Transcortical Direct Current Potential Shift Reflects Immediate Signaling of Systemic Insulin to the Human Brain

Manfred Hallschmid,[1] Bernd Schultes,[2] Lisa Marshall,[1] Matthias Mölle,[1] Werner Kern,[2] Julia Bredthauer,[1] Horst L. Fehm,[2] and Jan Born[1]

Circulating insulin is thought to provide a major feedback signal for the hypothalamic regulation of energy homeostasis and food intake, although this signaling appears to be slowed by a time-consuming blood-to-brain transport. Here we show, by recording direct current potentials, a rapid onset of the effects of circulating insulin on human brain activity. Recordings were obtained from 27 men who were intravenously injected with insulin (0.1 mU/kg body wt as bolus) and placebo. In a euglycemic condition, hypoglycemia was prevented, while in the hypoglycemic condition, plasma glucose reached a postinjection nadir of 43 mg/dl. Insulin injection induced a marked negative direct current (DC) potential shift starting within 7 min in all subjects. With euglycemic conditions, the DC potential at 10–60 min postinsulin injection averaged −621.3 μV (compared with preinjection baseline). Hypoglycemia reduced this potential to an average of −331.2 μV. While insulin per se did not affect oscillatory electroencephalographic activity, hypoglycemia peaking 25 min after insulin injection was accompanied by an immediate increase in θ activity. The rapid emergence of the DC potential shift, reflecting gross ionic changes in brain tissues, indicates that systemic insulin can serve as an immediate feedback signal in the control of hypothalamic and higher brain functions. *Diabetes* 53:2202–2208, 2004

Systemic insulin plays an important role as a neuromodulator in the brain, which itself does not synthesize insulin (1,2). In particular, circulating insulin has been proposed to serve as a major negative feedback signal to the brain in the regulation of energy homeostasis and food intake behaviors (2–4). Research in animals consistently supports the notion that insulin after intracerebroventricular administration acts to inhibit feeding behaviors (5) and the expression of neuropeptide Y in the hypothalamus (6). A recent study (7) demonstrated that hypothalamic insulin signaling is even necessary for the inhibition of hepatic glucose production.

Systemic insulin predominantly accesses the brain via a saturable active transendothelial transport across the blood-brain barrier (BBB) (8). Measures of insulin uptake into cerebrospinal fluid (CSF) after intravenous administration in dogs and humans have indicated a slow passage to the brain compartment over periods of several hours, with a detectable increase in CSF insulin levels following the systemic increase by 15-30 min (9,10). Hence, the question arises regarding the temporal dynamics of the impact of insulin on brain function: Can insulin provide an immediate feedback signal that may, for example, be involved in the acute regulation of eating behavior and satiety? Here, by measuring scalp-recorded direct current (DC) potentials, we provide evidence that systemic insulin induces gross changes in brain activity within minutes. These findings indicate that insulin may be a rapidly acting feedback signal in humans that acutely reports food intake to the brain, thereby possibly contributing to feelings of satiety and suppression of hunger. Apart from that, recording oscillatory electroencephalogram (EEG) activity revealed an increase in θ activity induced by hypoglycemia.

We examined the effects of insulin in healthy men after an intravenous bolus injection of 0.1 mU/kg body wt while blood glucose was maintained (insulin-eu) or allowed to drop (insulin-hypo) to separate the effects of insulin from those of hypoglycemia. A third group of subjects received placebo. The EEG was recorded because it has proven to be highly sensitive to hypoglycemic influences in many previous studies (11,12). DC potential recordings were of particular interest because they are able to reveal the gradual modulation of brain activity that arises from widespread ionic changes in extracellular space, including major contributions of glial origin (13,14). Also, DC potentials were found to be sensitive to food intake in hungry men (15).

RESEARCH DESIGN AND METHODS

A total of 27 right-handed, healthy men participated in the experiments. They gave written informed consent before the experiments, which were approved by the local ethics committee. We randomly assigned subjects to one of three experimental groups (n = 9 each): insulin-eu (aged 24.3 ± 1.2 years, BMI 22.0 ± 0.6 kg/m^2 [means ± SE]), insulin-hypo (aged 25.4 ± 1.0 years, BMI 23.8 ± 0.7 kg/m^2), and placebo (aged 26.7 ± 1.0 years, BMI 23.3 ± 0.7 kg/m^2).

Volunteers reported to the laboratory at 0800 after an overnight fast of 10 h. During recordings, subjects sat in a reclining chair in a sound-attenuated room of constant temperature with their heads stabilized by a cushion. We inserted venous cannulas into the subjects' arms and connected them to tubes enabling

From the [1]Institute of Neuroendocrinology, University of Lübeck, Lübeck, Germany; and the [2]Department of Internal Medicine I, University of Lübeck, Lübeck, Germany.

Address correspondence and reprint requests to Manfred Hallschmid, University of Lübeck, Institute of Neuroendocrinology, Ratzeburger Allee 160, Haus 23a, 23538 Lübeck, Germany. E-mail: hallschmid@kfg.mu-luebeck.de.

Received for publication 6 February 2004 and accepted in revised form 27 May 2004.

BBB, blood-brain barrier; CSF, cerebrospinal fluid; DC, direct current; EEG, electroencephalogram; EMG, electromyogram; EOG, electrooculogram.

infusion and blood sampling from an adjacent room without awareness of the subject. The arm from which samples were taken was positioned in a heated box (55°C) to enable drawing of arterialized venous blood. We instructed subjects to relax, to remain still during recordings, and to fixate their gaze on the wall in front of them. Subjects pressed a button every (estimated) 30 s to induce a constant state of mental activity. We monitored constant pressing of the button (one to two times per minute) to assure that each subject was alert and did not doze off during the recording epoch. Analysis of button press responses did not reveal differences in time estimation among the conditions. Furthermore, any signs of sleep were excluded by online monitoring of the EEG recordings.

Recordings of DC potentials and EEG started at 0930 with a baseline phase of 30 min, after which a bolus of insulin (0.1 mU/kg body wt; H-insulin; Aventis Pharma, Bad Soden, Germany) or placebo was injected ($t = 0$). In the additional experiments using intranasal administration, subjects received six puffs (three in each nostril), each containing 0.1 ml (10 IU) of insulin or vehicle, with a nasal spray atomizer over a period of 45 s. The total dose of insulin was 60 IU. Recordings were continued for 90 min after insulin administration. Arterialized blood was drawn at 5-min intervals to monitor plasma glucose concentration (Glucose Analyser; Beckman Coulter, Munich, Germany). After insulin injection, the insulin-eu group intravenously received a 20% glucose solution at a variable rate to maintain normal plasma glucose levels. In the insulin-hypo condition, we allowed plasma glucose levels to drop to ~40 mg/dl and then reconstituted them by infusing glucose. In the placebo condition, we infused saline solution. We collected blood samples for the determination of hormonal parameters every 15 min from −30 to 0 min, every 5 min between 0 and 60 min, and again every 15 min thereafter.

Recordings. Standard recordings of DC potentials, EEG, electrooculogram (EOG), and electromyogram (EMG) were performed as described previously (16,17). For DC potential and EEG recordings, removing the outer layers of the skin with abrasive paste and puncturing the scalp at three points prevented contamination from skin potentials. Nonpolarizable Ag/AgCl electrodes (8 mm diameter; In Vivo Metric, Healdsburg, CA) were mounted onto clip-on electrode sockets attached with collodion and filled with electrode gel (Electrode Electrolyte; TECA, Pleasantville, NY). Electrodes had been connected pairwise via electrode gel for 1 h to reduce bias potentials.

We obtained EEG recordings from left and right frontal (F3, F4), frontocentral (FC3, FC4), and central (C3, C4) electrodes referenced to linked electrodes at the mastoids. A SynAmps DC amplifier (low-pass filter, 30 Hz; sampling rate of 100 Hz; NeuroScan, Herndon, VA) was used. DC potential drifts with short-circuited input were constantly <5 μV/h. Electrode impedance, measured before and after recordings, never exceeded 5 kΩ. The EOG was registered from electrodes attached at the outer canthi of the eyes (horizontal EOG) and 1 cm below and above the right eye (vertical EOG). Filter setting was as for DC recordings. The EMG was recorded from submental electrodes with high- and low-pass filters at 10 and 70 Hz, respectively.

Average DC potential values were determined offline for subsequent 30-s intervals. We removed linear potential drifts during the 30-min baseline period extending into the 90-min postinjection period using a linear regression method. Applying this procedure over such extended recording intervals is presumed to minimize the risk of type I or II errors and to result in improved signal-to-noise ratio (18). Periods where EMG or EOG indicated increased muscular activity or eye movements were excluded from analysis. The average DC potential during baseline was set to 0 μV, and potential shifts during treatment were expressed as difference values. Analysis of rhythmic EEG activity was based on power spectral analyses using a fast fourier transformation. Spectra were determined for subsequent 30-s epochs of EEG activity throughout the total recordings. The power spectrum for every 30-s epoch was calculated by averaging the fast fourier transformation of five segments of 1,024 EEG data points, each corresponding to 10 s of EEG recorded at a sampling rate of 100 Hz. The segments overlapped by half the data points (i.e., 512 points). To reduce errors induced by edge effects, the signal was tapered toward zero at the extremes of each data segment with a raised cosine window. Average log power values for the δ (0.5–4 Hz), θ (4–8 Hz), α (8–12 Hz), and β (12–25 Hz) frequency bands were standardized with reference to average power during the 30-min baseline and combined for every 5 min of experimental time.

Blood hormone concentrations. We used routine assays to determine concentrations of serum insulin (Pharmacia Insulin RIA 100; Pharmacia Diagnostics, Uppsala, Sweden), growth hormone (human GH radioimmunoassay; Biermann, Bad Nauheim, Germany), and plasma ACTH (LUMI test ACTH; Brahms Diagnostica, Berlin, Germany). Plasma epinephrine was determined by high-performance liquid chromatography with electrochemical detection.

Statistical analysis. Initially, we evaluated differences in DC potential values and EEG band power among conditions on an exploratory basis by pointwise comparisons using t tests to identify the time ranges with the most consistent differences, as indicated by an accumulation of statistical significances in the pointwise comparisons for at least one electrode site (19,20). Values for these time intervals were then subjected to ANOVA, including a group factor of condition and a repeated measures factor for topography (electrode locations). Degrees of freedom were corrected using the Greenhouse-Geisser procedure. Post hoc contrasts were used to specify significant ANOVA main effects and interactions. The time ranges selected for analyses turned out to cover 10–60 min and 25–50 min postinjection for the DC potentials and 25–30 min postinjection for EEG activity. For the EEG band power analysis, two subjects from the insulin-hypo group and, for the DC potential analysis, one subject from the insulin-eu group, two subjects from the insulin-hypo group, and two subjects of the placebo group had to be excluded due to a technical failure and artifacts of apparent nonbiological origin. Baseline-adjusted hormonal parameters were analyzed with t tests. A P value ≤0.05 was considered significant.

RESULTS

Plasma insulin and glucose levels following insulin injection. Injection of insulin induced a sharp rise in plasma insulin levels to peak values of 394.2 ± 32.3 μU/ml (insulin-eu) and 369.1 ± 32.9 μU/ml (insulin-hypo) 5 min after injection (Fig. 1*A*). Subsequently, insulin levels dropped quickly and recovered within 45 min, although they were still higher than after placebo at the end of the recording (insulin-eu 11.0 ± 1.39 μU/ml, insulin-hypo 11.9 ± 1.7 μU/ml, placebo 6.3 ± 1.0 μU/ml, $P < 0.02$). Glucose concentrations in the insulin-eu condition remained constant and comparable with levels after placebo injection ($P > 0.17$). In the insulin-hypo condition, glucose concentrations were decreased 15–35 min after insulin administration, falling to nadir values of 43.6 ± 1.8 mg/dl 30 min postinjection ($P < 0.001$, for comparisons with insulin-eu and placebo).

Strong negative DC potential shift upon insulin injection. The DC potential showed a marked negative shift shortly after insulin injection, which reached maximum values exceeding −600 μV (in the insulin-eu condition) ~40–50 min postinjection and proceeded to decline only marginally toward the end of the recording epoch (Fig. 1*B*). While this negative DC shift was observed in both the insulin-eu and the insulin-hypo condition, it was more pronounced when euglycemia was maintained.

The analyses of average DC potential levels for the 10–60 min postinjection interval confirmed a distinctly greater negative potential level in the insulin-eu condition than in the placebo [$F(1,13) = 22.42$, $P < 0.001$] or insulin-hypo conditions [$F(1,13) = 4.84$, $P < 0.05$; $F(2,19) = 9.06$, $P < 0.01$, for the effect of treatment] (Table 1). The average negative potential in the insulin-hypo condition also differed significantly from the placebo level 25–50 min postinjection [$F(1,12) = 6.06$, $P < 0.05$]. Comparisons with preinjection baseline levels confirmed significance for the negative DC potential 10–60 min after injection of insulin, when euglycemia was maintained [$F(1,7) = 44.16$, $P < 0.0003$], and for the negativity in the insulin-hypo condition 25–50 min postinjection [$F(1,6) = 8.98$, $P < 0.03$]. Although the negative shift in the insulin-hypo condition appeared to be more pronounced on the right than left side, topographical differences among the electrode locations were nonsignificant ($P > 0.3$, for effects of topography). In the placebo condition, there were no significant changes in the DC potential during the intervals of interest ($P > 0.22$, for respective comparisons with baseline values). Analyses of single time points revealed that (at the frontal leads) the negative DC poten-

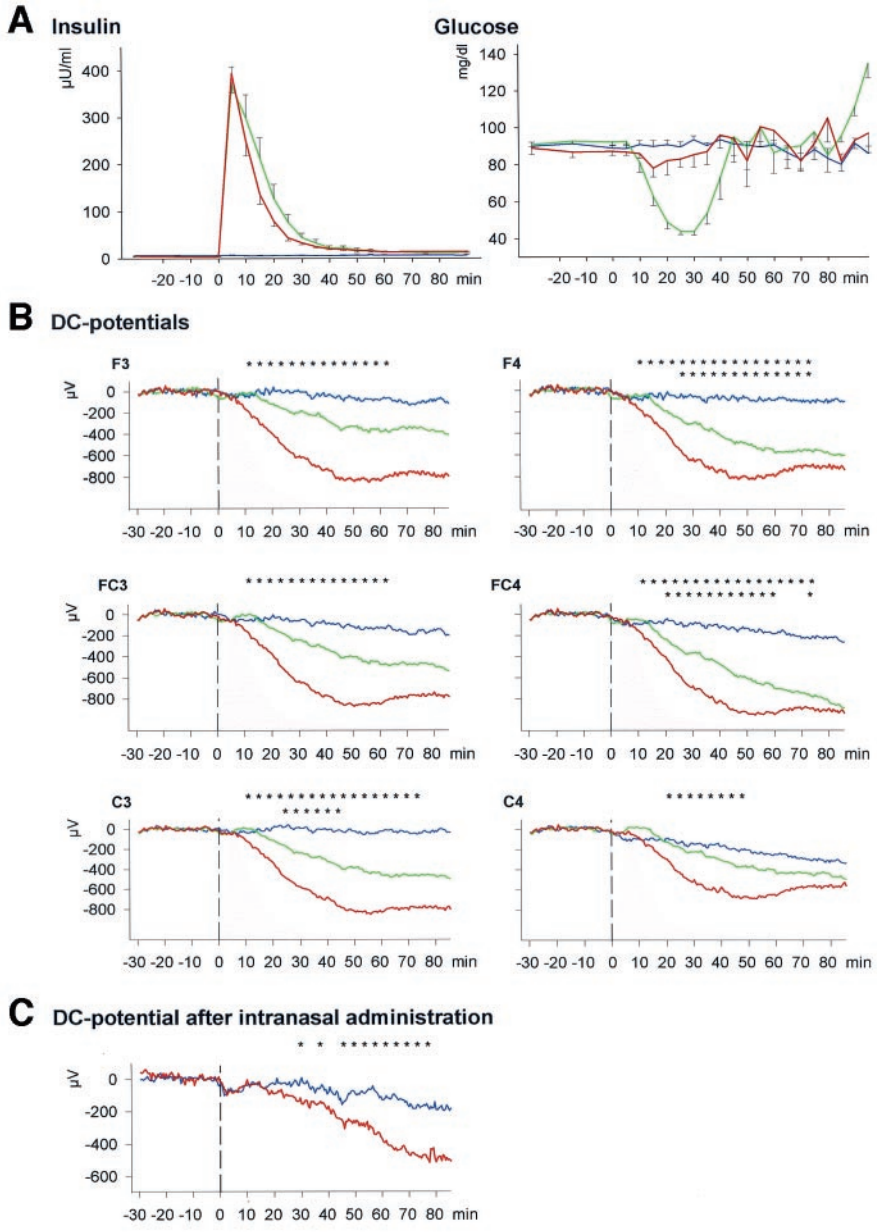

FIG. 1. Plasma insulin and glucose levels and DC potentials. *A*: Mean (±SE) plasma concentrations of insulin (*left*) and glucose (*right*) between 30 min before and 90 min after injection of insulin and placebo (at 0 min). *B*: Average DC potentials (over all subjects) recorded from left and right electrodes over frontal (F3, F4, respectively), frontocentral (FC3, FC4), and central (C3, C4) cortical areas for the same time interval. In one condition (insulin-eu, red line), the effects of insulin on plasma glucose concentration were compensated by infusion of glucose ($n = 8$). In the other condition (insulin-hypo, green line), hypoglycemia upon insulin injection was not prevented ($n = 7$). The placebo (blue line) injection of saline solution also was at $t = 0$ ($n = 7$). The average potential during baseline was set to 0 µV. Rows of asterisks indicate significance ($P < 0.05$) for pointwise comparisons between the potential levels in the placebo condition and the insulin-eu condition (*upper row*) and the insulin-hypo condition, respectively (*lower row*). Shaded areas indicate the time course of insulin plasma concentrations during the insulin-eu condition. *C*: DC potential (averaged over all recording sites and subjects) following intranasal administration ($t = 0$) of 0.6 ml (60 IU) insulin (red line) and placebo (blue line) in seven subjects. Significant differences among conditions are indicated ($P < 0.05$, one-sided t test).

tial shift in the insulin-eu condition attained significance within 7 min after insulin injection compared with baseline and after 10 min compared with placebo potential levels (Fig. 1). In the insulin-hypo condition, the negative DC shift did not reach significance (at F4) until 17 min postinjection compared with baseline levels.

TABLE 1
DC potential levels after insulin injection

Site	Insulin-eu	Insulin-hypo	Placebo	*P* (insulin-eu vs. placebo)	*P* (insulin-eu vs. insulin-hypo)
F3	−629.5 ± 120.5	−230.7 ± 108.0	−82.0 ± 85.6	<0.003	<0.03
FC3	−648.1 ± 95.4	−279.5 ± 141.8	−182.8 ± 75.2	<0.0006	<0.05
C3	−598.7 ± 96.4	−264.7 ± 121.1	−102.0 ± 59.9	<0.0002	<0.05
F4	−634.6 ± 103.8	−357.0 ± 110.3	−72.3 ± 56.0	<0.0003	<0.09
FC4	−704.5 ± 105.8	−425.5 ± 133.4	−114.8 ± 57.4	<0.0002	<0.12
C4	−515.3 ± 90.5	−254.9 ± 134.5	−167.1 ± 65.7	<0.02	<0.12

Data are means ± SE. Average DC potential levels (in µV) over left and right frontal (F3, F4), frontocentral (FC3, FC4), and central (C3, C4) cortical areas 10–60 min following intravenous injection of insulin. In the euglycemic condition (insulin-eu, $n = 8$), plasma glucose levels were held constant subsequent to insulin injection. In the hypoglycemic condition (insulin-hypo, $n = 7$), plasma glucose levels were allowed to drop to 40 mg/dl. In the placebo condition ($n = 7$), saline solution was injected. DC potential values indicate differences from baseline (set to 0 µV). The two right columns indicate significance for differences between insulin-eu and placebo (left) and between insulin-eu and insulin-hypo (right).

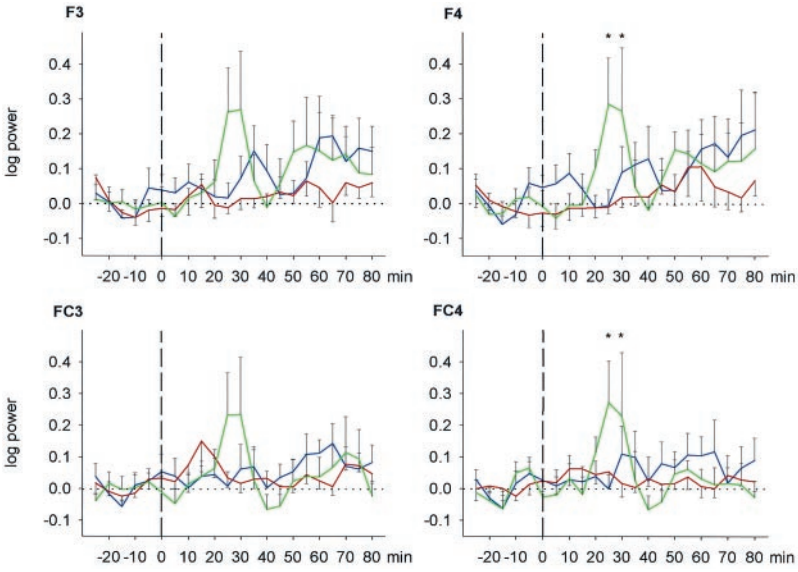

FIG. 2. EEG θ power. Mean (±SE) log θ power for frontal (F3, F4) and frontocentral (FC3, FC4) electrode positions during a 30-min baseline phase and a 90-min postinjection interval for three treatment conditions: insulin-eu (red line), injection of insulin at $t = 0$ with plasma glucose levels kept constant by glucose infusion ($n = 9$); insulin-hypo (green line), insulin injection at $t = 0$ followed by hypoglycemia ($n = 7$); and placebo (blue line), injection of saline solution at $t = 0$ ($n = 9$). Average power during baseline is set to zero. *$P < 0.05$, insulin-hypo vs. placebo.

Stimulation of EEG θ activity by hypoglycemia. Analyses of oscillatory EEG activity yielded consistent effects for the θ frequency range (4–8 Hz) only. θ power selectively increased in the insulin-hypo condition during the time of maximum hypoglycemia, i.e., 25–30 min after insulin injection [$F(2,22) = 3.68$, $P < 0.04$, for treatment effect; $P < 0.05$ for pairwise comparisons with insulin-eu and placebo conditions] (Fig. 2). In comparisons at single electrode sites, the increase in θ activity accompanying hypoglycemia appeared to be most robust at the frontal leads [compared with placebo at F4 and FC4, $F(1,14) = 5.3$ and 4.8, respectively, $P < 0.05$; vs. insulin-eu at F3 and F4, $F(1,14) = 5.9$ and 6.3, respectively, $P < 0.03$]. For the δ frequency range (0.5–4 Hz), a similar pattern of hypoglycemia-induced increase was obtained but did not reach significance [$F(2,22) = 1.43$, $P = 0.26$, for treatment effect].

Counterregulatory hormonal response to hypoglycemia. Insulin-induced hypoglycemia stimulated a rise in plasma concentrations of epinephrine starting at 15 min and reaching maximum concentrations 25–55 min postinjection (insulin-hypo 76.6 ± 15.7 pg/ml, insulin-eu 23.6 ± 3.4 pg/ml, placebo 13.3 ± 1.6 pg/ml; $P < 0.01$ for all). Also, increases in ACTH and growth hormone plasma concentrations commenced at 30 min and peaked at 40–60 min postinjection (ACTH: insulin-hypo 27.8 ± 6.9 pg/ml, insulin-eu 4.3 ± 0.6 pg/ml, placebo 7.5 ± 2.3 pg/ml; $P < 0.05$ for all; growth hormone: insulin-hypo 11.42 ± 4.01 ng/ml, insulin-eu 2.0 ± 0.6 ng/ml, placebo 2.34 ± 0.9 ng/ml; $P < 0.05$ for all) (Fig. 3).

DC potential shift after intranasal insulin administration. In supplementary experiments, we examined whether negative DC potential shifts also occurred following the intranasal administration of 60 IU insulin in seven men. Intranasal administration of insulin has been shown in humans to provide direct access of the molecule to the CSF within 30 min without substantial resorption into the blood stream (21). Here, compared with intranasal administration of placebo, insulin induced a marked negative DC potential shift at the frontal leads, developing ~30 min after administration and continuing for 50 min (averaging 45–75 min postadministration, −390.9 ± 106.1 μV vs. −140.6 ± 188.2 μV; $P < 0.05$ by one-sided t test, $P < 0.01$ vs. preadministration baseline level) (Fig. 1*C*). Plasma glucose levels were comparable between the insulin and placebo conditions of the intranasal sessions, averaging during the postadministration interval 86.7 ± 1.1 mg/dl after insulin and 89.3 ± 1.8 mg/dl after placebo ($P > 0.31$).

DISCUSSION

The brain is a major target of circulating insulin (1,2,4). Here, the intravenous injection of insulin induced a sus-

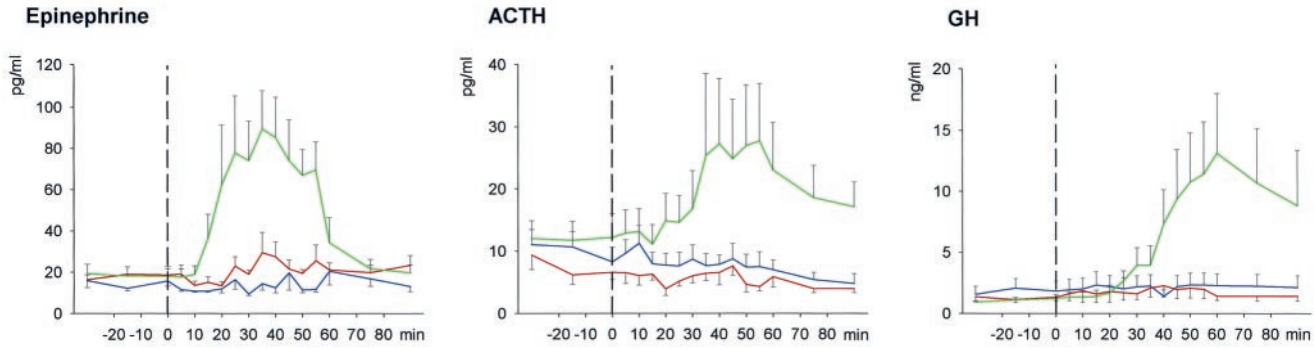

FIG. 3. Plasma epinephrine, ACTH, and growth hormone (GH). Mean (±SE) plasma concentrations of epinephrine (*left*), ACTH (*middle*), and GH (*right*) during a 30-min baseline phase and 90-min postinjection phase for the three conditions: insulin-eu (red line, $n = 9$), insulin-hypo (green line, $n = 9$), and placebo (blue line, $n = 9$).

tained negative shift in the transcortical DC potential recorded from the human scalp, which developed within 7 min after insulin administration. The shift was most pronounced when glucose levels were kept constant (i.e., under euglycemic conditions), but it also emerged when glucose concentrations were allowed to drop (i.e., in the insulin-hypo condition). This reason and the fact that in the latter condition, the DC potential shift developed at least 10 min before any significant hypoglycemia and associated counterregulation speak for a direct effect of insulin on brain activity independent of blood glucose concentration. On the other hand, an increase in oscillatory EEG θ activity coinciding with maximum hypoglycemia was found in the insulin-hypo condition.

In contrast to the DC potential shifts that were observed in both the insulin-hypo and the insulin-eu conditions, the increase in θ power occurred only during hypoglycemia and was absent when the glucose concentration was maintained at a normal level. Hence, it can be considered to be caused by the drop in plasma glucose concentration. The size and timing of the increase in θ activity observed here over frontocortical areas during hypoglycemia correspond well with previous reports (22). Frontocortical θ EEG activity is considered to reflect, at least in part, the activity of generating mechanisms in the hippocampus, where this type of oscillatory activity is most prominent (23,24). Of interest here are the findings of a recent study examining glucose concentrations in the hippocampal extracellular fluid of epileptic patients. In these patients, a substantial decrease in hippocampal extracellular fluid glucose concentration did not develop until 30 min after the onset of hypoglycemia (25). Assuming that extracellular fluid concentrations in these patients reflect tissue conditions comparable with those in our healthy subjects, these data lend themselves to conclude that the increase in θ in the insulin-hypo condition started before any substantial hypoglycemia developed in the hippocampus. This implies that, rather than from a localized lack in brain glucose, the increase in θ activity probably originates from a stimulation of glucose sensors (e.g., in the carotid body glomus cells), signaling blood glucose levels to the brain and limbic structures (26). However, this interpretation is tentative and in need of further validation, especially since a link between (sympathetic) glucose signaling at the carotid level and frontal EEG changes is, as yet, hypothetical. Developing hypoglycemia in the insulin-hypo condition also attenuated the insulin-induced negative DC potential shift. A counteracting influence on this negative shift is, indeed, buttressed by previous observations where increases in θ and lower α activity were found to be closely connected to DC potential shifts in a positive direction (27,28).

The DC potential commenced to shift toward negativity within 5–15 min after insulin injection in each subject. This early onset of central nervous changes fits with findings in rodents, in which insulin accumulated in brain tissue within 5 min after intravenous administration (29). Access of systemic insulin to the brain within a few minutes also corroborates the notion that effects of insulin on brain functions are regulated independently of its influence on systemic glucose concentration (30). However, a slower access of insulin to the brain compartment has been inferred from studies of CSF levels after intravenous administration. In dogs, a lag of 15 min occurred before any significant changes in CSF insulin levels were found (9), regardless of the clamped plasma insulin level. A similarly substantial delay between rises in plasma insulin and respective CSF levels has been reported in humans (31). Yet, rises in CSF insulin levels typically remain smaller than 5% of those in plasma (32), whereas insulin concentrations in rat brain extracts were found to be 30–40% of plasma levels (33), suggesting a preferential uptake of insulin in brain parenchyma. Schwartz et al. (10) concluded that the transport of circulating insulin into both brain and CSF occurs primarily across the BBB endothelium rather than the blood-CSF barrier and that most of the insulin is relatively rapidly extracted from interstitial fluid by binding to neuronal and glial tissue receptors.

DC potential levels after insulin injection had still not reached placebo values at the end of the 90-min recording epoch. This could represent a continuing effect of insulin, since plasma insulin concentrations at this time were still higher in the treatment than in the control conditions, although a contribution of mechanisms secondary to the action of insulin cannot be excluded. Given that the DC potential negativity after insulin also persisted in the euglycemic condition, hypoglycemic counterregulatory hormonal release does not appear to play a role in this context. It should be added that it is a common phenomenon that receptor-mediated effects of neuropeptides in the course of intracellular signal processing persist for some time once the substance has cleared the system.

The exact mechanisms behind the strong negative DC potential shift after intravenous injection of insulin cannot be derived from our data. The use of standard DC potential recording methodology excludes a number of potential sources from outside the brain. Recording the differential potential prevents confounding influences from potentials emerging similarly in tissues nearby both active and reference electrodes like skin and adipose tissue. Moreover, the skin underneath the electrodes was punctured, which abolishes skin potential shifts generated locally (34). To prevent biasing influences from muscle activity known to be associated by slow potential shifts (35), care was taken that subjects were relaxed during the recordings, and any periods were excluded where EMG recording indicated increased muscular activity. EOG monitoring excluded any ocular contributions to the observed shifts. The multiple controls performed here in conjunction with previous studies focusing on methodological issues of scalp-recorded DC potentials (15–17,35,36) provide solid evidence for the notion that the DC potential shift observed following insulin administration is of intracranial origin.

Further support for this conclusion is derived from our supplementary experiments employing the intransal administration of insulin. After intranasal administration, insulin has been shown to directly enter the brain CSF compartment without substantial absorption into the blood stream, thereby allowing the evaluation of direct effects on the CNS and preventing confounding influences of insulin acting on peripheral afferents to the brain (21). Here, following intranasal administration of insulin, basically the

same negative DC potential shift as after intravenous injection was observed. Plasma glucose concentration remained unchanged, confirming that intranasally administered insulin did not reach the blood stream in substantial amounts. This pattern indicates a negative shift in the DC potential resulting from a direct action of intranasal insulin on the brain, which could likewise be the mechanism inducing the same DC potential shift following systemic administration of insulin. Also, a possible contribution of electrical potentials generated in the course of the receptor-mediated transport of circulating insulin across the BBB has to be considered. Processes such as the binding of insulin to endothelial receptors, intracellular acidification during membrane transport, and others may involve ionic activity that over extended regions can sum up to substantial potential shifts at the cortical surface and scalp (37,38). On the other hand, intranasal administration of insulin, which bypasses the BBB, yielded a negative DC potential shift similar to that seen after intravenous injection. This argues against a primary role of the BBB in the generation of this shift but does not exclude additional contributions of BBB-related mechanisms, e.g., via pCO_2-dependent potential differences to the genesis of DC potential shifts, as has been emphasized in previous studies (39,40).

Assuming a primary central nervous origin, transcortical DC potential shifts of this amplitude reflect gross changes in extracellular ionic concentrations that most likely stem from glial functioning. Glial membranes are endowed with receptors for insulin and IGF (41), and insulin stimulates glucose uptake in glial cells (42). Through their widespread interconnections, glial cells form an ionic buffer mainly for K+, but also for Ca^{2+}, and thereby can generate substantial negative cortical DC potentials (43–46). Additional contributions of neuronal activity (e.g., from neocortical pyramidal cells) cannot be excluded but appear unlikely in light of the size and long duration of the shift and the lack of any substantial changes in oscillatory EEG activity (47).

In conclusion, the pronounced negative shift in the transcortical DC potential after insulin administration indicates that circulating insulin can rapidly act on brain activity independent from its systemic hypoglycemic action. To the best of our knowledge, a fast and direct action of systemic insulin on human central nervous activity of this type has not been demonstrated. While peak concentrations of insulin after the bolus injection used here were supraphysiological, the total amount of insulin administered was comparable to endogenous postprandial release. Based on this background, our data provide support for the notion that insulin can even serve as a rather short-term feedback signal in the hypothalamic control of food intake and satiety (48) and put a new complexion on the view of merely tonic signaling of insulin to the brain (5,49). It is of interest that euglycemic insulin infusion in men over several hours reduces rated hunger dose dependently (50). However, since in the present study indicators of hunger and food intake were not assessed, correlations of insulin-induced DC potential negativity with eating-related behaviors remain to be determined. In a previous study (15), a negative DC potential shift was found during food intake in humans, which, however, shifted toward positivity in conjunction with the termination of eating. In this context, it seems unlikely that the insulin signal is relevant for acutely inhibiting food intake but rather may add to the persistence of satiety after a meal preventing reinitiation of consumatory behavior. Considering the widespread distribution of insulin receptors in different brain structures, including hippocampus and neocortex (1), the feedback action of insulin, as reflected by the transcortical negative DC potential shift probably also pertains to extrahypothalamic neurocognitive functions (50).

ACKNOWLEDGMENTS

We thank C. Lühken and K. Trompf for their expert technical assistance and C. Otten for her invaluable laboratory work. Aero Pump (Hochheim, Germany) generously provided us with precision nasal air pumps.

REFERENCES

1. Unger JW, Livingston JN, Moss AM: Insulin receptors in the central nervous system: localization, signalling mechanisms and functional aspects. *Prog Neurobiol* 36:343–362, 1991
2. Schwartz MW, Figlewicz DP, Baskin DG, Woods SC, Porte D Jr: Insulin in the brain: a hormonal regulator of energy balance. *Endocr Rev* 13:387–414, 1992
3. Schwartz MW, Woods SC, Porte D Jr, Seeley RJ, Baskin DG: Central nervous system control of food intake. *Nature* 404:661–671, 2000
4. Obici S, Feng Z, Karkanias G, Baskin DG, Rossetti L: Decreasing hypothalamic insulin receptors causes hyperphagia and insulin resistance in rats. *Nat Neurosci* 5:566–572, 2002
5. Woods SC, Seeley RJ, Porte D Jr, Schwartz MW: Signals that regulate food intake and energy homeostasis. *Science* 280:1378–1383, 1998
6. Sipols AJ, Baskin DG, Schwartz MW: Effect of intracerebroventricular insulin infusion on diabetic hyperphagia and hypothalamic neuropeptide gene expression. *Diabetes* 44:147–151, 1995
7. Obici S, Zhang BB, Karkanias G, Rossetti L: Hypothalamic insulin signaling is required for inhibition of glucose production. *Nat Med* 8:1376–1382, 2002
8. Woods SC, Seeley RJ, Baskin DG, Schwartz MW: Insulin and the blood-brain barrier. *Curr Pharm Des* 9:795–800, 2003
9. Schwartz MW, Sipols A, Kahn SE, Lattemann DF, Taborsky GJ Jr, Bergman RN, Woods SC, Porte D Jr: Kinetics and specificity of insulin uptake from plasma into cerebrospinal fluid. *Am J Physiol* 259:E378–E383, 1990
10. Schwartz MW, Bergman RN, Kahn SE, Taborsky GJ Jr, Fisher LD, Sipols AJ, Woods SC, Steil GM, Porte D Jr: Evidence for entry of plasma insulin into cerebrospinal fluid through an intermediate compartment in dogs: quantitative aspects and implications for transport. *J Clin Invest* 88:1272–1281, 1991
11. Tribl G, Howorka K, Heger G, Anderer P, Thoma H, Zeitlhofer J: EEG topography during insulin-induced hypoglycemia in patients with insulin-dependent diabetes mellitus. *Eur Neurol* 36:303–309, 1996
12. Bjorgaas M, Sand T, Vik T, Jorde R: Quantitative EEG during controlled hypoglycaemia in diabetic and non-diabetic children. *Diabet Med* 15:30–37, 1998
13. Amzica F, Massimini M, Manfridi A: Spatial buffering during slow and paroxysmal sleep oscillations in cortical networks of glial cells in vivo. *J Neurosci* 22:1042–1053, 2002
14. Birbaumer N, Elbert T, Canavan AG, Rockstroh B: Slow potentials of the cerebral cortex and behavior. *Physiol Rev* 70:1–41, 1990
15. Schmitt B, Mölle M, Marshall L, Hallschmid M, Born J: Scalp recorded direct current (DC) potential shifts associated with food intake in hungry humans. *Behav Brain Res* 119:85–92, 2001
16. Marshall L, Mölle M, Fehm HL, Born J: Scalp recorded direct current brain potentials during human sleep. *Eur J Neurosci* 10:1167–1178, 1998
17. Schmitt B, Mölle M, Marshall L, Born J: Scalp recorded direct current potential shifts associated with quenching thirst in humans. *Psychophysiology* 37:766–776, 2000
18. Hennighausen E, Heil M, Rösler F: A correction method for DC drift artifacts. *Electroencephalogr Clin Neurophysiol* 86:199–204, 1993
19. Abt K: Significance testing of many variables: problems and solutions. *Neuropsychobiology* 9:47–51, 1983
20. Guthrie D, Buchwald JS: Significance testing of difference potentials. *Psychophysiology* 28:240–244, 1991

21. Born J, Lange T, Kern W, McGregor GP, Bickel U, Fehm HL: Sniffing neuropeptides: a transnasal approach to the human brain. *Nat Neurosci* 5:514–516, 2002
22. Tallroth G, Lindgren M, Stenberg G, Rosen I, Agardh CD: Neurophysiological changes during insulin-induced hypoglycaemia and in the recovery period following glucose infusion in type 1 (insulin-dependent) diabetes mellitus and in normal man. *Diabetologia* 33:319–323, 1990
23. Tesche CD, Karhu J: Theta oscillations index human hippocampal activation during a working memory task. *Proc Natl Acad Sci U S A* 97:919–924, 2000
24. Kahana MJ, Sekuler R, Caplan JB, Kirschen M, Madsen JR: Human theta oscillations exhibit task dependence during virtual maze navigation. *Nature* 399:781–784, 1999
25. Abi-Saab WM, Maggs DG, Jones T, Jacob R, Srihari V, Thompson J, Kerr D, Leone P, Krystal JH, Spencer DD, During MJ, Sherwin RS: Striking differences in glucose and lactate levels between brain extracellular fluid and plasma in conscious human subjects: effects of hyperglycemia and hypoglycemia. *J Cereb Blood Flow Metab* 22:271–279, 2002
26. Pardal R, Lopez-Barneo J: Low glucose-sensing cells in the carotid body. *Nat Neurosci* 5:197–198, 2002
27. Hallschmid M, Mölle M, Fischer S, Born J: EEG synchronization upon reward in man. *Clin Neurophysiol* 113:1059–1065, 2002
28. Marczynski TJ, York JL, Allen SL, Rick JH, Sherry CJ: Steady potential correlates of positive reinforcement and sleep onset in the cat: reward contingent positive variation (RCPV). *Brain Res* 26:305–332, 1971
29. Banks WA, Jaspan JB, Huang W, Kastin AJ: Transport of insulin across the blood-brain barrier: saturability at euglycemic doses of insulin. *Peptides* 18:1423–1429, 1997
30. Hasselbalch SG, Knudsen GM, Videbaek C, Pinborg LH, Schmidt JF, Holm S, Paulson OB: No effect of insulin on glucose blood-brain barrier transport and cerebral metabolism in humans. *Diabetes* 48:1915–1921, 1999
31. Wallum BJ, Taborsky GJ Jr, Porte D Jr, Figlewicz DP, Jacobson L, Beard JC, Ward WK, Dorsa D: Cerebrospinal fluid insulin levels increase during intravenous insulin infusions in man. *J Clin Endocrinol Metab* 64:190–194, 1987
32. Baura GD, Foster DM, Porte D, Jr, Kahn SE, Bergman RN, Cobelli C, Schwartz MW: Saturable transport of insulin from plasma into the central nervous system of dogs in vivo: a mechanism for regulated insulin delivery to the brain. *J Clin Invest* 92:1824–1830, 1993
33. Yalow RS, Eng J: Insulin in the central nervous system. *Adv Metab Disord* 10:341–354, 1983
34. Picton TW, Hillyard SA: Cephalic skin potentials in electroencephalography. *Electroencephalogr Clin Neurophysiol* 33:419–424, 1972
35. Rockstroh B, McCallum WC: Theories and significance of SPs. In *Slow Potential Changes in the Human Brain.* McCallum WC, Curry SH, Eds. New York, Plenum, 1993, p. 229–234
36. Rebert CS: Electrogenesis of slow potential changes in the central nervous system: a summary of issues. In *Multidisciplinary Perspectives in Event Related Brain Potential Research.* Otto D, Ed. Cincinnati, OH, Environmental Protection Agency, 1978, p. 3–11
37. Qian ZM, Li H, Sun H, Ho K: Targeted drug delivery via the transferrin receptor-mediated endocytosis pathway. *Pharmacol Rev* 54:561–587, 2002
38. Pardridge WM: Transport of insulin-related peptides and glucose across the blood-brain barrier. *Ann N Y Acad Sci* 692:126–137, 1993
39. Woody CD, Marshall WH, Besson JM, Thompson HK, Aleonard P, Albe-Fessard D: Brain potential shift with respiratory acidosis in the cat and monkey. *Am J Physiol* 218:275–283, 1970
40. Voipio J, Tallgren P, Heinonen E, Vanhatalo S, Kaila K: Millivolt-scale DC shifts in the human scalp EEG: evidence for a nonneuronal generator. *J Neurophysiol* 89:2208–2214, 2003
41. Kum W, Zhu SQ, Ho SK, Young JD, Cockram CS: Effect of insulin on glucose and glycogen metabolism and leucine incorporation into protein in cultured mouse astrocytes. *Glia* 6:264–268, 1992
42. Hamai M, Minokoshi Y, Shimazu T: L-glutamate and insulin enhance glycogen synthesis in cultured astrocytes from the rat brain through different intracellular mechanisms. *J Neurochem* 73:400–407, 1999
43. Kuffler SW, Nicholls JG: The physiology of neuroglial cells. *Ergeb Physiol* 57:1–90, 1966
44. Laming PR, Kimelberg H, Robinson S, Salm A, Hawrylak N, Muller C, Roots B, Ng K: Neuronal-glial interactions and behaviour. *Neurosci Biobehav Rev* 24:295–340, 2000
45. Fields RD, Stevens-Graham B: New insights into neuron-glia communication. *Science* 298:556–562, 2002
46. Heinemann U, Lux HD, Marciani MG, Hofmeier G: Slow potentials in relation to changes in extracellular potassium activity in the cortex of cats. In *Origin of Cerebral Field Potentials.* Speckmann EJ, Caspers H, Eds. Stuttgart, Germany, Georg Thieme Publishers, 1979, p. 33–43
47. Steriade M: Impact of network activities on neuronal properties in corticothalamic systems. *J Neurophysiol* 86:1–39, 2001
48. Air EL, Benoit SC, Blake Smith KA, Clegg DJ, Woods SC: Acute third ventricular administration of insulin decreases food intake in two paradigms. *Pharmacol Biochem Behav* 72:423–429, 2002
49. Baskin DG, Figlewicz LD, Seeley RJ, Woods SC, Porte D Jr, Schwartz MW: Insulin and leptin: dual adiposity signals to the brain for the regulation of food intake and body weight. *Brain Res* 848:114–123, 1999
50. Kern W, Peters A, Fruehwald-Schultes B, Deininger E, Born J, Fehm HL: Improving influence of insulin on cognitive functions in humans. *Neuroendocrinology* 74:270–280, 2001