Glucagon-Like Peptide-1 Inhibits Blood-Brain Glucose Transfer in Humans

Susanne Lerche,[1] Birgitte Brock,[1] Jørgen Rungby,[1] Hans E. Bøtker,[2] Niels Møller,[3] Anders Rodell,[4] Bo Martin Bibby,[5] Jens J. Holst,[6] Ole Schmitz,[1] and Albert Gjedde[4]

OBJECTIVE—Glucagon-like peptide-1 (GLP-1) has many effects on glucose homeostasis, and GLP-1 receptors are broadly represented in many tissues including the brain. Recent research in rodents suggests a protective effect of GLP-1 on brain tissue. The mechanism is unknown. We therefore tested whether these neuroprotective effects could relate to changes of glucose transport and consumption.

RESEARCH DESIGN AND METHODS—We studied 10 healthy men in a randomized, double-blinded, placebo-controlled cross-over experiment. We used positron emission tomography to determine the acute insulin-independent effect of GLP-1 on unidirectional glucose transport into the brain during a pituitary-pancreatic normoglycemic (plasma glucose ~4.5 mmol/l) clamp with 18-fluoro-deoxy-glucose as tracer.

RESULTS—On average, GLP-1 reduced cerebral glucose transport by 27% in total cerebral gray matter (P = 0.05) and by 25–30% in individual gray matter regions (P = 0.02–0.06). The same regions revealed a uniform trend toward similarly reduced cerebral glucose metabolism. Consequently, the intracerebral glucose concentration remained constant in all regions, with and without GLP-1.

CONCLUSIONS—We have demonstrated that a hormone involved in postprandial glucose regulation also limits glucose delivery to brain tissue and hence provides a possible regulatory mechanism for the link between plasma glucose and brain glucose. Because GLP-1 reduces glucose uptake across the intact blood-brain barrier at normal glycemia, GLP-1 may also protect the brain by limiting intracerebral glucose fluctuation when plasma glucose is increased. ***Diabetes* 57:325–331, 2008**

Glucagon-like peptide-1 (GLP-1) is an incretin hormone with several beneficial effects on glucose homeostasis. GLP-1 is not solely produced by the entero-endocrine L-cells in the gut but also in the brain by neurons in the nucleus of the tractus solitarius and thus functions as a neuropeptide (1). It stimulates insulin secretion and inhibits glucagon secretion in the presence of glucose, inhibits gastric emptying, and reduces appetite and food intake (2,3). Furthermore, it acts as a β-cell growth factor by stimulating β-cell proliferation and islet neogenesis and by reducing β-cell apoptosis (4). The glucose dependency of GLP-1 action on insulin and glucagon secretion results in a low risk of hypoglycemic episodes (5). These effects make GLP-1 attractive as an anti-diabetes medication, and several studies prove the beneficial effect on glucose homeostasis in the type 2 diabetic patient (6,7).

Although GLP-1 receptors (GLP-1Rs) are predominantly located on the β-, δ-, and presumably α-cells of the pancreas, receptors in other tissues may mediate GLP-1's effects outside the pancreas (8,9), where gastric emptying and appetite are well-known targets, as are other tissues including heart, kidney, and lungs (8). GLP-1R expression has been demonstrated in human brain (10), where receptors are particularly abundant in the paraventricular nucleus and the arcuate nucleus in the hypothalamus (11). Infusion of GLP-1 dose-dependently enhances satiety and reduces food intake in normal and obese subjects, including those with type 2 diabetes, and neural mechanisms appear to be involved (3).

The abundance of receptors in other locations in the brain, i.e., the thalamus, brainstem, lateral septum, subfornical organ, area postrema, the cerebral cortex, cerebellum, and caudate-putamen, as well as in the hippocampus, suggests that GLP-1 may have additional effects in the central nervous system as a whole (10,12). This is also evident from GLP-1R– knock-out mice, who have seriously disturbed learning abilities (13).

Recent studies in rodents provide evidence of both neuroprotective and neurotrophic effects of GLP-1 (13,14), including improvement of learning and memory (13). The mechanism of these putative protective effects of GLP-1 in the central nervous system is unknown. Applied directly to the rodent brain, GLP-1 affects peripheral glucose metabolism (15), implying that interaction exists among GLP-1, brain, and glucose homeostasis.

In the brain, intracellular and interstitial glucose concentrations are maintained by the stereospecific and non–energy-demanding mechanism of facilitated diffusion, mediated by the GLUT1 transporter, across the blood-brain barrier (BBB). BBB glucose transfer is downregulated during hyperglycemia (16,17) and stimulated during hypoglycemia (18). As insulin has no measurable effect on blood-brain glucose transfer (19,20), it remains uncertain how the non–insulin-dependent glucose transport across the BBB is regulated. GLP-1 itself has been shown to cross the BBB by passive diffusion (21).

Because the role of glucose delivery and metabolism in neuroprotection is unclear, we tested the hypothesis that

From the [1]Institute of Pharmacology, University of Aarhus, Aarhus, Denmark; the [2]Department of Cardiology, Aarhus University Hospital, Aarhus, Denmark; the [3]Department of Endocrinology M, Aarhus University Hospital, Aarhus, Denmark; the [4]PET-Center, Aarhus University Hospital, Aarhus, Denmark; the [5]Department of Biostatistics, University of Aarhus, Aarhus, Denmark; and the [6]Department of Medical Physiology, University of Copenhagen, Panum Institute, Copenhagen, Denmark.

Address correspondence and reprint requests to Susanne Lerche, MD, Institute of Pharmacology, University of Aarhus, The Bartholin Building, University Park 1240, 8000 Aarhus C, Denmark. E-mail: lerche@ki.au.dk.

Received for publication 16 August 2007 and accepted in revised form 3 November 2007.

Published ahead of print at http://diabetes.diabetesjournals.org on 8 November 2007. DOI: 10.2337/db07-1162. Clinical trial reg. no. NCT00256256, clinicaltrials.gov.

BBB, blood-brain barrier; FDG, 18-fluoro-deoxy-glucose; GLP-1, glucagon-like peptide 1; GLP-1R, GLP-1 receptor; PET, positron emission tomography; rGLP-1, recombinant GLP-1.

© 2008 by the American Diabetes Association.

The costs of publication of this article were defrayed in part by the payment of page charges. This article must therefore be hereby marked "advertisement" in accordance with 18 U.S.C. Section 1734 solely to indicate this fact.

an interaction exists between GLP-1 and delivery of glucose by measuring the transport and metabolism of the glucose tracer 18-fluoro-deoxy-glucose (FDG) during positron emission tomography (PET) under the influence of GLP-1.

RESEARCH DESIGN AND METHODS

Eleven nonsmoking, healthy, Caucasian, male subjects were included in the study; one subject was withdrawn due to uncompleted PET scan. Mean (± SD) age was 25 ± 3 years, and BMI was 22.6 ± 0.6 kg/m^2. They had no history of diabetes or cardiovascular disease and received no medication. All had a normal physical examination. The study was conducted in accordance with the Declaration of Helsinki, and the protocol was approved by the local ethics committee of the County of Aarhus. All participants received both oral and written information and signed an approved informed consent form before entering the study.

The study was carried out as a randomized, double-blinded, placebo-controlled cross-over study. Each subject was studied twice in random order with GLP-1 and placebo infusion. PET sessions were separated by an interval of 4–6 weeks. Both sessions commenced at 0800 h after an overnight fast, and subjects did not exercise 24 h before the sessions. Subjects were placed in bed, and two catheters were inserted for infusion of clamp hormones and for the infusion of GLP-1 or placebo. A third catheter was placed in an arterialized (heated) dorsal hand vein for blood sampling.

The randomization was performed by the pharmacy at Aarhus University Hospital, and the randomization list was kept at the pharmacy during the study. Sealed envelopes containing information about the code were delivered together with the study medication at day 1 and kept unbroken in the case report form. The code was broken after all data analysis was completed.

A pancreatic-pituitary clamp was performed according to principles previously described (22). In brief, somatostatin (D-24109 Kiel; Ferring) was infused at a rate of 300 μg/h to suppress endogenous insulin, glucagon, growth hormone, and GLP-1 production. Human glucagon (0.6 ng · kg^{-1} · min^{-1}) (Glucagen; Novo Nordisk, Copenhagen, Denmark), growth hormone (2 ng · kg^{-1} · min^{-1}) (Genotropin Miniquik, 0.2 mg; Pfizer, Ballerup, Denmark), and insulin (0.12 mU · kg^{-1} · min^{-1}) (Actrapid; Novo Nordisk) were infused with the aim of maintaining near-basal levels (0–360 min). Glucose (200 g/l) was infused at a variable rate to clamp plasma glucose close to 4.5 mmol/l. Euglycemia was maintained in order to assess a physiological role of GLP-1 at the BBB independent of insulin and glucagon levels and independent of a direct effect of plasma glucose on GLP-1 action.

The subjects received either intravenous recombinant GLP-1 (rGLP-1)(7-36 amide) or placebo at a rate of 1.2 pmol · kg^{-1} · min^{-1} (60–360 min) (23). rGLP-1(7-36 amide) was tested and found positive for sterility and negative for bacterial endotoxins. It was dissolved in a sterile buffer containing 600 mg acetoacid, 50.7 g mannitol, and sterile water added up to 1,000 g and had a pH of 4.5. The concentration of rGLP-1(7-36) was 1 mg/ml and stored frozen in vials of 0.25 ml. The test solution consisted of 0.25 ml rGLP-1 (1 mg/ml), 20 ml human albumin ZLB 5%, and NaCl (9 g/l) up to 100 ml of solution. The placebo solution consisted of the above-mentioned buffer solution containing human albumin and NaCl.

Plasma glucose was measured in duplicate every 10 min. Blood for measuring insulin, C-peptide, glucagon, GLP-1 (total and intact), growth hormone, free fatty acids, cortisol, and ghrelin was drawn every 30 min. Blood for measuring epinephrine and norepinephrine was drawn at 0, 150, and 240 min and every 30 min during the PET scan.

Assays. Plasma glucose was measured immediately after sampling on a Beckman glucose analyzer (Beckman, Palo Alto, CA). All other blood samples were stored at −20°C (C-peptide at −80°C) until assay.

The assays used for measuring serum insulin, serum C-peptide, serum free fatty acid, serum growth hormone, serum cortisol, plasma epinephrine, and plasma norepinephrine are described previously (24). S-ghrelin was measured with an in-house assay (25).

The assay for intact GLP-1 is an enzyme-linked immunosorbent assay using unextracted plasma, which was collected and stored in the presence of a dipeptidyl peptidase-IV inhibitor (valine-pyrrolidide, 0.01 mmol/l, final concentration added to the blood sample immediately after collection). Total GLP-1 was analyzed using a C-terminal radioimmunoassay for amidated GLP-1 (26). Glucagon was measured using a previously described assay (27).

PET. FDG was used as a tracer for brain glucose uptake and was produced in house according to Drug Master File, Danish national marketing authorization number 2165. We used a whole-body PET model EXACT HR47 (Siemens Medical, Knoxville, TN) with a 15-cm field of view and an acquisition capacity of 47 transaxial planes with a spatial resolution of 4–5 mm at the center of the field of view. All PET data were acquired in three-dimensional mode.

A 15-min transmission scan was performed to correct photon attenuation. Five hours after clamp start and 4 h after the GLP-1 or placebo infusion was started, a bolus of 200 MBq of FDG in 10 ml saline was injected intravenously over 10 s. Dynamic acquisition commenced at the beginning of tracer injection and continued for 45 min to acquire 23 frames (6 × 30 s and 7 × 1, 5 × 2, and 5 × 5 min).

For the extraction of region-specific PET data results, regions of interest were automatically extracted using a model-based segmentation approach (28) based solely on the registration of PET images to a standardized anatomic atlas. Regional tissue time-activity curves for FDG uptake were extracted for total cerebral gray matter, cerebral cortex, thalamus, striatum, cerebellar cortex, brainstem, and white matter.

Image-derived arterial input functions were automatically obtained by localizing the carotic artery on the PET images for each subject from each study day. An automated method for identification of the most probable voxel positions for the arteries was developed. Using a three-compartment model, we obtained values of unidirectional clearance (K_1*), efflux coefficient (k_2*), and phosphorylation rate constant (k_3*) for FDG in each region of interest. The rate of dephosphorylation and the absolute quantity of FDG were both considered negligible during the scanning period and are therefore not part of the model. The lumped constant is the conversion factor between the net uptakes of FDG and glucose as functions of transport across the BBB and the phosphorylation. In the model of competition between native glucose and FDG in brain, both transport across the BBB (K_1*, k_2*), and phosphorylation by hexokinase (k_3*) can be described by the Michaelis-Menten equation. This permits the use of fixed transport ($\tau = K_1^*/K_1$) and phosphorylation ($\varphi = k_3^*/k_3$) rate ratios for tracer and native glucose. (Those marked with * indicate FDG.)

By substituting transfer constants of FDG for those of glucose, using τ and φ, the lumped constant was determined directly for each subject in each region of interest as described previously (29). In this study we used the value of $\tau = 1.48$ and $\varphi = 0.39$ (30).

The net clearance of FDG was calculated as:

$$K^* = K1^* \times k3^*/k2^* + k3^*$$

The unidirectional glucose transfer was calculated as:

$$J\text{glc} = K1^* \times \text{Ca}/\tau$$

where J_{glc} is the unidirectional transport of glucose from blood to brain (influx of glucose), Ca equals the arterial steady-state plasma glucose concentration (300–360 min), and (as above) those marked with * indicate FDG.

The lumped constant (LC) was calculated as:

$$\varphi + (\tau - \varphi) \times (k3^*/k2^* + k3^*)$$

The net clearance of glucose was calculated as:

$$K\text{glc} = K^*/\text{LC}$$

The cerebral metabolic rate of glucose (CMR_{glc}) was calculated as:

$$K^* \times \text{Ca}/\text{LC}$$

And the cerebral tissue glucose concentration C_t was calculated as:

$$\tau \times (J\text{glc} - CMR\text{glc})/k2^*$$

Calculations and statistics. Primary end points were transfer coefficients of BBB glucose transport and unidirectional glucose transfer across the BBB at normal glycemia. Secondary end points were cerebral metabolic rate for glucose, cerebral tissue glucose concentration, and lumped constant.

The PET data were analyzed using a linear mixed-effects model with subject and all interactions involving subject, i.e., the interaction between subject and area and the interaction between subject and treatment, as random effects. Treatment (GLP-1/placebo), region, and the interaction between the two were included in the analysis as fixed effects. Data were transformed when appropriate based on an inspection of the residuals, and results are therefore presented as back-transformed estimated means with 95% CIs. The statistical software used was PROC MIXED in SAS (SAS Institute, Cary, NC) with a significance level of 5%. To compare the two treatment groups regarding circulating hormones and metabolites, a similar statistical model was used with time instead of region. Data are presented as means ± SEM.

RESULTS

The infusion of GLP-1 at a constant rate of 1.2 pmol · kg^{-1} · min^{-1} resulted in pharmacologically relevant plasma concentrations of the intact hormone (31) (Fig. 1).

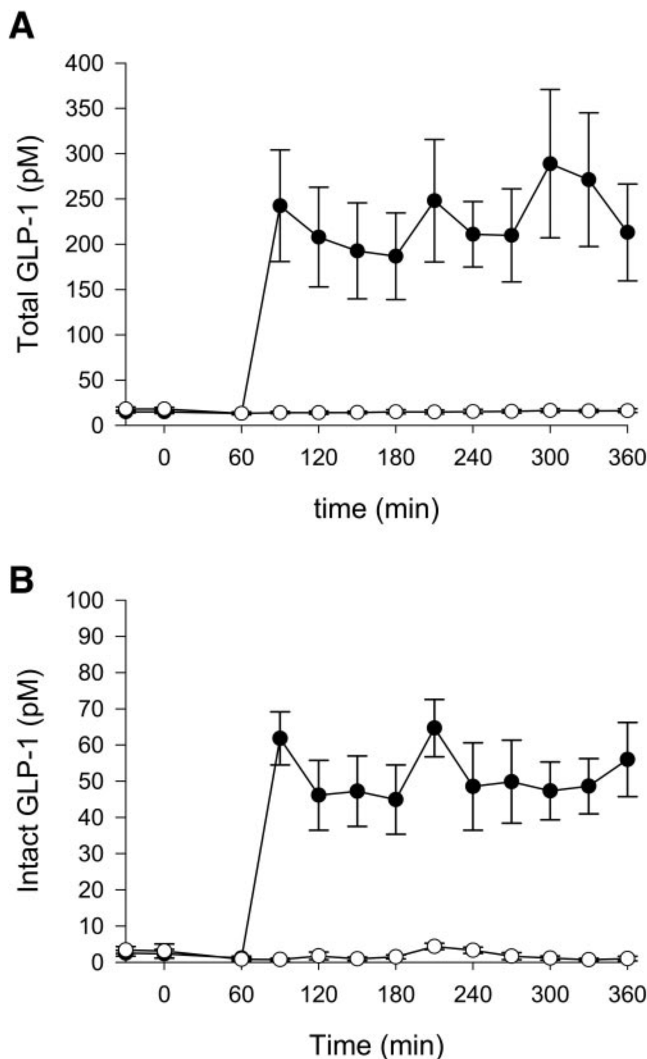

FIG. 1. Total (*A*) and intact (*B*) GLP-1 concentrations during GLP-1 (●) and placebo (○) infusion. GLP-1 infusion was initiated at t = 60 min. PET was performed from 300 to 360 min. Data are means ± SEM.

During PET, the plasma glucose concentration was successfully maintained at 4.6 ± 0.04 (GLP-1) and 4.6 ± 0.03 (placebo) mmol/l (P = 0.74) with similar glucose infusion rates (1.81 ± 0.30 vs. 1.55 ± 0.32 $mg \cdot kg^{-1} \cdot min^{-1}$ for GLP1 vs. placebo, P = 0.22) (Fig. 2). The plasma C-peptide level was suppressed (P = 0.66). As expected, there were no significant differences in circulating hormones and metabolites between sessions. Insulin, glucagons, and growth hormone infusions resulted in low, close to basal levels of these hormones ($P > 0.24$) (Fig. 3). Ghrelin, norepinephrine, cortisol (data not shown), and epinephrine levels were also low and did not change between sessions ($P > 0.14$). Only plasma GLP-1 levels differed between sessions ($P < 0.001$).

There was an overall significant effect of GLP-1 on the unidirectional clearance of glucose (K_{1glc}), but it was not the same in all regions (P = 0.015 for the interaction between region and treatment) (Table 1). K_{1glc} was decreased by 20–27% (P = 0.02–0.08) in the seven brain regions; the efflux coefficient for FDG (k_2*, data not shown) was decreased by 26–39% (P = 0.03) with GLP-1 infusion compared with placebo.

No significant change in the phosphorylation coefficient for FDG (k_3*, data not shown) was observed (P = 0.39), and the net clearance of FDG (K*, data not shown) (P = 0.58) and of glucose (K_{glc}) (P = 0.25) was unchanged with GLP-1 infusion. A tendency toward increased lumped constant with GLP-1 infusion was seen in all regions (P = 0.08).

The unidirectional glucose transfer (J_{glc}) across the BBB was reduced by GLP-1 infusion in all brain regions but not by the same amount (P = 0.01 for the interaction between region and treatment) (Fig. 4*A*). In specific brain regions, J_{glc} was reduced by 23–30% (P = 0.02–0.06) with GLP-1 infusion. The cerebral metabolic rate for glucose (CMR_{glc}) was reduced by 12–18% with GLP-1 infusion, but the difference was not significant (P = 0.28) (Fig. 4*B*). The brain tissue glucose concentration remained the same in all regions with and without GLP-1 infusion (P = 0.89) (Fig. 4*C*). Two subjects experienced transient nausea starting after ~1 and 3 h and with a duration of 5 and 1 h, respectively.

DISCUSSION

In general, the search for a regulatory mechanism of BBB glucose transfer operating in the brain vasculature has failed (32). The only known factor affecting BBB glucose transfer therefore remains the plasma glucose level itself. In vitro, animal studies have shown that GLP-1 signaling is involved in neuronal plasticity and neuroprotection (13), and GLP-1R activation has effects in the nervous system comparable with those of nerve growth factor (14). No human studies have linked GLP-1, cerebral glucose transport, and neuroprotection. Yet, maintaining a constant glucose concentration in brain tissue is important to the preservation of neuronal functioning. Several studies have shown characteristic electroencephalogram changes during hypoglycemia (plasma glucose <2.0 mmol/l) in both type 1 diabetic and healthy subjects (33,34). Hyperglycemia with concomitantly increased glucose concentration in brain tissue accelerates ischemic damage and worsens the outcome after ischemic stroke (35). Although, physiologically, the BBB glucose clearance is lower in acute hyperglycaemia, the absolute flux is of course elevated (17), and it still remains unclear whether the reduction of plasma glucose during stroke is neuroprotective, as trials are ongoing (36).

To our knowledge, the current study for the first time reveals that GLP-1 potentially regulates BBB glucose transfer. Independently of the insulin and glucagon levels, GLP-1 reduces unidirectional glucose delivery across the intact BBB at normoglycaemia. The cerebral glucose metabolism tended also to decline, but the tissue glucose concentration remained constant. Together the data implies that GLP-1 maintains a constant glucose concentration in the brain at normal glycaemia by limiting the glucose delivery across the BBB. We anticipate that if the effect is due to a decline of the maximal transport (T_{max}), it would be even more pronounced during hyperglycaemia.

However, the cerebral glucose metabolism does not depend strongly on the glucose concentration in the brain intracellular and interstitial fluids because brain hexokinase is saturated at the prevailing glucose concentration. Since the tissue glucose concentration depends on the glucose phosphorylation rate, the BBB transfer assumes a very important role in the maintenance of glucose concentration in the brain (37). In the brain, the unidirectional

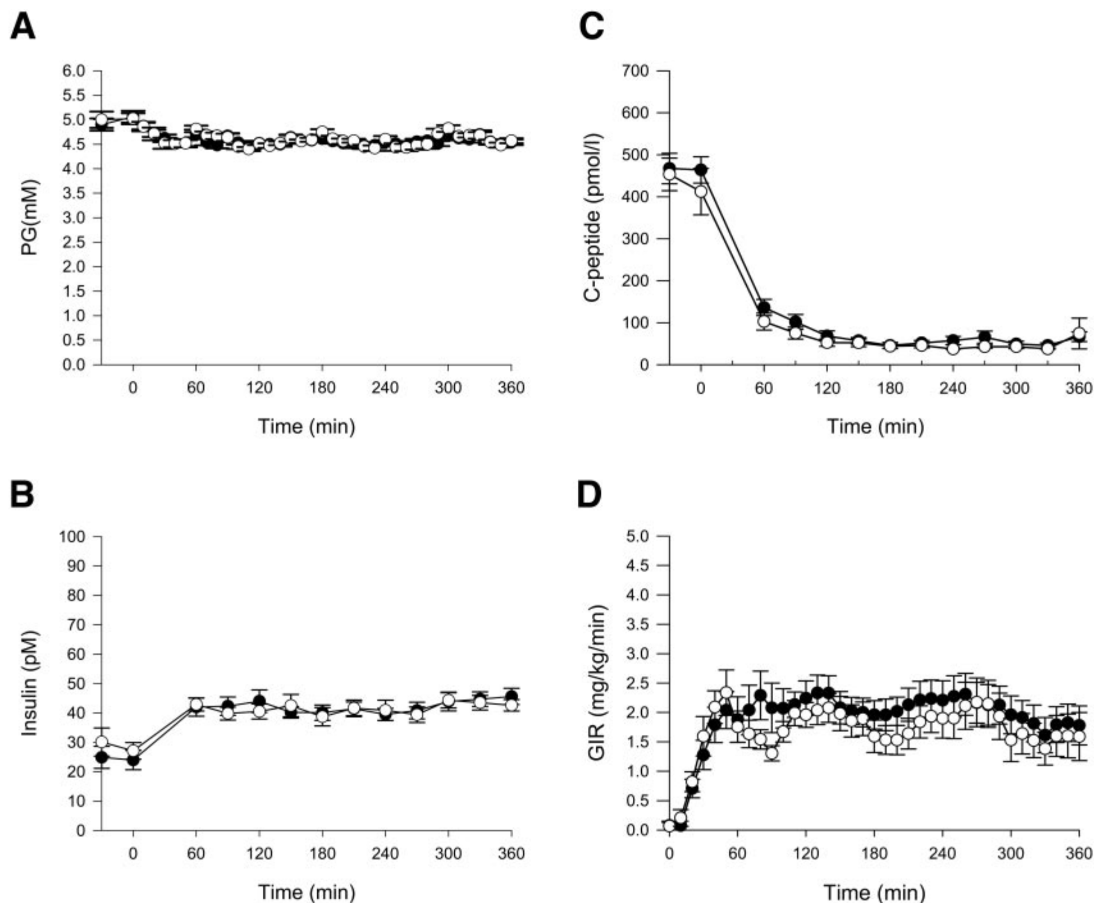

FIG. 2. Plasma glucose (PG) (*A*), insulin (*B*), and C-peptide (*C*) concentrations and glucose infusion rate (GIR) (*D*) during GLP-1 (●) and placebo (○) infusion. Data are means ± SEM.

glucose transport rate increases concurrently with increasing glycemia. Because the transport is only half saturated at ~4 mmol/l (38), the fraction of glucose that passes from blood to brain continues to decrease with increasing glycemia.

The net clearance of FDG (K^*) in the current study was 4.4 ml/100 cm^3 per min (95% CI 3.5–5.6), which is comparable to values found in the literature (4.2 ml · 100 g^{-1} · min^{-1}) (1 g brain tissue approximates a volume of 1 cm^3). However, in the current study, the unidirectional glucose transport in total cerebral gray matter was 93 μmol/100 cm^3 per min (range 77–114), which is approximately twice as high as previously reported values for whole brain, mostly explained by the focus on gray matter in the present study and the still imperfect methods of computing whole-brain glucose transport rates from regional values with PET. Values of the lumped constant for humans ranges from 0.50 to 0.80, and in the present study we obtained a lumped constant for gray matter of 0.55 (range 0.52–0.58) (30). The cerebral metabolic rate for glucose we obtained in total cerebral gray matter was 37 μmol/100 cm^3 per min (range 30–46), which is somewhat higher than the whole brain value of 25–30 μmol · 100 g^{-1} · min^{-1} (39).

Cerebral blood flow was not measured in this study; however, clearance (K) in principle is a measure that includes any blood flow effect, as it is the product of an extraction fraction (unidirectional or net extraction) and the plasma flow. Therefore, any blood flow effect is included in the clearance, which in turn is the measure that describes the flux when multiplied with the concentration. This description does not of course exclude the possibility that the change in clearance is the result of a blood flow change, but the effect is the effect measured as the clearance.

The effect of GLP-1 on the unidirectional clearance of glucose was not the same in all regions. But indeed a trend was observed in all regions, indicating that the effect is potentially uniformly seen in all brain regions with an intact BBB. In the brain, facilitated glucose diffusion across the BBB maintains a glucose concentration in brain tissue that is lower than in plasma and similar to that in cerebrospinal fluid (40). Calculated levels in the present brain regions are consistent with the highest levels in the most active part of gray matter and the lowest levels in white matter.

Individual lumped constants were calculated for each brain region. The lumped constant tended to increase during GLP-1 infusion in all brain regions. Because of the Michaelis-Menten kinetic competition for blood-brain transfer of glucose and glucose analogues, the lumped constant is in fact a variable that depends on the plasma glucose concentration and the relation between brain and plasma glucose.

In a single FDG-PET study, GLP-1 receptor activation reduced glucose metabolism in areas of the hypothalamus and brainstem involved in feeding and glucose sensing, where receptors are abundant (41). However, it was not apparent whether the effect was due to reduced glucose transport across the BBB and/or reduced phosphorylation

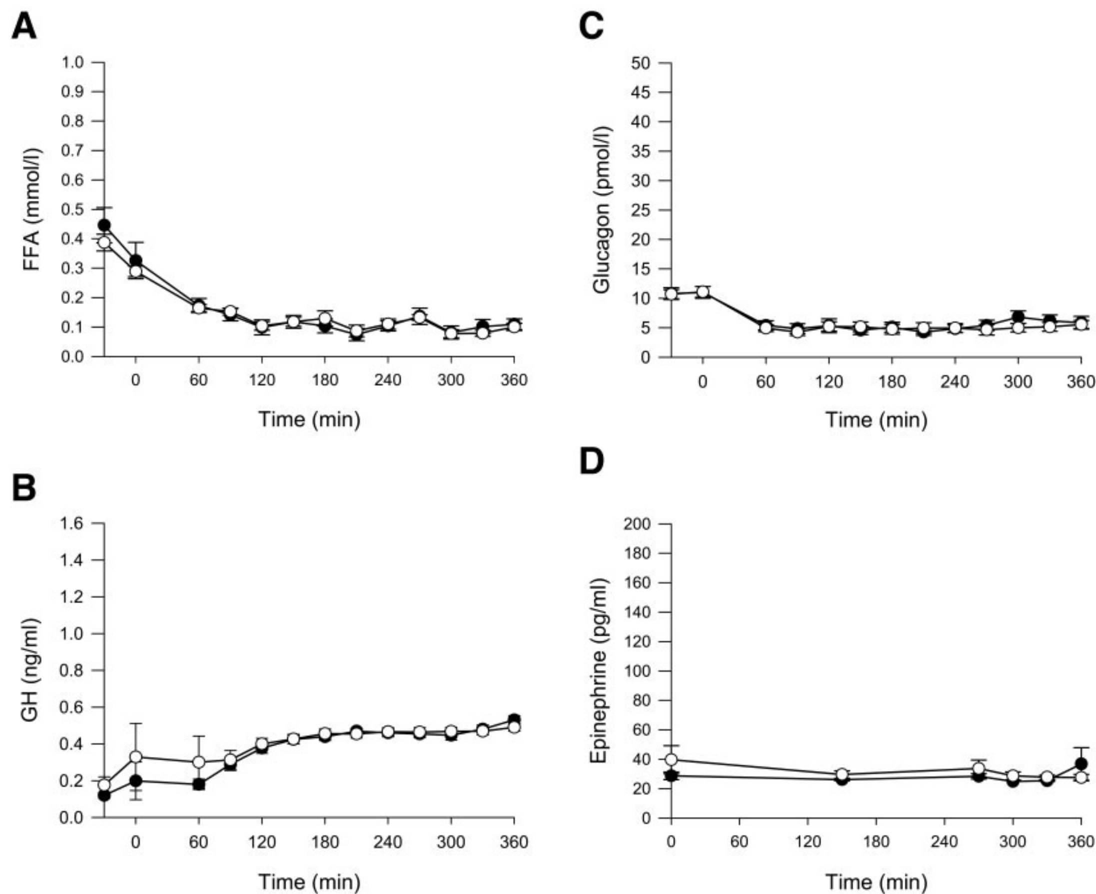

FIG. 3. Free fatty acid (FFA) (*A*), growth hormone (GH) (*B*), glucagons (*C*), and epinephrine (*D*) concentrations during GLP-1 (●) and placebo (○) infusion. Data are means ± SEM.

of glucose by hexokinase. No effect was observed in other brain areas. Caveats of the study noted are that circulating levels of glucose, insulin, and glucagon were uncontrolled during and after the short (30 min) GLP-1 exposure and that PET commenced 10 min after the end of GLP-1 infusion. As the half-life of GLP-1 is <2 min due to enzymatic degradation by ubiquitously represented dipeptidyl peptidase-IV (42), the true plasma GLP-1 concentrations are uncertain, and it is unknown whether pharmacologically relevant levels were reached during the PET examination.

In the present study, we demonstrate that GLP-1 reduces BBB transport of glucose in general in the brain. The interaction of GLP-1 with the normal human BBB is therefore a novel finding. The glucose transport across the BBB is mediated by the transporter GLUT1. We speculate that GLP-1 interacts with the GLUT1 transporter and the GLP-1 receptor in the BBB.

GLP-1 has a role in the maintenance of cerebral glucose balance and may have neuroprotective effects linked to both peripheral and cerebral glucose metabolism. During hyperglycemia, GLP-1 is secreted from the gut into the blood stream and may also be produced in the brain, as central GLP-1 signaling appears to be linked to the control of blood glucose concentrations (15,43). GLP-1 efficiently promotes disposal of glucose, and circulating glucose is reduced by means of increased insulin secretion and decreased glucagon secretion, thereby limiting glucose transport across the BBB. Here, we show that GLP-1 has an important effect that could be independent of insulin

TABLE 1
Initial clearance of glucose (K_{1glc}), net clearance of glucose (K_{glc}), and lumped constant (LC) during GLP-1 and placebo infusion

	K_{1glc} (ml/100 cm^3 per min)			K_{glc} (ml/100 cm^3 per min)		LC	
	Placebo	GLP-1	P	Placebo	GLP-1	Placebo	GLP-1
Grey matter	20 (16–25)	15 (13–18)	0.07	8.0(6.5–9.8)	6.8(5.5–8.3)	0.55 (0.52–0.58)	0.59 (0.56–0.62)
Cerebral cortex	20 (16–24)	15 (12–18)	0.08	8.2(6.6–10.0)	6.9(5.6–8.6)	0.56 (0.53–0.59)	0.60 (0.57–0.64)
Thalamus	22 (18–28)	16 (14–19)	0.07	7.9(6.4–9.7)	6.8(5.5–8.4)	0.53 (0.50–0.55)	0.57 (0.54–0.60)
Striatum	20 (16–25)	15 (13–18)	0.08	8.5(6.9–10.5)	7.2(5.9–8.9)	0.57 (0.54–0.60)	0.61 (0.57–0.64)
Cerebellar cortex	23 (19–30)	17 (14–21)	0.08	7.1(5.7–8.7)	6.0(4.9–7.4)	0.50 (0.48–0.53)	0.53 (0.50–0.55)
Brain stem	15 (13–18)	11 (9–13)	0.02	4.8(3.9–5.9)	3.9(3.2–4.8)	0.51 (0.48–0.53)	0.54 (0.51–0.57)
White matter	10 (9–12)	8 (7–9)	0.04	3.7(3.0–4.6)	3.3(2.6–4.0)	0.54 (0.51–0.57)	0.56 (0.53–0.59)
P for all regions				0.25		0.08	

Data are medians (95% CI). P for overall GLP-1 effect on K_{1glc} = 0.015. For calculation of K_{glc} and LC, see RESEARCH DESIGN AND METHODS.

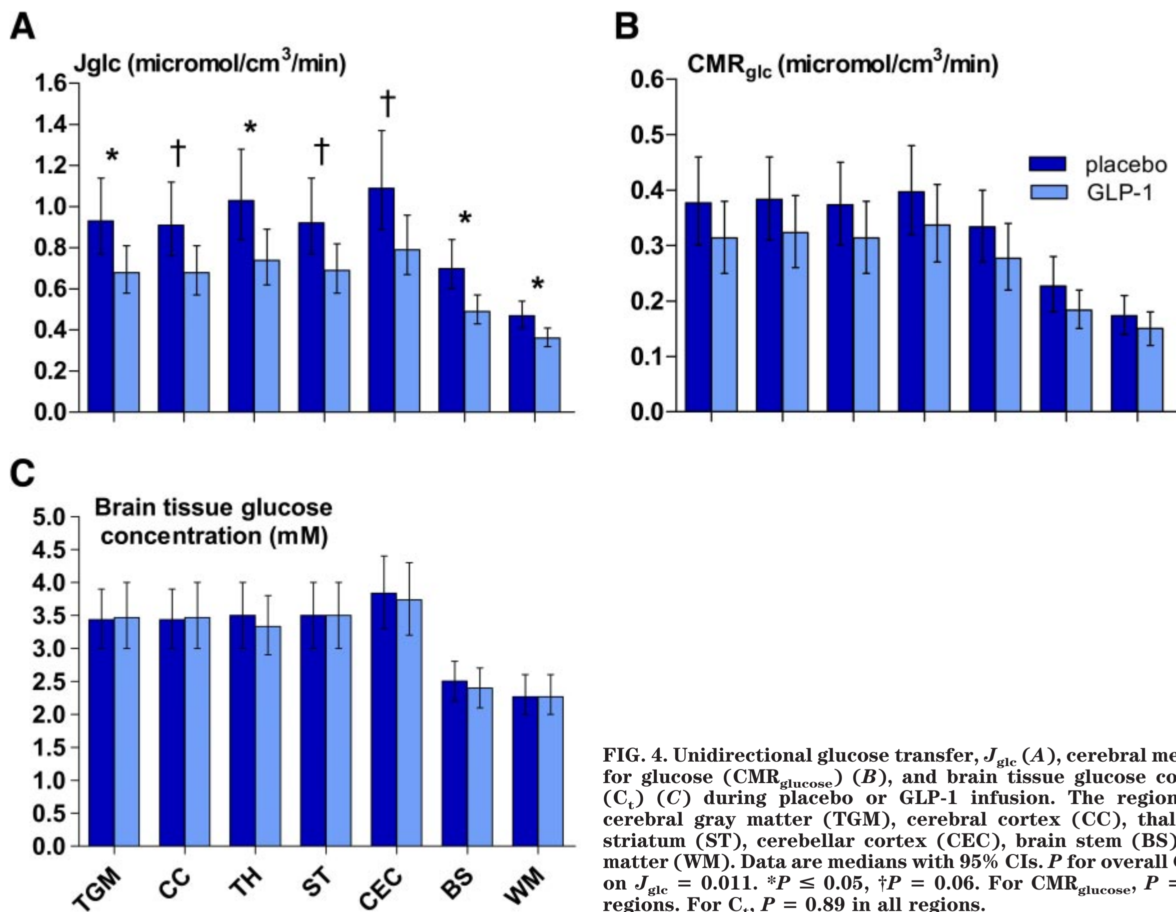

FIG. 4. Unidirectional glucose transfer, J_{glc} (*A*), cerebral metabolic rate for glucose ($CMR_{glucose}$) (*B*), and brain tissue glucose concentration (C_t) (*C*) during placebo or GLP-1 infusion. The regions are total cerebral gray matter (TGM), cerebral cortex (CC), thalamus (TH), striatum (ST), cerebellar cortex (CEC), brain stem (BS), and white matter (WM). Data are medians with 95% CIs. *P* for overall GLP-1 effect on J_{glc} = 0.011. *$P \leq 0.05$, †$P = 0.06$. For $CMR_{glucose}$, $P = 0.28$ in all regions. For C_t, $P = 0.89$ in all regions.

action. Hence, both peripheral and central actions of GLP-1 efficiently limit glucose fluctuations in the brain that may be of neuroprotective significance. Thus, under conditions with pharmacologically elevated levels of GLP-1, we propose a new extrapancreatic effect of GLP-1 in the brain that provides a link between brain glucose transport and neuroprotection.

ACKNOWLEDGMENTS

This study was supported by the Lundbeck Foundation, the Novo Nordisk Foundation, the Danish Diabetes Association, the Danish Medical Research Council, the Danish National Science Foundation, the FOOD Study Group/Danish Ministry of Food, Agriculture and Fisheries & Ministry of Family and Consumer Affairs (grant no. 2101-05-0044), and the Danish Clinical Intervention and Research Academy.

Parts of this study were presented at the 67th annual meeting of the American Diabetes Association, Chicago, Illinois, 22–26 June 2007.

We thank Annette Mengel and the staff at the PET Center, Aarhus University Hospital, for their outstanding technical assistance.

REFERENCES

1. Brubaker PL, Drucker DJ: Minireview: Glucagon-like peptides regulate cell proliferation and apoptosis in the pancreas, gut, and central nervous system. *Endocrinology* 145:2653–2659, 2004
2. Drucker DJ, Nauck MA: The incretin system: glucagon-like peptide-1 receptor agonists and dipeptidyl peptidase-4 inhibitors in type 2 diabetes. *Lancet* 368:1696–1705, 2006
3. Holst JJ: On the physiology of GIP and GLP-1. *Horm Metab Res* 36:747–754, 2004
4. Baggio LL, Drucker DJ: Biology of incretins: GLP-1 and GIP. *Gastroenterology* 132:2131–2157, 2007
5. Nauck MA, Heimesaat MM, Behle K, Holst JJ, Nauck MS, Ritzel R, Hufner M, Schmiegel WH: Effects of glucagon-like peptide 1 on counterregulatory hormone responses, cognitive functions, and insulin secretion during hyperinsulinemic, stepped hypoglycemic clamp experiments in healthy volunteers. *J Clin Endocrinol Metab* 87:1239–1246, 2002
6. Ahren B, Schmitz O: GLP-1 receptor agonists and DPP-4 inhibitors in the treatment of type 2 diabetes. *Horm Metab Res* 36:867–876, 2004
7. Drucker DJ: The evidence for achieving glycemic control with incretin mimetics. *Diabetes Educ* 32 (Suppl. 2):72S–81S, 2006
8. Ahren B: GLP-1 and Extra-islet Effects. *Horm Metab Res* 36:842–845, 2004
9. Vella A, Camilleri M, Rizza RA: The gastrointestinal tract and glucose tolerance. *Curr Opin Clin Nutr Metab Care* 7:479–484, 2004
10. Wei Y, Mojsov S: Tissue-specific expression of the human receptor for glucagon-like peptide-I: brain, heart and pancreatic forms have the same deduced amino acid sequences. *FEBS Lett* 358:219–224, 1995
11. Goke R, Larsen PJ, Mikkelsen JD, Sheikh SP: Distribution of GLP-1 binding sites in the rat brain: evidence that exendin-4 is a ligand of brain GLP-1 binding sites. *Eur J Neurosci* 7:2294–2300, 1995
12. Perry T, Greig NH: The glucagon-like peptides: a new genre in therapeutic targets for intervention in Alzheimer's disease. *J Alzheimers Dis* 4:487–496, 2002
13. During MJ, Cao L, Zuzga DS, Francis JS, Fitzsimons HL, Jiao X, Bland RJ, Klugmann M, Banks WA, Drucker DJ, Haile CN: Glucagon-like peptide-1 receptor is involved in learning and neuroprotection. *Nat Med* 9:1173–1179, 2003
14. Perry T, Lahiri DK, Chen D, Zhou J, Shaw KT, Egan JM, Greig NH: A novel neurotrophic property of glucagon-like peptide 1: a promoter of nerve growth factor-mediated differentiation in PC12 cells. *J Pharmacol Exp Ther* 300:958–966, 2002
15. Knauf C, Cani PD, Perrin C, Iglesias MA, Maury JF, Bernard E, Benhamed F, Gremeaux T, Drucker DJ, Kahn CR, Girard J, Tanti JF, Delzenne NM, Postic C, Burcelin R: Brain glucagon-like peptide-1 increases insulin secretion and muscle insulin resistance to favor hepatic glycogen storage. *J Clin Invest* 115:3554–3563, 2005

16. Gjedde A, Crone C: Blood-brain glucose transfer: repression in chronic hyperglycemia. *Science* 214:456–457, 1981
17. Hasselbalch SG, Knudsen GM, Capaldo B, Postiglione A, Paulson OB: Blood-brain barrier transport and brain metabolism of glucose during acute hyperglycemia in humans. *J Clin Endocrinol Metab* 86:1986–1990, 2001
18. Blomqvist G, Gjedde A, Gutniak M, Grill V, Widen L, Stone-Elander S, Hellstrand E: Facilitated transport of glucose from blood to brain in man and the effect of moderate hypoglycaemia on cerebral glucose utilization. *Eur J Nucl Med* 18:834–837, 1991
19. 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
20. Knudsen GM, Hasselbalch SG, Hertz MM, Paulson OB: High dose insulin does not increase glucose transfer across the blood-brain barrier in humans: a re-evaluation. *Eur J Clin Invest* 29:687–691, 1999
21. Kastin AJ, Akerstrom V, Pan W: Interactions of glucagon-like peptide-1 (GLP-1) with the blood-brain barrier. *J Mol Neurosci* 18:7–14, 2002
22. Nielsen MF, Nyholm B, Caumo A, Chandramouli V, Schumann WC, Cobelli C, Landau BR, Rizza RA, Schmitz O: Prandial glucose effectiveness and fasting gluconeogenesis in insulin-resistant first-degree relatives of patients with type 2 diabetes. *Diabetes* 49:2135–2141, 2000
23. Toft-Nielsen MB, Madsbad S, Holst JJ: Continuous subcutaneous infusion of glucagon-like peptide 1 lowers plasma glucose and reduces appetite in type 2 diabetic patients. *Diabetes Care* 22:1137–1143, 1999
24. Degn KB, Brock B, Juhl CB, Djurhuus CB, Grubert J, Kim D, Han J, Taylor K, Fineman M, Schmitz O: Effect of intravenous infusion of exenatide (synthetic exendin-4) on glucose-dependent insulin secretion and counterregulation during hypoglycemia. *Diabetes* 53:2397–2403, 2004
25. Gormsen LC, Nielsen C, Gjedsted J, Gjedde S, Vestergaard ET, Christiansen JS, Jorgensen JO, Moller N: Effects of free fatty acids, growth hormone and growth hormone receptor blockade on serum ghrelin levels in humans. *Clin Endocrinol (Oxf)* 66:641–645, 2007
26. Orskov C, Rabenhoj L, Wettergren A, Kofod H, Holst JJ: Tissue and plasma concentrations of amidated and glycine-extended glucagon-like peptide I in humans. *Diabetes* 43:535–539, 1994
27. Holst JJ: Evidence that enteroglucagon (II) is identical with the C-terminal sequence (residues 33–69) of glicentin. *Biochem J* 207:381–388, 1982
28. Collins DL, Holmes CJ, Peters TM, Evans AC: Automatic 3D model-based neuroanatomical segmentation. In *Human Brain Mapping*. Vol. 3, issue 3. New York, Wiley-Liss, Inc., 1995, p. 190–208
29. Kuwabara H, Evans AC, Gjedde A: Michaelis-Menten constraints improved cerebral glucose metabolism and regional lumped constant measurements with [18F]fluorodeoxyglucose. *J Cereb Blood Flow Metab* 10:180–189, 1990
30. Hasselbalch SG, Madsen PL, Knudsen GM, Holm S, Paulson OB: Calculation of the FDG lumped constant by simultaneous measurements of global glucose and FDG metabolism in humans. *J Cereb Blood Flow Metab* 18:154–160, 1998
31. Zander M, Madsbad S, Madsen JL, Holst JJ: Effect of 6-week course of glucagon-like peptide 1 on glycaemic control, insulin sensitivity, and beta-cell function in type 2 diabetes: a parallel-group study. *Lancet* 359:824–830, 2002
32. Gjedde A: Modulation of substrate transport to the brain. *Acta Neurol Scand* 67:3–25, 1983
33. Pramming S, Thorsteinsson B, Stigsby B, Binder C: Glycaemic threshold for changes in electroencephalograms during hypoglycaemia in patients with insulin dependent diabetes. *Br Med J (Clin Res Ed)* 296:665–667, 1988
34. Amiel SA, Pottinger RC, Archibald HR, Chusney G, Cunnah DT, Prior PF, Gale EA: Effect of antecedent glucose control on cerebral function during hypoglycemia. *Diabetes Care* 14:109–118, 1991
35. Lindsberg PJ, Roine RO: Hyperglycemia in acute stroke. *Stroke* 35:363–364, 2004
36. Gilmore RM, Stead LG: The role of hyperglycemia in acute ischemic stroke. *Neurocrit Care* 5:153–158, 2006
37. Pardridge WM: Brain metabolism: a perspective from the blood-brain barrier. *Physiol Rev* 63:1481–1535, 1983
38. Crone C: Facilitated transfer of glucose from blood into brain tissue. *J Physiol* 181:103–113, 1965
39. Hasselbalch SG, Holm S, Pedersen HS, Svarer C, Knudsen GM, Madsen PL, Paulson OB: The (18)F-fluorodeoxyglucose lumped constant determined in human brain from extraction fractions of (18)F-fluorodeoxyglucose and glucose. *J Cereb Blood Flow Metab* 21:995–1002, 2001
40. Leybaert L: Neurobarrier coupling in the brain: a partner of neurovascular and neurometabolic coupling? *J CerebBlood Flow Metab* 25:2–16, 2005
41. Alvarez E, Martinez MD, Roncero I, Chowen JA, Garcia-Cuartero B, Gispert JD, Sanz C, Vazquez P, Maldonado A, de Caceres J, Desco M, Pozo MA, Blazquez E: The expression of GLP-1 receptor mRNA and protein allows the effect of GLP-1 on glucose metabolism in the human hypothalamus and brainstem. *J Neurochem* 92:798–806, 2005
42. Deacon CF: Therapeutic strategies based on glucagon-like peptide 1. *Diabetes* 53:2181–2189, 2004
43. D'Alessio DA, Sandoval DA, Seeley RJ: New ways in which GLP-1 can regulate glucose homeostasis. *J Clin Invest* 115:3406–3408, 2005