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Central Nervous Insulin Administration Does Not Potentiate the Acute Glucoregulatory Impact of Concurrent Mild Hyperinsulinemia

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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 international units [IU] (10 and 20 IU every 15 min) of the insulin analog aspart (ins-asp) and placebo, respectively, during experimental sessions lasting 6 h. The use of ins-asp 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-asp into the circulation. In control experiments mimicking the systemic but not the central nervous uptake of the IN 210 IU dose via intravenous infusion of ins-asp at a dose of 0.12 IU/kg/24 h (*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 via not only direct effects on peripheral organs but also 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 h 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 h (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). In this study, 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

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insulin administration that directly targets the brain (6,7) and effectively modulates central nervous system (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-asp) 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 nondominant arm for venous blood drawings. From 0845 to 1345 h, ins-asp (NovoRapid; Novo Nordisk, Bagsvaerd, Denmark) at a cumulative dose of 210 and 420 international units (IU) and placebo, respectively, were IN administered in 21 individual doses of 10, 20, and 0 IU at 15-min intervals. Thus, one 0.1-mL puff of placebo and one 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 (Fig. 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 five 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, for which setup was identical to experiment I except that instead of IN administration, ins-asp compared with placebo (0.9% NaCl) was IV administered at a body weight–adjusted dose of 0.12 IU/kg/24 h from 0845 to 1345 h.

Table 1—Subject characteristics

	Experiment I (n = 20)	Experiment II (n = 10)	P value
Age (years)	24.4 ± 0.8	25.5 ± 0.9	0.55
BMI (kg/m^2)	22.8 ± 0.5	22.9 ± 0.4	0.63
Fat mass (kg)	13.4 ± 1.0	15.3 ± 1.4	0.60
Lean body mass (kg)	60.6 ± 0.7	60.5 ± 2.5	0.95
HOMA-IR	1.4 ± 0.2	1.2 ± 0.2	0.48

Data are means ± SEM. P values derive from paired, two-tailed t tests for independent samples and Mann–Whitney U tests as appropriate. HOMA-IR, HOMA of insulin resistance.

Analyses of Plasma Glucose and Hormonal Parameters

Blood samples were centrifuged and supernatants stored at −80°C. Blood for the measurement of glucagon was pretreated with aprotinin (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), and glucagon (radioimmunoassay; 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 posttreatment values. Depending on their distribution, data were subjected to ANOVA (degrees of freedom corrected using the Greenhouse-Geisser procedure) or nonparametric tests. A P value <0.05 was considered significant.

RESULTS

Experiment I: IN 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; Fig. 1*A*). In parallel, endogenous insulin concentrations decreased throughout the experiment ($P < 0.0001$), with a trend toward even lower concentrations when ins-asp was administered ($P = 0.089$ for treatment; Fig. 1*B*). In accordance, ins-asp compared with placebo decreased serum C-peptide ($P < 0.002$, Friedman tests; Fig. 1*C*). IN ins-asp treatment, moreover, increased GH concentrations and in the 420 IU condition attenuated the circadian decline in cortisol, whereas a slight

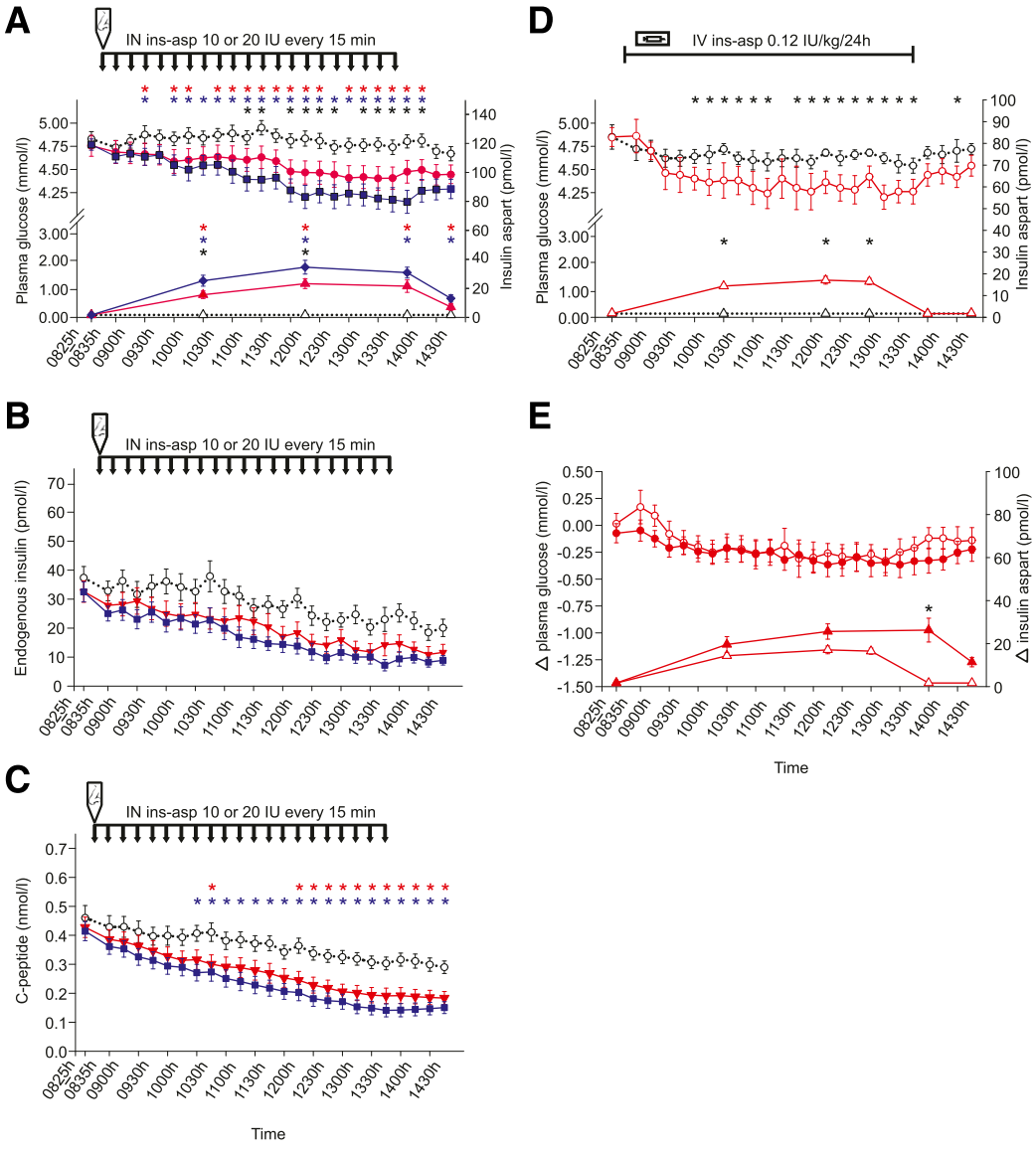

Figure 1—Circulating concentrations of glucose, endogenous insulin, C-peptide, and ins-asp. Experiment I: Mean (± SEM) concentrations of plasma glucose (top lines) and ins-asp (bottom lines) (*A*), endogenous insulin in serum (*B*), and serum C-peptide (*C*) assessed before (averaged across the 0825- and 0835-h baseline values) and during/after recurrent 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 open symbols/dotted lines) from 0845 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 open symbols/dotted lines) and ins-asp (red open symbols/lines) at a concentration of 0.12 IU/kg/24 h 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 (IN 210 IU and IV 0.12 IU/kg/24 h, respectively) and placebo for concentrations of plasma glucose (circles) and serum ins-asp (triangles) during IN (closed symbols; experiment I) and IV (open symbols; experiment II) administration of ins-asp. 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. *$P < 0.05$ (pairwise *t* tests with Bonferroni corrections for multiple comparisons).

increase in glucagon was not significant (Table 2). During IN ins-asp treatment, a dose-dependent increase in serum ins-asp emerged (area under the curve $[AUC]_{placebo}$ 0 ± 0 vs. $AUC_{ins\text{-}asp\text{-}in210}$ 5,936 ± 949 vs. $AUC_{ins\text{-}asp\text{-}in420}$ 9,229 ± 1194 pmol · L^{-1} · min; $P < 0.0001$; Fig. 1*A*) 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

Table 2—Responses of counterregulatory hormones

	Placebo	210 IU ins-asp	420 IU ins-asp	*P* value
Glucagon (10^3 ng · L^{-1} · min)	63 ± 3	65 ± 3	67 ± 3	0.43
Cortisol (10^3 nmol · L^{-1} · min)	97 ± 5	98 ± 4	115 ± 6	**0.007**
GH (10^3 μg · L^{-1} · min)	327 ± 77	826 ± 183	1,172 ± 232	**0.0002**

Data are means ± SEM. *P* values derive from ANOVA with the within-subject factor treatment for glucagon and cortisol and from the nonparametric Friedman test for GH. Significant *P* values are in boldface.

infusion ($AUC_{placebo}$ 0 ± 0 vs. $AUC_{ins\text{-}asp\text{-}iv}$ 3,726 ± 267 pmol · L^{-1} · min; $P < 0.03$; Fig. 1*D*) significantly decreased plasma glucose concentrations ($P < 0.02$ for treatment × time, Fig. 1*D*). 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 ($AUC_{ins\text{-}asp\text{-}iv}$ 3,726 ± 267 vs. $AUC_{ins\text{-}asp\text{-}in210}$ 5,936 ± 949 pmol · L^{-1} · min; $P > 0.2$), with this difference increasing toward the end of experiments (Fig. 1*E*). However, respective suppressive effects on plasma glucose were completely identical ($P > 0.57$ for time × group; Fig. 1*E*), indicating that the enhanced brain transport of ins-asp during IN as compared with 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 with 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 h before a 4-h euglycemic pancreatic clamp decreased endogenous glucose production during the last 2 h 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-h 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 cerebrospinal fluid (CSF) concentrations of ins-asp in this study, 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 420 IU dose of IN insulin produced robust and, because of the recurrent delivery, prolonged increases in CSF ins-asp levels. Furthermore, electroencephalography/functional magnetic resonance imaging–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 ~50 pmol/L, which leaves open the question of peripheral versus 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 contributes to setting the basal tone of peripheral glucose homeostasis in humans.

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Author Contributions. V.O. designed the study, enrolled subjects and carried out experiments for the study, analyzed the data, discussed the results, and wrote the paper. H.L. and J.B. discussed the results. J.S. and K.W. enrolled subjects and carried out experiments for the study. M.H. designed the study, analyzed the data, discussed the results, and wrote the paper. M.H. 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.

References

1. Obici S, Zhang BB, Karkanias G, Rossetti L. Hypothalamic insulin signaling is required for inhibition of glucose production. Nat Med 2002;8:1376–1382

2. Obici S, Feng Z, Karkanias G, Baskin DG, Rossetti L. Decreasing hypothalamic insulin receptors causes hyperphagia and insulin resistance in rats. Nat Neurosci 2002;5:566–572

3. Pocai A, Lam TK, Gutierrez-Juarez R, et al. Hypothalamic K(ATP) channels control hepatic glucose production. Nature 2005;434:1026–1031

4. Edgerton DS, Lautz M, Scott M, et al. Insulin's direct effects on the liver dominate the control of hepatic glucose production. J Clin Invest 2006;116:521–527

5. Ramnanan CJ, Kraft G, Smith MS, et al. Interaction between the central and peripheral effects of insulin in controlling hepatic glucose metabolism in the conscious dog. Diabetes 2013;62:74–84

6. Born J, Lange T, Kern W, McGregor GP, Bickel U, Fehm HL. Sniffing neuropeptides: a transnasal approach to the human brain. Nat Neurosci 2002;5:514–516

7. Dhuria SV, Hanson LR, Frey WH 2nd. Intranasal delivery to the central nervous system: mechanisms and experimental considerations. J Pharm Sci 2010;99:1654–1673

8. Benedict C, Kern W, Schultes B, Born J, Hallschmid M. Differential sensitivity of men and women to anorexigenic and memory-improving effects of intranasal insulin. J Clin Endocrinol Metab 2008;93:1339–1344

9. Benedict C, Brede S, Schiöth HB, et al. Intranasal insulin enhances postprandial thermogenesis and lowers postprandial serum insulin levels in healthy men. Diabetes 2011;60:114–118

10. Hallschmid M, Schultes B, Marshall L, et al. Transcortical direct current potential shift reflects immediate signaling of systemic insulin to the human brain. Diabetes 2004;53:2202–2208

11. Reger MA, Watson GS, Green PS, et al. Intranasal insulin improves cognition and modulates beta-amyloid in early AD. Neurology 2008;70:440–448

12. Benedict C, Hallschmid M, Schmitz K, et al. Intranasal insulin improves memory in humans: superiority of insulin aspart. Neuropsychopharmacology 2007;32:239–243

13. Kurtzhals P, Schäffer L, Sørensen A, et al. Correlations of receptor binding and metabolic and mitogenic potencies of insulin analogs designed for clinical use. Diabetes 2000;49:999–1005

14. Brange J, Owens DR, Kang S, Vølund A. Monomeric insulins and their experimental and clinical implications. Diabetes Care 1990;13:923–954

15. Bouche C, Lopez X, Fleischman A, et al. Insulin enhances glucose-stimulated insulin secretion in healthy humans. Proc Natl Acad Sci U S A 2010;107:4770–4775

16. Halperin F, Lopez X, Manning R, Kahn CR, Kulkarni RN, Goldfine AB. Insulin augmentation of glucose-stimulated insulin secretion is impaired in insulin-resistant humans. Diabetes 2012;61:301–309

17. Kishore P, Boucai L, Zhang K, et al. Activation of K(ATP) channels suppresses glucose production in humans. J Clin Invest 2011;121:4916–4920

18. Akopova OV, Nosar VI, Bouryi VA, Mankovskaya IN, Sagach VF. Influence of ATP-dependent K(+)-channel opener on K(+)-cycle and oxygen consumption in rat liver mitochondria. Biochemistry (Mosc) 2010;75:1139–1147

19. Perseghin G, Regalia E, Battezzati A, et al. Regulation of glucose homeostasis in humans with denervated livers. J Clin Invest 1997;100:931–941

20. Wallum BJ, Taborsky GJ Jr, Porte D Jr, et al. Cerebrospinal fluid insulin levels increase during intravenous insulin infusions in man. J Clin Endocrinol Metab 1987;64:190–194
21. Fanelli C, Pampanelli S, Epifano L, et al. Relative roles of insulin and hypoglycaemia on induction of neuroendocrine responses to, symptoms of, and deterioration of cognitive function in hypoglycaemia in male and female humans. Diabetologia 1994;37:797–807
22. Ramnanan CJ, Edgerton DS, Cherrington AD. Evidence against a physiologic role for acute changes in CNS insulin action in the rapid regulation of hepatic glucose production. Cell Metab 2012;15:656–664
23. Ramnanan CJ, Saraswathi V, Smith MS, et al. Brain insulin action augments hepatic glycogen synthesis without suppressing glucose production or gluconeogenesis in dogs. J Clin Invest 2011;121:3713–3723
24. Chowers I, Lavy S, Halpern L. Effect of insulin administered intracisternally on the glucose level of the blood and the cerebrospinal fluid in vagotomized dogs. Exp Neurol 1966;14:383–389
25. Heni M, Wagner R, Kullmann S, et al. Central insulin administration improves whole-body insulin sensitivity via hypothalamus and parasympathetic outputs in men. Diabetes 2014;63:4083–4088