Original Articles

Evidence for Effects of Insulin on Sensory Processing in Humans

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Systemic insulin passes the blood-brain barrier and insulin receptors have been detected in various brain regions. Yet, the biological significance of insulin acting on the brain remains rather unclear. Reports of different awareness of hypoglycemic symptoms during hypoglycemia induced by human insulin (HI) and porcine insulin (PI) suggest a modulatory influence of insulin on sensory processing. In a double-blind, within-subject, crossover comparison, we recorded visual-evoked potentials (VEP) in 30 healthy men during euglycemia and after 20 or 50 min of constant hypoglycemia of 2.66 mM (47.9 mg/dl) induced by HI and PI. Blood glucose and serum insulin levels were identical in both sessions. Hypoglycemia reduced amplitudes of the VEP components P1 and N2 and increased latencies of N1, P1, and N2. However, hypoglycemia-induced changes in VEP amplitudes and latencies were significantly stronger during PI than HI infusion: P1-N2 difference amplitude decreased from (means ± SE) 11.9 ± 0.9 to 10.7 ± 0.8 μV during HI and from 12.4 ± 0.9 to 8.7 ± 0.7 μV during PI infusion ($P < 0.002$). P1 latency increased from 112.0 ± 3.2 to 118.8 ± 3.2 ms during HI and from 114.0 ± 3.3 to 126.3 ± 4.6 ms during PI infusion ($P < 0.05$). Differences between the effects of the insulins were consistently apparent after 20 min of hypoglycemia, which indicates a short-term action of the hormone. The results add to those of a foregoing study demonstrating differential effects of HI- and PI-induced hypoglycemia on auditory evoked potentials. The changes in sensory processing during hypoglycemia, depending on the type of insulin, suggest a direct modulation of these brain functions by insulin. *Diabetes* 43:351–56, 1994

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HI, human insulin; PI, porcine insulin; VEP, visual-evoked potentials; BBB, blood-brain barrier; AEP, auditory evoked potentials; ANOVA, analysis of variance.

In the last few years, neurophysiological and behavioral effects of insulin on the brain have been documented (1–3). Insulin has rapid access to the brain either via the circumventricular organs lacking the blood-brain barrier (BBB) or via a receptor-mediated transport system located in endothelial cells of brain microvessels (4–8). In several species, including humans, parallel changes in plasma and brain interstitial and cerebrospinal fluid-insulin concentrations are well documented (9–11). In the brain, insulin receptors are widely distributed with the highest concentrations in the olfactory bulb, in the hypothalamus, and throughout the limbic system (12–15).

At the cellular level, insulin exerts a variety of actions on neurons (1,16); for example, it inhibits firing of neurons in the hippocampus (17) and hypothalamus (18). Through an influence on membrane sodium transport in choroid plexus (19), insulin could cause widespread neuronal hyperpolarization (20). Moreover, insulin has been found to inhibit re-uptake of norepinephrine in dissociated rat brain cells (21) and to alter catecholamine turnover in the hypothalamus (22,23). Insulin also has been shown to stimulate uptake of glucose by rat hypothalamus (24,25) and to increase glucose transporter mRNA in neurons and glia cells (26).

However, effects of insulin on single cells cannot define the functional significance of central nervous insulin for information processing and for behavioral adaptation. Direct central nervous effects of insulin are difficult to distinguish from alterations in neuronal functions attributable to the insulin-induced decrease of blood glucose concentrations. Therefore, this study evaluated the effects of human insulin (HI) and porcine insulin (PI) on sensory processing with both insulins inducing hypoglycemia of identical strength. PI is more lipophilic than HI (28); thus, the entry of these insulins into the brain and the kinetic pathways within the brain may be different. Be-

cause the potency of both insulins is comparable with regard to inducing hypoglycemia (29), different effects of HI- and PI-induced hypoglycemia on human brain functions would indicate an afferent action of insulin on these brain functions. In support of this view, the awareness of hypoglycemic symptoms has been reported to differ between HI- and PI-induced hypoglycemia (36–43). In a foregoing study, differences in sensory processing reflected by auditory evoked potentials (AEP) during HI- and PI-induced hypoglycemia have already been demonstrated (44).

Extending on this previous auditory study, this study aimed to establish afferent influences of insulin on human brain functions using visual-evoked brain potentials (VEP). Evoked potentials representing physiological indicators of sensory processing have been shown to reflect different influences of insulin-induced hypoglycemia on sensory processing more precisely than subjective reports of hypoglycemia awareness (44).

RESEARCH DESIGN AND METHODS

The study subjects consisted of 30 healthy male volunteers (18–32 years of age) of normal body weight (± 10%) and without a personal or family history of diabetes. All subjects were nonsmokers and not under current medication. Subjects fasted for 12 h before testing and abstained 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 committee on research involving human subjects at the University of Lübeck, Lübeck, Germany, and written informed consent was obtained from all subjects.

Procedures. The double-blind experiments were held and designed according to a within-subject crossover comparison. Each subject was tested twice, with an interval of at least one week between both sessions. On one of these occasions, subjects received biosynthetic HI (Velasulin H, Novo-Nordisk, Bethesda, MD). On the other occasion, purified PI (Velasulin, Novo-Nordisk) was given. The order of administration was counterbalanced across subjects. Experiments took place in a sound-attenuated and electrically shielded room between 0900 and 1300, with the subjects sitting in a supine position in bed. One hour before testing, two catheters were inserted into veins of the dorsal hand, which was warmed to 55°C. With this procedure, arterialized blood could be sampled for continuous blood glucose monitoring with the glucose analyzer of a Biostator-Glucose Controller (Life Science Instruments, Elkhart, IN) (45) and for determination of serum insulin. 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 and for blood glucose (Beckman Glucose Analyzer II, Fullerton, CA) to calibrate the biostator.

After a baseline of 60 min, an initial bolus of insulin (30 mU/kg) was administered intravenously, and, thereafter, insulin was infused at a constant rate of 1.5 $mU \cdot kg^{-1} \cdot min^{-1}$. Hypoglycemia was maintained at a plasma glucose concentration of about 2.66 mM (47.9 mg/dl) by an additional manually controlled glucose infusion (20% solution) for ~1.5 h. Thereafter, blood glucose was increased to baseline level.

VEP were recorded during stimulation with checkerboard pattern reversals delivered at a rate of 1.1 Hz on a video screen with constant background illumination (100 lux at the subject's eye). Luminance of the white squares was 400 cd/m^2, and luminance of the black squares was 4.5 cd/m^2, which resulted in a contrast of 97.5%. Pattern size was 2.3 cm, resulting in a visual angle of 1° at a distance of 1.4 m between the subject's eyes and the screen. To avoid eye movements, subjects fixated on a dot, which was always visible in the center of the screen.

VEP were recorded during the baseline phase and also at constant hypoglycemia (2.66 mM [47.9 mg/dl]), after 20 min ($n = 15$), and 50 min ($n = 15$) of steady-state conditions.

Recordings and apparatus. VEP were measured with Ag/AgCl electrodes attached to the vertex (Cz, reference), at Oz (active), and at Fpz (ground). VEP signals were amplified (filter bandpass between 1 and 100 Hz, 12 dB/octave) and averaged on-line from a series of 100 artifact-free sweeps (sweep time 250 ms; sampling rate 20,000 Hz) by a Nicolet Compact 4 (Madison, WI). Sweeps were excluded if the voltage of any data point exceeded 90 μV.

Serum insulin was determined by radioimmunoassay (Pharmacia Insulin RIA 100, Pharmacia Diagnostics, Uppsala, Sweden) with an interassay error and measured as coefficient of variation, below 5.4%. Intra-assay variation was <4.5% in all cases. The same kit was used for all samples of an individual subject.

Data reduction and analysis. VEP components were determined visually by two experimenters, who were blind with respect to the treatment conditions. Latencies and amplitudes of N1, P1, and N2 components and peak-to-peak amplitudes of the N1-P1 and the P1-N2 difference were measured. Effects on VEP measures were statistically assessed by repeated analysis of variance (ANOVA) measures, which contained the factors glucose level (baseline versus hypoglycemia) and type of insulin (HI versus PI). ANOVAs were also performed with an additional group factor to assess whether effects of hypoglycemia depended on the time since onset of the steady hypoglycemic state (20 vs. 50 min). Measures of blood glucose and serum insulin levels of both sessions were assessed by ANOVAs.

RESULTS

Blood glucose and serum insulin. Mean plasma glucose and serum insulin levels during the HI and PI sessions are shown in Fig. 1. In both groups, blood glucose concentrations (means ± SE) (HI: 4.87 ± 0.07 mM [87.7 ± 1.3 mg/dl]; PI: 4.88 ± 0.07mM [87.9 ± 1.3 mg/dl]) and serum insulin values (HI: 59.3 ± 8.1 pM [9.9 μU/ml ± 1.4]; PI: 57.9 ± 9.3 pM [9.7 μU/ml ± 1.6]) during baseline were almost identical. After continuous infusion of 1.5 $mU \cdot kg^{-1} \cdot min^{-1}$ HI and PI, respectively, nearly the same serum insulin levels were obtained (HI:

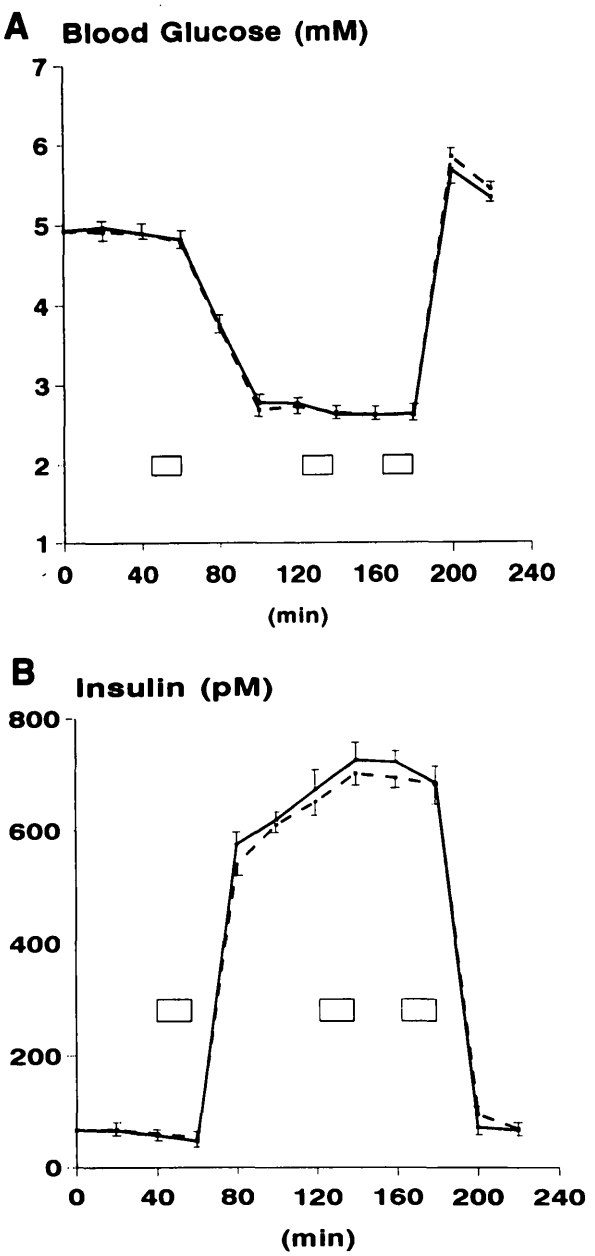

FIG. 1. *A*: Means ± SE plasma glucose concentrations during sessions with HI (——) and PI (- - - -) treatment. *B*: Means ± SE of serum insulin during fasting baseline condition and during infusion of 1.5 mU · kg^{-1} · min^{-1} PI (- - - -) and HI (——). Occasions of VEP recordings (□) are indicated.

681.3 ± 43.9 pM [113.6 μU/ml ± 7.3]; PI: 674.1 ± 40.8 pM [112.4 μU/ml ± 6.8]). During this phase, blood glucose concentrations were reduced to nearly the same extent by HI and PI (HI: 2.67 ± 0.06 mM [48.1 ± 1.1 mg/dl]; PI: 2.65 ± 0.06mM [47.8 ± 1.1 mg/dl]).

VEP. Table 1 summarizes the effects of HI- and PI-induced hypoglycemia on VEP components. Latencies of all VEP components were increased during hypoglycemia ($P < 0.001$). The latencies of the P1 component were influenced differently by PI- and HI-induced hypoglycemia, which increased from 112.0 ± 3.2 to 118.8 ± 3.2 ms (i.e., 6.1%) during HI infusion and from 114.0 ± 3.3 to 126.3 ± 4.6 ms (i.e., 10.8%) during PI infusion (hypoglycemia × insulin: $P < 0.05$).

Amplitudes of P1 and N2 were significantly reduced during hypoglycemia ($P < 0.005$). The amplitude reductions following PI-induced hypoglycemia were more distinct (P1: −29.2%; N2: −30.8%) than after HI-induced hypoglycemia (P1: −11.6%, hypoglycemia × insulin: $P < 0.005$; N2: −8.0%, hypoglycemia × insulin: $P < 0.05$). N1-P1 difference amplitude decreased during hypoglycemia ($P < 0.01$). Again, this reduction tended to be stronger during PI- than HI-induced hypoglycemia ($P < 0.06$). Hypoglycemia-induced amplitude reductions were most pronounced for P1-N2 difference amplitude ($P < 0.001$), and this effect was markedly stronger during PI- (−29.8%) than HI- (−10.1%) induced hypoglycemia (hypoglycemia × insulin: $P < 0.002$) (Fig. 2). The stronger effect of PI-induced hypoglycemia as compared with HI-induced hypoglycemia on the N1-P2 difference amplitude was somewhat more prominent in recordings obtained after 20 min than after 50 min of steady-state hypoglycemia (hypoglycemia × insulin × group: $P < 0.09$) (Fig. 3).

DISCUSSION

As expected, VEP proved to be sensitive to the influences of hypoglycemia. Lowering blood glucose to 2.66 mM (47.9 mg/dl) decreased P1 and N2 baseline-to-peak amplitudes, as well as N1-P1 and P1-N2 difference amplitudes, and increased latencies of all VEP components. These findings are in line with previous reports of decreased amplitudes of VEP (46) and prolonged latencies of AEP components generated at the cortical (44,47) and brainstem level (48,49).

In this study, we compared the effects of HI- and PI-induced hypoglycemia on sensory function within the visual pathways. The insulins differed in their modulating effects on hypoglycemia-induced VEP changes, i.e., changes during HI- and PI-induced hypoglycemia were in the same direction, but changes during HI-induced hypoglycemia were significantly less pronounced than those during PI treatment, which indicates a greater decay of sensory functions during PI-induced hypoglycemia. Thus, the P1-N2 difference amplitude, indexing a stimulus-induced cortical arousal mediated by collateral activation of mesencephalic reticular structures, was distinctly reduced during hypoglycemia. This reduction was more pronounced during PI than HI treatment.

Because blood glucose and serum insulin levels were almost identical in both sessions, the differential effects on VEP suggest a direct but diverging influence of both insulins on central nervous functioning. Although of moderate size, the differences in the effects of HI and PI complement previous results of a differential effect of HI- and PI-induced hypoglycemia on human-evoked potentials components in the auditory modality (44). Like these effects on VEP, effects of hypoglycemia on AEP have been found to be weaker after HI than PI. Also, magnitude and time course of these effects on AEP bear great resemblance to those found in this study. The coherence of this pattern makes it very unlikely that these effects represent just random changes.

TABLE 1
Latencies and baseline-to-peak amplitudes of VEP-components N1, P1, and N2 as well as difference amplitudes for N1-P1 and P1-N2 during the HI- and PI-sessions averaged across all subjects

	HI session		PI session			
	Baseline	Hypoglycemia	Baseline	Hypoglycemia	Hypoglycemia* (*P*)	PI – HI† (*P*)
Latencies (ms)						
N1	73.4 ± 1.1	75.9 ± 1.2	74.1 ± 1.1	76.7 ± 1.3	+2.55 ± 0.40 (<0.001)	+0.16 ± 0.98 (NS)
P1	112.0 ± 3.2	118.8 ± 3.2	114.0 ± 3.3	126.3 ± 4.6	+9.55 ± 1.20 (<0.001)	+5.54 ± 2.31 (<0.046)
N2	189.8 ± 3.1	197.8 ± 3.6	190.2 ± 2.9	200.3 ± 3.6	+9.05 ± 2.40 (<0.001)	+2.12 ± 2.58 (NS)
Amplitudes (μV)						
N1	−3.9 ± 0.6	−3.9 ± 0.7	−4.0 ± 0.5	−4.2 ± 0.7	+0.14 ± 0.28 (NS)	−0.17 ± 0.35 (NS)
P1	6.9 ± 0.7	6.1 ± 0.6	7.2 ± 0.4	5.1 ± 0.4	−1.46 ± 0.30 (<0.001)	−1.28 ± 0.41 (<0.005)
N2	−5.0 ± 0.5	−4.6 ± 0.4	−5.2 ± 0.4	−3.6 ± 0.5	−1.03 ± 0.33 (<0.005)	−1.24 ± 0.44 (<0.046)
N1-P1	10.8 ± 0.8	10.0 ± 0.8	11.2 ± 0.7	9.3 ± 0.7	−1.35 ± 0.43 (<0.01)	−1.14 ± 0.53 (<0.060)
P1-N2	11.9 ± 0.9	10.7 ± 0.8	12.4 ± 0.9	8.7 ± 0.7	−2.45 ± 0.58 (<0.001)	−2.51 ± 0.73 (<0.001)

Data are means ± SE.
*Increase (+) in latencies and decrease (−) in amplitudes during hypoglycemia compared with baseline and the significance of this effect.
†Difference between the effect of PI minus HI, which indicates the more pronounced effect on latencies and amplitudes during PI and respective significances.

Because this study aims at demonstrating differences between the effects of HI and PI on hypoglycemia-induced VEP changes, it cannot be decided whether PI enhanced or HI diminished hypoglycemic impairment of sensory processing. However, a dose-dependent inhibition of insulin on the firing rate of neurons of the hippocampus (17) and the hypothalamus has been reported (50–57), and a stimulation of membrane ion transport, which causes hyperpolarization (58), has been demonstrated. These findings hint at a direct inhibitory action of insulin on neuronal activity and may be a basis to explain the reduction in P1-N2 amplitude and the increase in P1 latency, which was particularly prominent during PI-induced hypoglycemia.

The structure of HI and PI differs only in one amino acid. However, PI has been reported to be more lipophilic than HI (28). Thus, PI probably crosses the BBB more easily than HI, which results in higher concentrations of PI in the brain during the earlier phase of hypoglycemia. Once in the brain, PI concentrations, being higher than those of HI, could exert a stronger inhibitory influence on neuronal activity than HI. This mechanism of a faster blood-brain transfer of PI than HI also explains two important aspects of this study. The transfer explains why the differential effects of HI and PI on hypoglycemic changes in the P1-N2 difference amplitude (and the P3 latency in a foregoing study [44]) were most prominent after 20 min of constant hypoglycemia and why, as a result of increasing effects of HI-induced hypoglycemia, these effects tended to disappear with time spent in hypoglycemia (Fig. 3). Rather than directly inhibiting neuronal activity, differential brain concentrations of HI and PI could also result in differences of local glucose uptake (24–26) and, thus, indirectly affect neuronal activity.

Although inconsistent (30–35), it has been reported

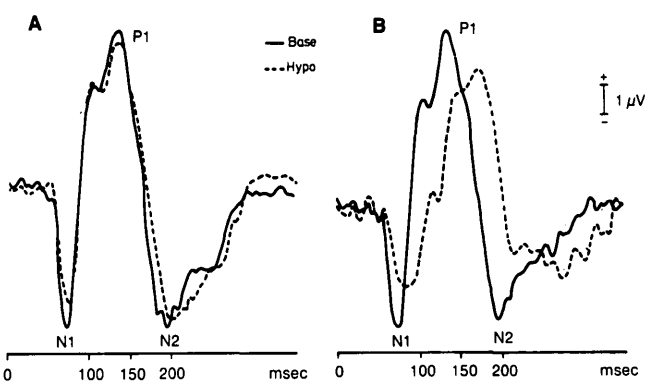

FIG. 2. VEP responses in one subject under baseline condition (——) and after 20 min of steady-state hypoglycemia (- - - -) during the HI (*A*) and PI (*B*) session. Note distinct changes in P1-N2 difference amplitude and latency only during PI-induced hypoglycemia.

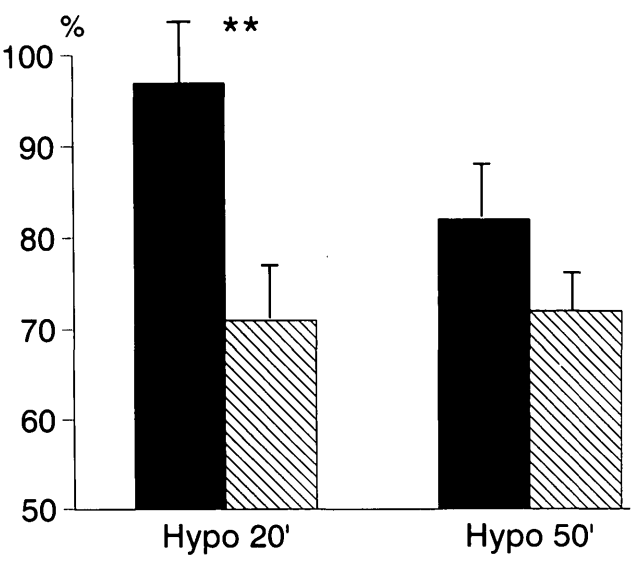

FIG. 3. Percentage (means ± SE) of reductions of P1-N2 difference amplitude of VEP recordings obtained after 20 min (Hypo 20') and after 50 min (Hypo 50') of steady-state hypoglycemia. Significant (= $P < 0.001$) differences between HI (■) and PI (▨) were distinctly apparent after 20 min of hypoglycemia, whereas differences disappeared with time on hypoglycemia.**

that the subjective recognition of early warning symptoms of increasing hypoglycemia is diminished or delayed during HI-induced hypoglycemia, as compared with PI-induced hypoglycemia. This phenomenon has been commonly referred to as increased hypoglycemia unawareness during treatment with HI (36–43). It corresponds to the less-pronounced changes in VEP during HI- than PI-induced hypoglycemia. Because peripheral hypoglycemia was identical after HI and PI, hypoglycemic signals from the body periphery to the brain probably did not differ between both conditions. Hence, differences in the awareness of hypoglycemic symptoms are very likely a consequence of a differential processing of signals within the nervous system. Here, a different processing of sensory stimuli during HI- and PI-induced hypoglycemia has been demonstrated using the method of stimulus evoked potentials. It is conceivable that the awareness of changes in central nervous stimulus processing (being stronger after PI than HI) may serve as a first subjective cue for an acute impending hypoglycemia.

An impaired or delayed recognition of hypoglycemic warning symptoms also has been reported to occur in patients with long duration of diabetes under intensified insulin therapy and in patients with an insulinoma (3). Because supraphysiological levels of serum insulin are common in these patients, peripheral insulin receptors appear to be downregulated. By contrast, the number of central nervous insulin receptors is maintained during persisting high insulin concentrations (59,60). The results of this study indicate that insulin directly affects central nervous stimulus processing. Thus, increased insulin concentrations in the brain, in the presence of an unchanged number of central nervous binding sites, may contribute to a pertained steady change in the brain's stimulus processing function, with an impairing influence also on the recognition of signs of impending hypoglycemia. Also, elevated serum insulin levels have been shown to reduce symptoms during hypoglycemia (27).

In line with the view that hyperinsulinemia contributes to changes in sensory processing and hypoglycemia unawareness are reports of related alterations in insulin-dependent diabetes mellitus and in non-insulin-dependent diabetes mellitus. These patients, who are often hyperinsulinemic, have been found to display reduced amplitudes and prolonged latencies of evoked potential components under euglycemic conditions (61–70), which are similar to those observed in this study in healthy subjects during insulin-induced hypoglycemia.

In summary, the results of this study demonstrate distinct effects of hypoglycemia on amplitudes and latencies of evoked potential components in humans. Most importantly, these results demonstrate that HI- and PI-induced hypoglycemia differ with respect to their patterns of action with the effects of PI-induced hypoglycemia in general exceeding those of HI-induced hypoglycemia, which indicates evidence for effects of insulin on neuronal functions in humans. Further research should clarify to what extent the differences are relevant for the awareness of hypoglycemia in diabetic patients.

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REFERENCES

1. Baskin DG, Figlewicz DP, Woods SC, Porte D Jr, Dorsa DM: Insulin in the brain. *Annu Rev Physiol* 49:334–47, 1987
2. Schwartz MW, Figlewicz DP, Baskin DG, Woods SC, Porte D: Insulin in the brain: a hormonal regulator of energy balance. *Endocr Rev* 13:387–413, 1992
3. Gerich JE, Mokan M, Veneman T, Korytkowski M, Mitrakou A: Hypoglycemia unawareness. *Endocr Rev* 12:356–71, 1991
4. Baskin DG, Porte D Jr, Guest K, Dorsa DM: Regional concentrations of insulin in the rat brain. *Endocrinology* 112:898–903, 1983
5. Van Houten M, Posner BI, Kopriwa BM, Brawer JR: Insulin binding sites localized to nerve terminals in rat median eminence. *Science* 207:1081–83, 1980
6. Van Houten M, Nance DM, Gauthier S, Posner BI: Origin of insulin-receptive nerve terminals in rat median eminence. *Endocrinology* 113:1393–99, 1983
7. Frank HJL, Pardridge WM: Insulin binding to brain microvessels. *Adv Metab Disord* 10:291–302, 1983
8. Pardridge WM, Eisenberg J, Yang J: Human blood-brain barrier insulin receptor. *J Neurochem* 44:1771–78, 1985
9. Wallum BJ, Porte D Jr, Figlewicz DP, Jacobson L, Dorsa D: Cerebrospinal fluid insulin levels increase during intravenous insulin infusion in man. *J Clin Endocrinol Metab* 64:190–94, 1987
10. Woods SC, Porte DJ, Bobbioni E, Ionescu E, Sauter JF, Rohner-Jeanrenaud F, Jeanrenaud B: Insulin: its relationship to the central nervous system and to the control of food intake and body weight. *Am J Clin Nutr* 42:1063–71, 1985
11. Yang YJ, Hope ID, Ader M, Bergman RN: Insulin transport across capillaries is rate limiting for insulin action in dogs. *J Clin Invest* 84:1620–28, 1989
12. Figlewicz DP, Dorsa DM, Stein LJ, Baskin DG, Paquette T, Greenwood MRC, Woods SC, Porte D Jr: Brain and liver insulin binding is decreased in Zucker rats carrying the "fa" gene. *Endocrinology* 117:1537–43, 1985
13. Kappy MS, Sellinger S, Raizada MK: Insulin binding in four regions of the developing brain. *J Neurochem* 42:198–203, 1984
14. Schlüter KJ, Andre J, Enzmann F, Kerp L: Insulin receptor binding in pork brain: different affinities of porcine and human insulin. *Horm Metabol Res* 16:411–14, 1984
15. Rees-Jones RW, Hendricks SA, Quarum M, Roth J: The insulin receptor of rat brain is coupled to tyrosine kinase activity. *J Biol Chem* 259:3470–74, 1984
16. Unger JW, Livingston JN, Moss AM: Insulin receptors in the central nervous system: localization, signalling mechanisms and functional aspects. *Prog Neurobiol* 36:343–62, 1991
17. Palovick RA, Philiphs MI, Kappy MS, Raizada MK: Insulin inhibits pyramidal neurons in hippocampal slices. *Brain Res* 309:187–91, 1984
18. Shibata S, Liou SY, Ueki S, Oomura Y: Inhibitory action of insulin on suprachiasmatic nucleus neurons in rat hypothalamic slice preparation. *Physiol Behav* 36:79–81, 1985
19. Johanson CE, Murphy VA: Acetazolamide and insulin alter choroid plexus epithelial cell [Na^+], pH and volume. *Am J Physiol* 258: F1538–46, 1990
20. Shapiro E, Brown SD, Saltiel AR, Schwartz JH: Short-term action of insulin on Aplysia neurons: generation of a possible novel modulator of ion channels. *J Neurobiol* 22:55–62, 1991
21. Masters BA, Shemer J, Judkins JH, Clarke DW, LeRoith D, Raizada MK: Insulin receptors and insulin action in dissociated brain cells. *Brain Res* 417:247–56, 1987
22. Oliver EH, Sartin JL, Dieberg G, Rahe CH, Marple DN, Kemppainen RJ: Effects of acute insulin deficiency on catecholamine and indolamine content and catecholamine turnover in microdissected hypothalamic nuclei in streptozotocin-diabetic rats. *Acta Endocrinol* 120:343–50, 1989
23. Peinado JM, Meyers RD: Norepinephrine release from PVN and lateral hypothalamus during perfusion with 2-DG or insulin in the sated and fasted rat. *Pharmacol Biochem Behav* 27:715–21, 1991
24. Lucignani G, Namba H, Nehlig A, Porrino LJ, Kennedy C, Solioloff L: Effects of insulin on local cerebral glucose utilization in the rat. *J Cereb Blood Flow Metab* 7:309–14, 1987
25. Kadekaro M, Sanaki H, Solioloff L: Metabolic mapping of neural pathways involved in gastrosecretory response to insulin hypoglycemia in the rat. *J Physiol (Lond)* 300:393–407, 1980
26. Dringen R, Hamprecht B: Glucose, insulin and insulin-like growth factor I regulate the glycogen content of astroglia-rich primary cultures. *J Neurochem* 58:511–17, 1992

27. Kerr D, Reza M, Smith N, Leatherdale B: Importance of insulin in subjective, cognitive, hormonal responses to hypoglycemia in patients with IDDM. *Diabetes* 40:1057–62, 1991
28. Markussen J, Damgaard U, Johansen NL, Sörgensen KM, Sorensen E, Thim L: Charakterisierung des aus Schweineinsulin präparierten humaninsulin. *Aktuel Endokrinol* 1:104–14, 1981
29. Klier M, Kerner W, Torres AA, Pfeiffer EF: Comparison of the biologic activity of biosynthetic human insulin and natural pork insulin in juvenile-onset diabetic subjects assessed by the glucose controlled insulin infusion system. *Diabetes Care* 4:193–95, 1981
30. Colagiuri S, Miller JJ, Petocz P: Double-blind crossover comparison of human and porcine insulins in patients reporting lack of hypoglycaemia awareness. *Lancet* 339:1432–35, 1992
31. Patrick AW, Bodmer CW, Tieszen KL, White MC, Williams G: Human insulin and awareness of acute hypoglycemic symptoms in insulin-dependent diabetes. *Lancet* 338:528–32, 1991
32. Jick H, Hall GC, Dean AD, Jick SS, Derby LE: A comparison of the risk of hypoglycemia between users of human and animal insulins. I. Experience in the United Kingdom. *Pharmacotherapy* 10:395–97, 1990
33. Jick SS, Derby LE, Gross KM, Jick H: Hospitalizations because of hypoglycemia in users of animal and human insulins. II. Experience in the United States. *Pharmacotherapy* 10:398–99, 1990
34. Orchard TJ, Maser RE, Becker DJ, Dorman JS, Drash AL: Human insulin use and hypoglycaemia: insights from the Pittsburgh Epidemiology of Diabetes Complication Study. *Diabetic Med* 8:469–74, 1991
35. Egger M, Smith GD, Teuscher A: Human insulin and awareness of hypoglycemia: need for a large randomised trial. *Br Med J* 305:351–55, 1992
36. Teuscher A, Berger WG: Hypoglycemia unawareness in diabetics transferred from beef/porcine insulin to human insulin. *Lancet* ii: 382–85, 1987
37. Berger W, Althaus B: Reduced awareness of hypoglycemia after changing from porcine to human insulin in IDDM. *Diabetes Care* 10:260–61, 1987
38. Berger W, Keller U, Honegger B, Jaeggi E: Warning symptoms of hypoglycemia during treatment with human and porcine insulin in diabetes mellitus. *Lancet* 13:1041–44, 1989
39. Lingenfelser T, Overkamp D, Renn W, Glück H, Maassen M, Eggstein M, Jakober B: Different awareness of hypoglycaemia induced by human or purified pork insulin in type I diabetic patients. *Diabetes Res Clin Pract* 13:29–36, 1991
40. Jakober B, Lingenfelser T, Glück H, Maassen M, Overkamp D, Renn W, Eggstein M: Symptoms of hypoglycemia: a comparison between porcine and human insulin. *Klin Wochenschr* 68:447–53, 1990
41. Egger M, Smith GD, Imhoof H, Teuscher A: Risk of severe hypoglycemia in insulin treated diabetic patients transferred to human insulin: a case control study. *Br Med J* 303:617–21, 1991
42. Egger M, Smith GD, Teuscher AU, Teuscher A: Influence of human insulin on symptoms and awareness of hypoglycaemia: a randomised double-blind crossover trial. *Br Med J* 303:622–26, 1991
43. Egger M, Smith GD, Teuscher A: Human insulin and awareness of hypoglycaemia: need for a large randomised trial. *Br Med J* 305: 351–55, 1992
44. Kern W, Lieb K, Kerner W, Born J, Fehm HL: Differential effects of human and pork insulin-induced hypoglycemia on neuronal functions in humans. *Diabetes* 39:1091–98, 1990
45. Pfeiffer EF, Thum Ch, Clemens AH: The artificial beta cell: a continuous control of blood sugar by external regulation of insulin infusion (glucose controlled insulin infusion system). *Horm Metab Res* 487:339–42, 1974
46. Harrad RA, Cockram CS, Plumb AP, Stone S, Fenwick P, Sönksen PH: The effect of hypoglycemia on visual function: a clinical and electrophysiological study. *Clin Science* 69:673–79, 1985
47. De Feo P, Gallai V, Mazzotta G, Crispino G, Torlone E, Perriello G, Ventura MM, Santeusanio F, Brunetti P, Bolli GB: Modest decrements in plasma glucose concentration cause early impairment in cognitive function and later activation of glucose counterregulation in the absence of hypoglycemic symptoms in normal man. *J Clin Invest* 82:436–44, 1988
48. Koh THHG, Aynsley-Green A, Tarbit M, Eyre JA: Neural dysfunction during hypoglycemia. *Arch Dis Child* 63:1353–58, 1988
49. Kern W, Born J, Kerner W, Fehm HL: Different effects of human and porcine insulin induced abnormalities of brainstem sensory function. *Clin Physiol Biochem* 8:122–27, 1990
50. Grundstein HS, James DE, Storlien LH, Smythe GA, Draegen EW: Hyperinsulinemia suppresses glucose utilization in specific brain regions: in vivo studies using the euglycemic clamp in the rat. *Endocrinology* 116:604–10, 1985
51. Shibata S, Liou SY, Ueki S, Oomura Y: Inhibitory action of insulin on suprachiasmatic nucleus neurons in rat hypothalamic slice preparation. *Physiol Behav* 36:79–81, 1986
52. Kessler JA, Spray DC, Saez JC, Bennett MVL: Determination of synaptic phenotype: Insulin and cAMP independently initiate development of electrotonic coupling between cultured sympathetic neurons. *Proc Natl Acad Sci USA* 81:6235–39, 1984
53. Wolinsky EJ, Patterson PH, Willard AL: Insulin promotes electrical coupling between cultured sympathetic neurons. *J Neurosci* 5:1675–79, 1985
54. Rhoads DE, Rocco RJ, Osburn LD, Peterson WA, Raghupathy E: Stimulation of synaptosomal uptake of neurotransmitter amino acids by insulin: possible role of insulin as a neuromodulator. *Biochem Biophys Res Commun* 119:1198–1204, 1984
55. Sauter A, Goldstein M, Engel J, Ueta K: Effect of insulin on central catecholamines. *Brain Res* 260:330–33, 1983
56. Boyd FT, Clarke DW, Muther TF, Raizada MK: Insulin receptors and insulin modulation of norepinephrine uptake in neuronal cultures from rat brain. *J Biol Chem* 260:15880–84, 1985
57. Bernstein HG, Poeggel G, Dorn A, Luppa H, Ziegler M: Insulin stimulates sodium potassium activated ATPase from rat hippocampus. *Experientia* 37:434–35, 1981
58. Lowe WL, Boyd FT, Clarke DW, Raizada MK, Hart C, LeRoith D: Development of brain insulin receptors: structural and functional studies of insulin receptors from whole brain and primary cell cultures. *Endocrinology* 119:25–35, 1986
59. Boyd FT, Raizada MK: Effects of insulin and tunicamycin on neuronal insulin receptors in culture. *Am J Physiol* 245:C283–87, 1983
60. Clarke DW, Boyd FT, Kappy MS, Raizada MK: Insulin stimulates macromolecular synthesis in cultured glial cells from brain. *Am J Physiol* 249:C484–89, 1985
61. Donald MW, Bird CE, Lawson JS, Letemendia FJJ, Monga TN, Surridge DHC, Varette-Cerre P, Williams DL, Williams DML, Wilson DL: Delayed auditory brainstem responses in diabetes mellitus. *Neurol Neurosurg Psychiatry* 44:641–44, 1981
62. Donald MW, Erdahl DLW, Surridge DHC, Monga TN, Lawson JS, Bird CE, Letemendia FJJ: Functional correlates of reduced central conduction velocity in diabetic subjects. *Diabetes* 33:627–33, 1984
63. Fedele D, Martini A, Cardone C, Chomaccio F, Bellavere F, Molinari G, Negrin P, Crepaldi G: Impaired auditory brainstem-evoked responses in insulin-dependent diabetic subjects. *Diabetes* 33: 1085–89, 1984
64. Harkins SW, Gardner DF, Anderson RA: Auditory and somatosensory far-field evoked potentials in diabetes mellitus. *Int J Neurosci* 28:41–47, 1985
65. Khardori R, Soler NG, Good DC, Devlesc Howard AB, Broughton D, Walbert J: Brainstem auditory and visual evoked potentials in type I (insulin-dependent) diabetic patients. *Diabetologia* 29:362–65, 1986
66. Bucher MB, Assai I, Leuenberger PM: Neuropathies oculaires et neuropathies systemiques diabetiques. *Klin Monatsbl Augenheilkd* 176:711–16, 1980
67. Puvanendran K, Devathasan G, Wong PK: Visual evoked responses in diabetes. *J Neurol Neurosurg Psychiatry* 46:643–47, 1983
68. Yamazaki H, DachiUsami E, Chiba J: Contrast threshold of diabetic patients determined by VECP and psychophysical measurement. *Acta Ophthalmol* 60:386–92, 1983
69. Cirillo D, Gonfiantini E, DeGrandis D, Bongiovanni L, Robert JJ, Pinelli L: Visual evoked potentials in diabetic children and adolescents. *Diabetes Care* 7:273–75, 1984
70. Anastasi M, Lauricella M, Giordano C: Visual evoked potentials in insulin dependent diabetics. *Acta Diabetol Lat* 22:343–49, 1985