Central nervous insulin administration does not potentiate the acute
glucoregulatory impact of concurrent mild hyperinsulinemia

Short title: Brain insulin and glucose homeostasis in humans

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Experiments in rodents suggest that hypothalamic insulin signaling essentially contributes to the acute control of peripheral glucose homeostasis. Against this background, we investigated in healthy humans whether intranasal (IN) insulin, which is known to effectively reach the brain compartment, impacts systemic glucose metabolism. Twenty overnight-fasted healthy, normal-weight men were IN administered 210 and 420 IU (10 and 20 IU every 15 min) of the insulin analogue aspart (ins-aspart) and placebo, respectively, during experimental sessions lasting 6 h. The use of ins-aspart rather than human insulin enabled us to disentangle exogenous and endogenous insulin kinetics. IN insulin dose-dependently decreased plasma glucose concentrations while reducing C-peptide and attenuating endogenous insulin levels. However, we also observed a slight dose-dependent permeation of ins-aspart into the circulation. In control experiments mimicking the systemic but not the central nervous uptake of the IN 210 IU dose via IV infusion of ins-aspart at a dose of 0.12 mIU/kg/24h (n=10), we obtained essentially identical effects on fasting plasma glucose concentrations. This pattern indicates that sustained IN insulin administration to the human brain to enhance central nervous insulin signaling does not acutely alter systemic glucose homeostasis beyond effects accounted for by concurrent mild hyperinsulinemia.
Studies in rodents indicate that insulin controls systemic glucose metabolism not only via direct effects on peripheral organs, but also via a brain-liver axis governed by the hypothalamus (1-3). Intracerebroventricular (ICV) infusion of insulin in rats induced a 44% reduction in hepatic glucose production within 4 hours of a peripheral insulin clamp, which could be blocked by ICV infusion of insulin antibodies (1). This effect is assumed to be mediated by the activation of hypothalamic ATP-sensitive potassium (K$_{ATP}^+$) channels and vagal inputs to the liver (3). However, in dogs receiving carotid artery insulin infusions to raise cerebral insulin concentrations, a fourfold increase in head insulin during peripheral hyperinsulinemia did not enhance hepatic insulin sensitivity within 3 hours (4). Moreover, the impact of hepatic portal vein insulin delivery on hepatic glucose flux during a pancreatic clamp in the conscious dog was not altered by the inhibition of hypothalamic insulin action (5). Here we investigated whether central nervous insulin signaling contributes to the acute control of peripheral glucose homeostasis in humans. We made use of the intranasal (IN) route of insulin administration that directly targets the brain (6,7) and effectively modulates CNS functions including the regulation of energy balance (8,9), electroencephalographic brain activity (10), and cognition (8,11,12). We administered IN insulin aspart (ins-aspart) that compared with regular insulin has an identical binding profile to the receptor (13) but a reduced tendency to form hexamers (14), which increases its bioavailability and functional impact (12) while the mechanism of action of regular insulin is preserved. This enabled us to distinguish exogenous from endogenous insulin and to estimate the relative contributions of CNS and systemic insulin to the acute control of glucose homeostasis.

**Research Design and Methods**

Two double-blind, placebo-controlled, balanced within-subject experiments (I and II) which conformed to the declaration of Helsinki and were approved by the local ethics committee were conducted in healthy men (Table 1) who gave written informed consent.
**Design and procedure.** In experiment I, 20 subjects participated in three sessions that were spaced apart at least 10 days and included the IN administration of placebo (vehicle) and two different doses of ins-asp, respectively. After a 12-h nocturnal fast of the subjects, the experiment started with the insertion of a venous cannula in the subject’s non-dominant arm for venous blood drawings. From 0845h-1345h, ins-asp (NovoRapid; Novo Nordisk, Bagsvaerd, Denmark) at a cumulative dose of 210 IU and 420 IU, and placebo, respectively, were intranasally administered in 21 individual doses of 10 IU, 20 IU, and 0 IU at 15 min-intervals. Thus, either one 0.1-ml puff of placebo and 1 puff of ins-asp (210 IU condition; 1 puff/nostril), two 0.1-ml puffs of ins-asp (420 IU condition) or two 0.1-ml puffs of placebo were given every 15 min. Subjects were monitored to ensure a constant state of vigilance, and blood was repeatedly sampled (see Figure 1).

In experiment I, IN ins-asp administration at both doses induced discernible increases in circulating ins-asp concentrations (see Results). Therefore, in experiment II we administered ins-asp intravenously (IV) to induce ins-asp plasma levels comparable to those found in the IN 210 IU condition of experiment I, albeit at a much lower degree of brain permeation than achieved by IN administration (6). In a dose-finding experiment in 5 healthy men, we first determined the dose of IV ins-asp mimicking the increase in serum ins-asp observed in the 210 IU condition of experiment I. Subsequently, 10 male subjects (Table 1) were examined in two experimental conditions, IV ins-asp and placebo, whose set-up was identical to experiment I except that instead of IN administration, ins-asp compared to placebo (0.9% NaCl) was IV administered at a body weight-adjusted dose of 0.12 mIU/kg/24h from 0845-1345 h.

**Analyses of plasma glucose and hormonal parameters.** Blood samples were centrifuged and supernatants stored at -80°C. Blood for the measurement of glucagon was pre-treated with aprotinine (370 kIU/ml; Roth GmbH, Karlsruhe, Germany). Plasma glucose was measured in fluoride plasma (hexokinase method; Aeroset, Abbott Diagnostics, North
Chicago, IL), and serum insulin by an ELISA assay (Dako Denmark A/S, Glostrup, Denmark) measuring endogenous insulin but not ins-asp (15,16). Serum ins-asp concentrations were determined by an ELISA assay containing monoclonal antibodies specific for ins-asp (Celerion AG, Fehraltorf, Switzerland). Routine assays were used to measure C-peptide, growth hormone (GH), cortisol (all Immulite, DPC, Los Angeles, CA, USA), and glucagon (RIA, IBL International, Hamburg, Germany).

**Statistical analyses.** Data are means ± SEM. Baseline adjustment was achieved by subtracting individual baseline values before insulin administration (0825-0835 h) from post-treatment values. Depending on their distribution, data were subjected to analyses of variance (ANOVA; degrees of freedom corrected using the Greenhouse-Geisser procedure) or non-parametric tests. A *P* value <0.05 was considered significant.

**Results**

**Experiment I: Intranasal ins-asp dose-dependently lowers blood glucose concentrations.** Baseline concentrations of all parameters were comparable between conditions (*P*>0.25). IN administration of ins-asp induced sustained dose-dependent decreases in plasma glucose concentrations within 45 min of treatment onset (*P*<0.0002 for Treatment × Time; Figure 1A). In parallel, endogenous insulin concentrations decreased throughout the experiment (*P*<0.0001), with a trend towards even lower concentrations when ins-asp was administered (*P*=0.089 for Treatment; Figure 1B). In accordance, ins-asp compared to placebo decreased serum C-peptide (*P*<0.002, Friedman tests; Figure 1C). IN ins-asp treatment moreover increased GH concentrations and in the 420 IU condition attenuated the circadian decline in cortisol whereas a slight increase in glucagon was not significant (Table 2). During IN ins-asp treatment a dose-dependent increase in serum ins-asp emerged (AUC\(_{\text{placebo}}\) 0±0 vs. AUC\(_{\text{ins-asp-in210}}\) 5936±949 vs. AUC\(_{\text{ins-asp-in420}}\) 9229±1194 pmol*l\(^{-1}\)*min; *P*<0.0001; Figure 1A) that most likely resulted from permeation of ins-asp into the bloodstream via the nasopharyngeal
mucosa. The potential contribution of this effect to the reduction in plasma glucose was assessed in experiment II.

**Experiment II: Comparable effects of IN and IV ins-asp on plasma glucose levels when similar increases in serum ins-asp are achieved.** Baseline glucose levels did not differ between conditions ($P>0.88$). Increasing serum ins-asp levels by IV ins-asp infusion ($\text{AUC}_{\text{placebo}} 0±0$ vs $\text{AUC}_{\text{ins-asp-iv}} 3726±267 \text{ pmol}\cdot\text{l}^{-1}\cdot\text{min}^{-1}; P<0.03$; Figure 1D) significantly decreased plasma glucose concentrations ($P<0.02$ for Treatment $\times$ Time, Figure 1D). The rise in ins-asp serum levels during IV administration of experiment II turned out to be comparable to but slightly less pronounced than that emerging during IN delivery of 210 IU insulin in experiment I ($\text{AUC}_{\text{ins-asp-iv}} 3726±267$ vs. $\text{AUC}_{\text{ins-asp-in210}} 5936±949 \text{ pmol}\cdot\text{l}^{-1}\cdot\text{min}^{-1}; P>0.2$), with this difference increasing toward the end of experiments (Figure 1E). However, respective suppressive effects on plasma glucose were completely identical ($P>0.57$ for Time $\times$ Group; Figure 1E), indicating that the enhanced brain transport of ins-asp during IN as compared to IV administration did not exert any additional impact on plasma glucose.

**Discussion**

Our experiments relying on IN and IV administration of ins-asp to fasted human subjects contradict the assumption that CNS insulin signaling acutely contributes to peripheral glucose homeostasis in humans. While repetitive IN administration of high cumulative doses of ins-asp to the brain dose-dependently reduced circulating glucose concentrations, it also resulted in a slight entry of ins-asp into the circulation. Mimicking the increase in serum ins-asp found after IN insulin delivery by means of IV administration induced a completely comparable reduction in plasma glucose. Although our study design does not permit further mechanistic conclusions on hepatic glucose fluxes, this pattern indicates that the decrease in plasma glucose triggered by IN ins-asp administration can be accounted for by the concurrent mild hyperinsulinemia induced by IN insulin delivery. Additional effects on glucose flux of
enhanced brain insulin signaling during IN in comparison to IV administration (6) were not observed.

Our findings contrast with rodent experiments on the role of CNS insulin in the control of whole-body glucose metabolism (1-3). In rats, blocking hypothalamic insulin signaling markedly impaired the inhibition of hepatic gluconeogenesis and, consequently, hepatic glucose production during an euglycemic pancreatic clamp (1), indicating that hypothalamic insulin signaling is required for the suppression of hepatic glucose production by circulating insulin. Subsequent studies implicated insulin’s effect on hypothalamic $K_{ATP}^+$ channels and efferent vagal mediation in this glucoregulatory brain-liver loop (3). In humans, oral administration of the $K_{ATP}^+$ channel activator diazoxide 3 hours before a 4-hour euglycemic pancreatic clamp decreased endogenous glucose production during the last two hours of the clamp, while in control experiments in rats this effect was abolished by ICV administration of the $K_{ATP}^+$ channel blocker glibenclamide (17). However, the identification of a centrally mediated diazoxide effect on glucose production in that study was complicated by the clamp-induced decrease in the physiological gradient between portal vein and systemic/cerebral artery insulin exposure, and by the fact that $K_{ATP}^+$ channels are also expressed in hepatic mitochondria (18). Moreover, the regulation of hepatic glucose production is maintained in liver-transplanted patients (19) which does not speak for a superordinate relevance of the brain-liver axis in humans.

In our experiments, glucose concentrations started to decrease almost simultaneously with the rise in systemic ins-asp levels. However, we could not find any differences in glucose concentrations between the group treated with IN and the group treated with IV ins-asp even in the final hour ensuing the 5-hour period of IN insulin administration. This outcome argues against a physiologically significant contribution of CNS insulin signaling to the short-term regulation of fasting glucose homeostasis in healthy humans. While we did not measure CSF concentrations of ins-asp here, our previous experiments unequivocally demonstrated that the
IN administration of 40 IU insulin approximately doubles CSF insulin levels in humans (6). Therefore it seems safe to assume that both the 210 IU and the 420 IU dose of IN insulin produced robust and, because of the recurrent delivery, prolonged increases in CSF ins-asp levels. Furthermore, EEG-/fMRI-based as well as neurobehavioral studies have repeatedly demonstrated the impact of IN insulin on cognitive and metabolic brain function (8-12). In contrast, a significant contribution to central nervous insulin signaling of peripheral ins-asp (re-)entering the brain compartment appears unlikely considering that much stronger elevations in plasma insulin are necessary to substantially raise CSF insulin concentrations (20) and induce changes in CNS function (10). Also, given that in the 210 IU ins-asp condition, glucagon and cortisol concentrations remained unaffected and mean nadir glucose levels of 4.4 mmol/l during this and the respective IV control experiment exceeded the counterregulatory threshold of 3.6-3.9 mmol/l (21), a modulatory role of counterregulatory hormones might be assumed to be of secondary relevance in this context, but should be explored in subsequent studies.

Our data obtained in healthy men are in line with findings in dogs indicating that direct insulin action predominates in the acute regulation of peripheral glucose homeostasis (4,5,22,23). A 200-fold elevation of central insulin concentrations by ICV insulin infusion in dogs failed to alter glycogenolysis or gluconeogenesis but induced an upregulation of hepatic glycogen synthesis that slightly reduced hepatic glucose balance during the last 60 min of a 240-min euglycemic, pancreatic clamp with basal portal vein insulin and glucagon infusion (23). Glucose-lowering effects of intracisternally administered insulin in dogs that appeared to depend on intact vagal nerve function could only be achieved with extremely high pharmacological doses of insulin (24). Divergent findings in rodents and larger mammals like dogs and humans might be related to the fact that such larger organisms display 5-10 times smaller rates of endogenous glucose production than rodents, with the latter moreover exhibiting reduced hepatic glycogen reserves (22). Given the greater physiological relevance
of the gluconeogenic pathway, its acute control by the CNS might be more pronounced in rats and mice. A recent study in humans reported on improved peripheral insulin sensitivity, assessed with a euglycemic, hyperinsulinemic clamp, after IN administration of 160 IU insulin (25). In accordance with our findings, however, IN insulin administration induced an elevation of circulating insulin concentrations peaking at around 50 pmol/l, which leaves open the question of peripheral vs. central contributions to the observed effects.

Taken together, our results indicate that in humans, sustained CNS insulin delivery via IN administration does not impact peripheral glucose homeostasis beyond effects accounted for by concurrent systemic hyperinsulinemia. Future studies should investigate whether brain insulin signaling rather contributes to setting the basal tone of peripheral glucose homeostasis in humans.

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VO and MH designed the study. VO, KW and JS enrolled subjects and carried out experiments for the study. VO and MH analyzed the data. VO, HL, JB and MH discussed the results. VO and MH wrote the paper. MH is the guarantor of this work and, as such, had full
access to all the data in the study and takes responsibility for the integrity of the data and the
accuracy of the data analysis.

The authors have no potential conflicts of interest relevant to this article.
References

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Table 1. Subject characteristics.

<table>
<thead>
<tr>
<th></th>
<th>Experiment I (n=20)</th>
<th>Experiment II (n=10)</th>
<th>P value</th>
</tr>
</thead>
<tbody>
<tr>
<td>Age (y)</td>
<td>24.4±0.8</td>
<td>25.5±0.9</td>
<td>0.55</td>
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<tr>
<td>BMI (kg/m²)</td>
<td>22.8±0.5</td>
<td>22.9±0.4</td>
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<tr>
<td>Fat mass (kg)</td>
<td>13.4±1.0</td>
<td>15.3±1.4</td>
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<tr>
<td>Lean body mass (kg)</td>
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<td>60.5±2.5</td>
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<tr>
<td>HOMA-IR</td>
<td>1.4±0.2</td>
<td>1.2±0.2</td>
<td>0.48</td>
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</tbody>
</table>

All values are means ± SEM. HOMA-IR, insulin resistance according to the Homeostasis Model Assessment. *P*-values derive from paired, two-tailed *t*-tests for independent samples and Mann-Whitney-U-tests as appropriate.
Table 2. Responses of counterregulatory hormones.

<table>
<thead>
<tr>
<th></th>
<th>Placebo</th>
<th>210 IU ins-asp</th>
<th>420 IU ins-asp</th>
<th>P value</th>
</tr>
</thead>
<tbody>
<tr>
<td>Glucagon (10^3 ng*min^-1)</td>
<td>63±3</td>
<td>65±3</td>
<td>67±3</td>
<td>0.43</td>
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<tr>
<td>Cortisol (10^-2 nmol*min^-1)</td>
<td>97±5</td>
<td>98±4</td>
<td>115±6</td>
<td>0.007</td>
</tr>
<tr>
<td>GH (10^-3 µg*min^-1)</td>
<td>327±77</td>
<td>826±183</td>
<td>1172±232</td>
<td>0.0002</td>
</tr>
</tbody>
</table>

All values are means ± SEM. GH, growth hormone. P values derive from ANOVA with the within-subject factor Treatment and, respectively, the non-parametric Friedman test (GH).
Figure 1. Circulating concentrations of glucose, endogenous insulin, C-peptide and insulin aspart (ins-asp). Experiment I: Mean (± SEM) concentrations of (A) plasma glucose (upper lines) and ins-asp (lower lines), (B) endogenous insulin in serum, and (C) serum C-peptide assessed before (averaged across the 0825 h and 0835 h baseline values) and during/after recurrent intranasal (IN) administration of ins-asp at doses of 10 IU/15 min (amounting to a total dose of 210 IU; red symbols/lines) and 20 IU/15 min (total dose of 420 IU; blue symbols/lines) as well as placebo (vehicle; black empty symbols/dotted lines) from 0845 h to 1345 h. n=20. (D) Experiment II: Mean (± SEM) concentrations of plasma glucose (circles) and serum ins-asp (triangles) during continuous IV infusion of placebo (saline; black empty symbols/dotted lines) and ins-asp (red empty symbols/lines) at a concentration of 0.12 IU per kg per 24h for 300 min. n=10, with ins-asp assessments restricted to 5 of the 10 subjects. (E) Experiment I vs. II: Mean (± SEM) differences between ins-asp (210 IU IN and 0.12 IU/kg/24h IV, respectively) and placebo for concentrations of plasma glucose (circles) and serum ins-asp (triangles) during IN (full symbols; experiment I) and IV (empty symbols; experiment II) administration of ins-asp. – * P<0.05 (pairwise t-tests with Bonferroni corrections for multiple comparisons). Red, blue and black asterisks in panels A and C indicate significant differences between placebo and 210 IU ins-asp, placebo and 420 IU ins-asp, and between the two ins-asp conditions, respectively. Black asterisks in panels D and E indicate significant differences between conditions and experiments I and II, respectively.
Figure 1. Circulating concentrations of glucose, endogenous insulin, C-peptide and insulin aspart (ins-asp).

Experiment I: Mean (± SEM) concentrations of (A) plasma glucose (upper lines) and ins-asp (lower lines), (B) endogenous insulin in serum, and (C) serum C-peptide assessed before (averaged across the 0825 h and 0835 h baseline values) and during/after recurrent intranasal (IN) administration of ins-asp at doses of 10 IU/15 min (amounting to a total dose of 210 IU; red symbols/lines) and 20 IU/15 min (total dose of 420 IU; blue symbols/lines) as well as placebo (vehicle; black empty symbols/dotted lines) from 0845 h to 1345 h. n=20. (D) Experiment II: Mean (± SEM) concentrations of plasma glucose (circles) and serum ins-asp (triangles) during continuous IV infusion of placebo (saline; black empty symbols/dotted lines) and ins-asp (red empty symbols/lines) at a concentration of 0.12 IU per kg per 24h for 300 min. n=10, with ins-asp assessments restricted to 5 of the 10 subjects. (E) Experiment I vs. II: Mean (± SEM) differences between ins-asp (210 IU IN and 0.12 IU/kg/24h IV, respectively) and placebo for concentrations of plasma glucose (circles) and serum ins-asp (triangles) during IN (full symbols; experiment I) and IV (empty symbols; experiment II) administration of ins-asp. – * P<0.05 (pairwise t-tests with Bonferroni corrections for
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