42.6 and 185.2 ± 18.0 pmol/l, $P > 0.3$).

TABLE 1

Baseline-to-peak amplitudes (in microvolts) of AEP components N1 and P2 for standard tone pips at frontal, central, and parietal recording sites during the baseline phase and during the phase of intranasal administration of placebo and insulin

	Baseline	Intranasal administration
N1		
Frontal		
Placebo	-8.82 ± 0.86	-8.90 ± 0.64
Insulin	-8.56 ± 0.71	-8.32 ± 0.70
Central		
Placebo	-9.42 ± 1.03	-9.43 ± 0.98
Insulin	-9.10 ± 1.01	$-8.43 \pm 0.98^*$
Parietal		
Placebo	-5.67 ± 0.67	-5.68 ± 0.73
Insulin	-5.59 ± 0.90	$-4.94 \pm 0.83^\dagger$
P2		
Frontal		
Placebo	3.15 ± 0.46	2.65 ± 0.44
Insulin	3.16 ± 0.79	3.61 ± 0.73
Central		
Placebo	5.56 ± 0.69	4.59 ± 0.53
Insulin	5.24 ± 0.81	5.29 ± 0.84
Parietal		
Placebo	3.76 ± 0.88	3.11 ± 0.48
Insulin	3.78 ± 0.41	3.60 ± 0.45

Data are means \pm SE averaged across all subjects. $^*P < 0.005$; $^\dagger P < 0.01$ (ANCOVA with baseline value as covariate).

Systolic and diastolic blood pressure and heart rate.

Measures of cardiovascular activity did not indicate any effect of intranasal insulin (systolic blood pressure before and during intranasal placebo, 124.3 ± 3.4 and 124.6 ± 2.9 mmHg; before and during intranasal insulin, 123.7 ± 2.5 and 124.8 ± 2.6 mmHg, $P > 0.8$; diastolic blood pressure before and during intranasal placebo, 75.7 ± 2.1 and 79.6 ± 2.5 mmHg; before and during intranasal insulin, 75.3 ± 2.2 and 79.2 ± 1.9 mmHg, $P > 0.8$; heart rate before and during intranasal placebo, 67.2 ± 2.5 and 66.9 ± 2.5 beats/min; before and during intranasal insulin, 65.5 ± 2.4 and 65.5 ± 2.3 beats/min, $P > 0.5$).

AEPs. There was no significant difference ($P > 0.2$) in any of the baseline parameters between the placebo and insulin condition. As expected, N1 and P2 showed maximum baseline-to-peak amplitudes in recordings from Cz, while the P3-component after target pips dominated at parietal recording sites (topography $P < 0.001$).

Tables 1 and 2 summarize the effects of intranasal administration of placebo and insulin on AEP components. Treatment with placebo left the baseline-to-peak amplitude of the N1 component virtually unaffected, whereas intranasal administration of insulin significantly reduced the N1 amplitude. This effect was most pronounced for electrode location Cz ($-0.67 \mu\text{V}$, $P < 0.005$), but was also obtained at Pz ($-0.65 \mu\text{V}$, $P < 0.01$). Baseline-to-peak amplitude of the P2 component did not differ between the placebo and insulin sessions. N1 and P2 latencies also remained unaffected by insulin treatment.

Target tones elicited a distinct P3 component. Intranasal administration of insulin significantly reduced baseline-to-peak amplitude as well as AUC (280–500 ms poststimulus) compared with the placebo recordings (Table 2). The effect on these measures was most pronounced over frontal recording sites and decreased toward the parietal regions (mean difference in P3 baseline-to-peak amplitude between placebo versus insulin: Fz, $-2.50 \mu\text{V}$, $P < 0.0005$; Cz, $-1.33 \mu\text{V}$, $P < 0.03$; Pz, $-1.22 \mu\text{V}$, $P < 0.02$). Comparable changes were observed for the AUC subcomponents determined separately for the 280–500 and 500–700 ms latency bins (AUC 280–500 ms poststimulus: Fz, $-436.8 \mu\text{V} \cdot \text{ms}$, $P < 0.01$; Cz, $-263.9 \mu\text{V} \cdot \text{ms}$, $P < 0.08$; Pz, $-281.7 \mu\text{V} \cdot \text{ms}$, $P < 0.004$; AUC 500–700 ms poststimulus: Fz, $-335.9 \mu\text{V} \cdot \text{ms}$, $P < 0.06$; Cz, $-138.3 \mu\text{V} \cdot \text{ms}$, NS; Pz, $-91.1 \mu\text{V} \cdot \text{ms}$, $P < 0.04$) (Fig. 2). P3 latency was prolonged at Pz by 25.2 ms during treatment with insulin compared with placebo ($P < 0.02$) (Table 3).

Mood and self-reported side effects. Assessment of mood with the adjective list EWL-N revealed that intranasal treatment with insulin reduced scores of introversion and increased extraversion ($P < 0.05$ for both vs. placebo). The subjective symptoms assessed by seven-point rating scales (hunger, sweating, etc.) were not different between intranasal placebo and insulin treatment. Other side effects were not reported. At the end of a session, subjects were unable to correctly identify whether they had received placebo or insulin.

DISCUSSION

Intranasally administered insulin without absorption enhancers is known to be hardly absorbed into the bloodstream (38–41). Accordingly, as expected from previous studies, the dose of insulin administered intranasally in this study was too low to produce a measurable and biologically relevant increase in insulin concentrations in the bloodstream, as indicated by both unchanged serum concentrations of

TABLE 2

Baseline-to-peak amplitudes (in microvolts) of the P3 component of the AEP and AUC 280–500 and 500–700 ms poststimulus (in microvolts per milliseconds) for target tone pips at frontal, central, and parietal recording sites during the baseline phase and the phase of intranasal administration of placebo and insulin

	P3		AUC 280–500 ms		AUC 500–700 ms	
	Baseline	Intranasal administration	Baseline	Intranasal administration	Baseline	Intranasal administration
Frontal						
Placebo	3.57 ± 0.82	4.92 ± 0.79	103.1 ± 154.4	270.8 ± 138.7	-705.4 ± 141.6	-571.3 ± 163.0
Insulin	3.66 ± 1.07	2.42 ± 1.00*	30.7 ± 188.9	-166.0 ± 183.8†	-791.5 ± 172.6	-907.2 ± 198.3‡
Central						
Placebo	6.65 ± 1.21	7.92 ± 1.18	654.9 ± 242.3	880.7 ± 232.3	84.6 ± 209.4	236.7 ± 231.2
Insulin	7.53 ± 1.46	6.59 ± 1.41§	710.2 ± 282.2	616.8 ± 249.8‡	222.6 ± 242.1	98.4 ± 212.3
Parietal						
Placebo	9.88 ± 1.09	11.18 ± 1.16	1,429.3 ± 176.6	1,650.3 ± 193.2	801.4 ± 205.6	930.0 ± 240.5
Insulin	10.11 ± 1.54	9.96 ± 1.38§	1,477.0 ± 233.1	1,368.6 ± 226.6*	1,000.1 ± 212.1	838.9 ± 216.1§

Data are means ± SE averaged across all subjects. * $P < 0.005$; † $P < 0.01$; ‡ $P < 0.1$; § $P < 0.05$ (ANCOVA with the baseline value as covariate).

insulin and identical blood glucose levels during intranasal administration of insulin, in comparison with the effects of placebo. Yet during intranasal administration of insulin, amplitudes of the N1 and P3 component of the AEP distinctly decreased and P3 latency increased compared with placebo treatment. These results exclude that the changes in AEPs during intranasal administration of insulin were mediated via the bloodstream. Converging evidence has been provided by previous studies for a direct pathway between nose and brain, along which substances can enter the brain after intranasal administration. As mentioned above, several viruses (21–27), metal ions (28–31), drugs (32), and proteins (33,34) have been shown to penetrate directly from the nose to the brain in animal studies. Moreover, in humans central nervous effects after intranasal administration of peptide

hormones like arginine vasopressin (35) and cholecystokinin (36) on stimulus-evoked brain responses have been found to be unrelated to plasma concentrations of these hormones. For ethical reasons, we did not assess changes in CSF insulin levels in our normal volunteers. Nevertheless, the earlier results, together with the present findings of distinct changes in AEP and mood after intranasal administration of insulin in the absence of substantial increases in blood insulin concentrations, conclusively support the view that for peptides such as insulin the nose–brain pathway provides a facilitated access to cerebral nervous functions.

A mechanism of nose–brain transport that has been extensively investigated is the retrograde intra-axonal spread (27,34). Passage via this route takes several days, however, which is too long to account for the effects of intranasal insulin in the present study that emerged within 60 min. Balin et al. (33) have provided conclusive evidence for a direct extracellular pathway from the nose mucosa to the brain CSF compartment. In those experiments involving nonprimates and primates, horseradish peroxidase after intranasal administration reached the fiber layer of the olfactory bulb within minutes after passing through patent intercellular clefts in the olfactory epithelium. Peroxidase diffused out of the olfactory fiber layer over time, presumably a consequence of bulk flow of cerebrospinal fluid. Thus, considering the relatively fast changes in AEP components after intranasal insulin in the present study, the effect must be assumed to result from an extracellular rather than intracellular transport of insulin to the brain. It is conceivable that binding sites at the olfactory bulbs, which show a remarkably high density of insulin receptors (2), contribute to the transport, but this remains to be elucidated.

After systemic administration of insulin, direct effects of the peptide on central nervous functions in humans are difficult to distinguish from alterations due to the associated decrease in blood glucose concentrations. For ethical reasons, direct intracerebroventricular administration of insulin in humans is not possible. Therefore, evidence regarding the effects of insulin on human brain functions provided from previous studies has been rather indirect. In such studies, we compared the effects of porcine and human insulin (PI and HI) (42–44).

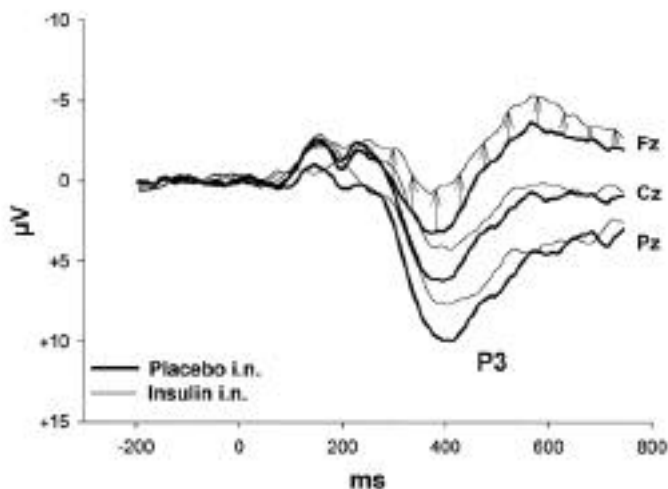


FIG. 2. Grand average AEP responses during intranasal treatment with placebo (—) and insulin (---) recorded at frontal (Fz), central (Cz), and parietal (Pz) electrode positions. To isolate the “pure” P3 component, the AEP curve for standard tones was subtracted from the AEP curve for target tones. Arrows indicate the broad negative potential shift over the frontal recording site during intranasal insulin compared with placebo. Negative is upward.

TABLE 3

Latency (in milliseconds) of the P3 component of the AEP for target tone pips at frontal, central, and parietal recording sites during the baseline phase and the phase of intranasal administration of placebo and insulin

	Baseline	Intranasal administration
Frontal		
Placebo	387.5 ± 10.4	377.5 ± 9.7
Insulin	378.9 ± 10.6	393.3 ± 11.8
Central		
Placebo	396.1 ± 10.7	395.6 ± 10.9
Insulin	408.9 ± 16.9	409.4 ± 12.0
Parietal		
Placebo	408.3 ± 11.1	388.9 ± 9.2
Insulin	413.8 ± 15.6	415.6 ± 9.2*

Data are means ± SE averaged across all subjects. * $P < 0.05$ (ANCOVA with the baseline value as covariate).

Assuming that PI, because of its higher lipophilicity, enters the brain more easily than HI, we expected that PI would exert stronger effects in the brain than HI. In fact, while hypoglycemia induced by both insulins was identical, reduction in amplitudes and prolongation in latencies of evoked brain responses were more pronounced during PI than HI administration. These more pronounced effects of PI- than HI-induced hypoglycemia provided a first hint at a direct effect of insulin on human brain functions, effects remarkably similar to those obtained during intranasal administration of insulin in the present study. Another approach to investigate direct central nervous effects of insulin in humans is to induce different levels of hyperinsulinemia while maintaining the same euglycemic blood glucose levels by infusion of glucose. With this approach as well, higher insulin levels (compared with lower insulin levels) resulted in AEP changes similar in shape and topography to those seen here after intranasal administration of insulin (48). That changes in evoked brain potentials show so much congruence across different experimental approaches supports the view that insulin alters the underlying mechanisms of cognitive processing via a direct effect on the human brain. Moreover, considering the many factors that potentially mask cerebral nervous effects after systemic insulin administration, intranasal administration appears to be the most effective way to unravel the central nervous effects of insulin in humans.

The changes in AEP components during intranasal administration of insulin resemble the pattern of reduced amplitudes and/or prolonged latencies of EP components that has been consistently observed during insulin-induced hypoglycemia (43,49–51). Thus, direct effects of insulin on central nervous functions may in part contribute to the AEP changes found during insulin-induced hypoglycemia. This view is further supported by findings of Lingens et al. (52). In that study, hypoglycemia-induced changes in AEP were more pronounced at higher serum insulin levels.

In this context, it seems noteworthy that several studies have reported reduced amplitudes and prolonged latencies of evoked potential components in patients with type 1 and type 2 diabetes under euglycemic conditions (17,18,53–57). These alterations did not correlate with duration of diabetes, HbA_{1c}, or later complications of diabetes (18,53,54,56,57).

Although in patients with type 1 and type 2 diabetes serum levels of insulin can be elevated persistently to supraphysiological levels, the number of central nervous insulin receptors may not be downregulated (58,59). Hence, it is tempting to speculate that chronically increased insulin concentrations in the brain in the presence of an unchanged number of central nervous insulin receptors may be, in part, responsible for the evoked potential changes observed in diabetic patients. Indeed, these alterations bear distinct similarities to those observed in the present study in healthy subjects during acute intranasal treatment with insulin.

Although the P3 amplitude showed the typical scalp distribution, with the most prominent amplitudes over the parietal recording site, the decrease in P3 amplitude during intranasal insulin was most pronounced over the frontal recording site. A similar decrease in frontal amplitudes has recently been observed in AEP during a euglycemic-hyperinsulinemic clamp (48) as well as in visual-evoked potentials during insulin-induced hypoglycemia in healthy subjects (50) and patients with type 2 diabetes (60). As in the present study, the frontal decrease in evoked potential amplitudes was accompanied by a broad negative potential shift 280–700 ms poststimulus, which also appeared to contribute in part to the reduction in the P3 baseline-to-peak amplitude. This negative shift, termed frontal negative slow wave, has been related, in the context of more complex test paradigms, to an increased effort-related allocation of further processing resources (61–64). In fact, in a recent study this frontal negative slow wave observed during euglycemic hyperinsulinemia was paralleled by an improvement in selective attention and memory functions (48).

Interestingly, Mecklinger et al. (65) found that the occurrence of the frontal negative slow wave is accompanied by an increased theta rhythm in the frontal EEG recordings. An increased frontal theta activity has been likewise reported in insulin-induced hypoglycemia (49,66). Frontal theta activity is very likely mediated by theta activity generated within the hippocampus (67), and the hippocampus also has been identified as an important generator of evoked potential positivity in the P3/slow wave latency bin (68–72). So it could well be that increased frontal theta activity, a frontal negative slow wave, and a reduction in the frontal P3 amplitude commonly derive from an inhibition of hippocampal neuronal activity after insulin administration. The hippocampus is one of the brain regions with a great number of insulin-binding sites, especially in the dendritic fields of CA1 pyramidal cells and in the molecular layer of the dentate gyrus (2). Insulin binding to these receptors decreases the firing rate of pyramidal neurons in the hippocampus (6), and such influences may well act to increase EEG theta rhythm and frontal slow wave negativity.

In sum, in the absence of any changes in serum concentrations of insulin and blood glucose, intranasal administration of insulin reduced amplitudes of the N1 and P3 component and increased latency of the P3 component of the AEP. These findings hint at a direct pathway for insulin from the nose to the brain. This route of peptide administration could provide a useful tool for the investigation of direct effects of insulin on central nervous function in humans. In this context, it may find a potential clinical application in the effort to promote weight loss in obese patients (2,73) and to improve cognitive function in patients with Alzheimer's dementia,

whose cognitive deficits seem to be related to a decrease in central nervous insulin concentration (74).

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