Journal of Molecular Neuroscience

ISSN0895-8696/02/18:7–14/$12.00

Interactions of Glucagon-Like Peptide-1 (GLP-1) with the Blood-Brain Barrier

Abba J. Kastin,* Victoria Akerstrom, and Weihong Pan

VA Medical Center and Tulane University School of Medicine, New Orleans, LA 70112-1262

Received July 5, 2001; Accepted July 14, 2001

Abstract

Glucagon-like peptide-1 (GLP-1) reduces insulin requirement in diabetes mellitus and promotes satiety. GLP-1 in the periphery (outside the CNS) has been shown to act on the brain to reduce food ingestion. As GLP-1 is readily degraded in blood, we focused on the interactions of [Ser^8]GLP-1, an analog with similar biological effects and greater stability, with the blood-brain barrier (BBB). The influx of radiolabeled [Ser^8]GLP-1 into brain has several distinctive characteristics:

1. A rapid influx rate of $8.867 \pm 0.798 \times 10^4$ mL/g-min as measured by multiple-time regression analysis after iv injection in mice.
2. Lack of self-inhibition by excess doses of the unlabeled [Ser^8]GLP-1 either iv or by *in situ* brain perfusion, indicating the absence of a saturable transport system at the BBB.
3. Lack of modulation by short-term fasting and some other ingestive peptides that may interact with GLP-1, including leptin, glucagon, insulin, neuropeptide Y, and melanin-concentrating hormone.
4. No inhibition of influx by the selective GLP-1 receptor antagonist exendin(9–39), suggesting that the GLP-1 receptor is not involved in the rapid entry into brain.

Similarly, there was no efflux system for [Ser^8]GLP-1 to exit the brain other than following the reabsorption of cerebrospinal fluid (CSF). The fast influx was not associated with high lipid solubility. Upon reaching the brain compartment, substantial amounts of [Ser^8]GLP-1 entered the brain parenchyma, but a large proportion was loosely associated with the vasculature at the BBB. Finally, the influx rate of [Ser^8]GLP-1 was compared with that of GLP-1 in a blood-free brain perfusion system; radiolabeled GLP-1 had a more rapid influx than its analog and neither peptide showed the self-inhibition indicative of a saturable transport system. Therefore, we conclude that [Ser^8]GLP-1 and the endogenous peptide GLP-1 can gain access to the brain from the periphery by simple diffusion and thus contribute to the regulation of feeding.

Index Entries: Feeding; blood-brain barrier; GLP-1; peptide.

Introduction

Currently undergoing clinical investigation for the treatment of diabetes mellitus, the preproglucagon-derived glucagon-like peptide-1 (7–36) NH_2(GLP-1) is secreted from the intestine after eating. The distribution of GLP-1 in rat brain has been investigated by various methods, including immunohistochemistry (Jin et al., 1988; Larsen et al., 1997), receptor binding (Shimizu et al., 1987; Kanse et al., 1988; Turton et al., 1996), radioimmunoassay, and chromatography (Shimizu et al., 1987; Kreymann et al., 1989). The presence of GLP-1 in specific brain regions is consistent with its role in the regulation of food ingestion. Nonetheless, in the arcuate nucleus where a high density of GLP-1 binding sites is present, the number of immunoreactive GLP-1 nerve fibers is

*Author to whom all correspondence and reprint requests should be addressed.

very low. This suggests that GLP-1 from the circulating blood, rather than from the CNS, is more likely to act on the receptors in this area (Larsen et al., 1997).

Central but not peripheral administration of GLP-1 in rats can reduce food intake robustly and nonadversely in behavioral tests (Turton et al., 1996; Tang-Christensen et al., 1996). On the other hand, GLP-1 administered peripherally suppresses appetite and reduces feeding in patients with type 2 diabetes mellitus (body mass index [BMI] 29.4 kg/m^2) (Gutzwiller et al., 1999; Toft-Nielsen et al., 1999), in obese men (BMI 45.5 kg/m^2) (Naslund et al., 1999), and in healthy, normal weight men (BMI 20.3–25.7 kg/m^2) (Flint et al., 1998). The discrepancy in the routes of action might lie in the method of peripheral injection; in the human studies, GLP-1 was infused for several hours whereas in the rat study it was given by bolus intraperitoneal injection. Therefore, the stability of GLP-1 may account for the different effects.

GLP-1 is rapidly metabolized in rat blood. At two min after injection of GLP-1, less than half of the injected peptide remains intact and by 10 min, no intact GLP-1 is detected (Kieffer et al., 1995). Because of this rapid metabolism, for most of our studies we used the more stable analog of GLP-1 in which Ala^8 is replaced with Ser^8, representing the addition of only a hydroxyl group with retention of strong biological activity and receptor specificity (Ritzel et al., 1998).

Interactions have been described between GLP-1 and several of the ingestive peptides/polypeptides. The distribution of leptin receptor mRNA overlaps with that of GLP-1 gene expression, particularly in the nucleus of the solitary tract (Goldstone et al., 1997; Mercer et al., 1998), and GLP-1 neurons there are activated by leptin (Elias et al., 2000). A specific GLP-1 receptor antagonist, exendin(9–39), blocks the reduction in food intake and body weight of rats pretreated with leptin (Goldstone et al., 1997). GLP-1 also is a potent stimulator of insulin release (Ritzel et al., 1998; Toft-Nielsen et al., 1999), and can inhibit glucagon secretion, contributing to its glucose-lowering effect (Creutzeldt et al., 1996). Furthermore, central administration of GLP-1 completely prevents the orexigenic effects of melanin-concentrating hormone (MCH) and neuropeptide Y (NPY) (Tritos et al., 1998). Accordingly, we also examined the possible inhibition of GLP-1 penetration of the BBB by leptin, exendin(9–39), insulin, glucagon, MCH, and NPY.

Materials and Methods

Adult male albino ICR mice (Charles River, Wilmington, MA), 6–10/group, weighing about 22 g were anesthetized with urethane (4 g/kg, ip). The procedures used were approved by the Institutional Animal Care and Use Committee. [Ser^8]glucagon-like peptide-1 (7–36) amide (human, MW 3313.7, Bachem, Torrance, CA) and GLP-1 were radiolabeled with ^{125}I by the chloramine-T method and purified on a column of Sephadex G-10. Acid precipitation showed 99% incorporation of ^{125}I into [Ser^8]GLP-1 and high performance liquid chromatography (HPLC) showed more than 95% purity. The specific activity of the ^{125}I-[Ser^8]GLP-1 was 170 Ci/mmol. For ^{125}I-GLP-1, acid precipitation showed 98% incorporation of ^{125}I, 90% purity by HPLC, and a specific activity of 166 Ci/mmol.

Test of Stability of Radiolabeled Peptide in Blood and Brain by HPLC

Blood and brain samples were obtained 5 and 10 min after injection of ^{125}I-[Ser^8]GLP-1. The brain was homogenized in phosphate-buffered saline with a glass homogenizer. To this was added a cocktail of enzyme inhibitors (P8340, Sigma, St. Louis, MO) as well as ethylene diamine-tetraacetic acid (EDTA) and 1,10 phenanthroline to minimize the liberation of enzymes resulting from homogenization of brain tissue after completion of the in vivo experiment. The enzyme inhibitors were not added to the serum samples so as to reflect the natural condition of the ^{125}I-[Ser^8]GLP-1 in blood before entry into brain.

After centrifugation at 4,000*g* at 4°C for 10 min, the supernatant was lyophilized and rehydrated 10 min before elution on a reversed phase C_{18} column. Recovery of processed ^{125}I-[Ser^8]GLP-1 at this step was about 85%. The gradient consisted of 0.1% trifluoroacetic acid in water with 0.1% trifluoroacetic acid in acetonitrile increasing from 0 to 30% for 3 min, 30% to 60% for 30 min, 60% for 5 min, and 60 to 80% for an additional 5 min. The flow rate was 1 mL/min. Values were corrected for processing as determined by addition of ^{125}I-[Ser^8]GLP-1 to blood and homogenized brain samples of uninjected mice.

Influx of ^{125}I-[Ser^8]GLP-1 Into Brain Determined by Multiple-Time Regression Analysis

The ^{125}I-[Ser^8]GLP-1 was injected in a dose of about 2.5 pmol/mouse (1.5×10^6 cpm) through the isolated left jugular vein along with 1 μCi/mouse (1.5×10^6 cpm) of ^{99m}Tc-albumin in 200 μL of lactated Ringer's

solution containing 1% albumin (n = 10/group). At various time intervals between 1 and 10 min after iv injection, blood was collected from a cut in the right carotid artery and the mouse was immediately decapitated. Serum and brain samples were obtained and counted in a dual channel γ-counter.

The ratio of the radioactivity of brain tissue and serum was calculated, and multiple-time regression analysis was applied to determine the relationship between the ratios and exposure time, the theoretical time for the steady-state concentration of circulating ^{125}I-[Ser^{8}]GLP-1 after correction for disappearance from blood. A linear regression correlation was present. The slope of the regression line between tissue/serum ratios and exposure time is K_i, the unidirectional influx constant reflecting entry rate.

To determine whether the entry of ^{125}I-[Ser^{8}]GLP-1 was saturable, self-inhibition was tested initially by addition of our standard dose of 5 µg/mouse (1.51 nmol) of unlabeled [Ser^{8}]GLP-1 in the injected solution. Cross-inhibition was tested with 5 µg/mouse of exendin(9–39) (1.48 nmol), glucagon (1–29) (1.43 nmol), insulin (0.865 nmol), leptin (313 pmol), NPY (1.18 nmol), and MCH (2 nmol). Smaller doses of [Ser^{8}]GLP-1 and leptin were also tested. The potential effect of fasting was studied in a group of mice with food removed 18 h before the iv study while water was freely available. Experiments testing self-inhibition by [Ser^{8}]GLP-1 and cross-inhibition by leptin were repeated five times.

Identification of ^{125}I-[Ser^{8}]GLP-1 in Brain Parenchyma by Capillary Depletion and Washout

The capillary depletion method was used to separate cerebral capillaries and vascular components from brain parenchyma. Each of eight mice received an injection of ^{125}I-[Ser^{8}]GLP-1 together with ^{99m}Tc-albumin in 200 µL of lactated Ringer's/1% bovine serum albumin at time 0. At 5 min, blood was collected from the abdominal aorta and four of the mice were perfused intracardially over 30 s with 20 mL of the Ringer's solution after the descending aorta was blocked and bilateral jugular veins were severed. The cerebral cortex, devoid of circumventricular organs, was homogenized in glass with physiological buffer and mixed thoroughly with 26% dextran. An aliquot of the homogenate was centrifuged at 5400g for 15 min at 4°C.

The pellet, containing the capillaries, and the supernatant, representing the brain parenchymal/interstitial fluid space, were carefully separated. The ratios of radioactivity of ^{125}I-[Ser^{8}]GLP-1 in the supernatant (parenchyma) or pellet (capillary) over serum, corrected by subtraction of ^{99m}Tc-albumin ratios of radioactivity representing vascular space, were used to determine the ^{125}I-[Ser^{8}]GLP-1 in three compartments of the cortex:

1. Tightly bound to vascular endothelial cells (after washout).
2. Loosely associated with the vascular endothelial cells or circulating cellular elements (difference in brain cortex before and after washout).
3. In the brain cortical parenchyma (after washout).

Perfusion in a Blood-Free Solution

^{125}I-[Ser^{8}]GLP-1 or ^{125}I-GLP-1, with and without an excess (2 µg/mL) of unlabeled [Ser^{8}]GLP-1, GLP-1, or exendin(9–39), together with ^{99m}Tc-albumin, were added to buffer and perfused through the left ventricle of the heart at a rate of 2 mL/min for up to 5 min. In each experiment, six anesthetized mice in which the thoracic aorta had been clamped and both jugular veins severed immediately before the perfusion started, were used. A post-wash of 20 mL of perfusion buffer was used to remove the ^{125}I-[Ser^{8}]GLP-1 or ^{125}I-GLP-1 in the cerebral vasculature in addition to the intrinsic perfusion. The radioactivity in both brain samples and perfusate was measured, and the brain/perfusate ratio of ^{99m}Tc-albumin was subtracted from that of ^{125}I-[Ser^{8}]GLP-1 or ^{125}I-GLP-1 as a correction of the vascular space.

Efflux from Brain

About 25,000 cpm of both ^{125}I-[Ser^{8}]GLP-1 and ^{99m}Tc-albumin were simultaneously injected into the brain of mice anesthetized with urethane at a site 1 mm lateral and 0.2 mm posterior to the bregma with a Hamilton syringe (Banks et al., 1997). Mice were studied (n = 5/group) at 0, 2, 5, 10, and 20 min after injection. The 0 min value was determined in mice overdosed with anesthesia before injection, as previously explained (Banks and Kastin, 1989). The half-time disappearance was determined from the regression line obtained from the plot of the logarithm of brain radioactivity against time.

Octanol/PBS Partition Coefficient

To a mixture of ^{125}I-[Ser^{8}]GLP-1 and 1 mL of octanol was added 1 mL of 0.25 M phosphate-buffered saline (PBS). After being vigorously mixed for 1 min and gently mixed for an additional 10 min, the two phases were separated by centrifugation at 4000g for 10 min. Aliquots were counted for radioactivity and the par-

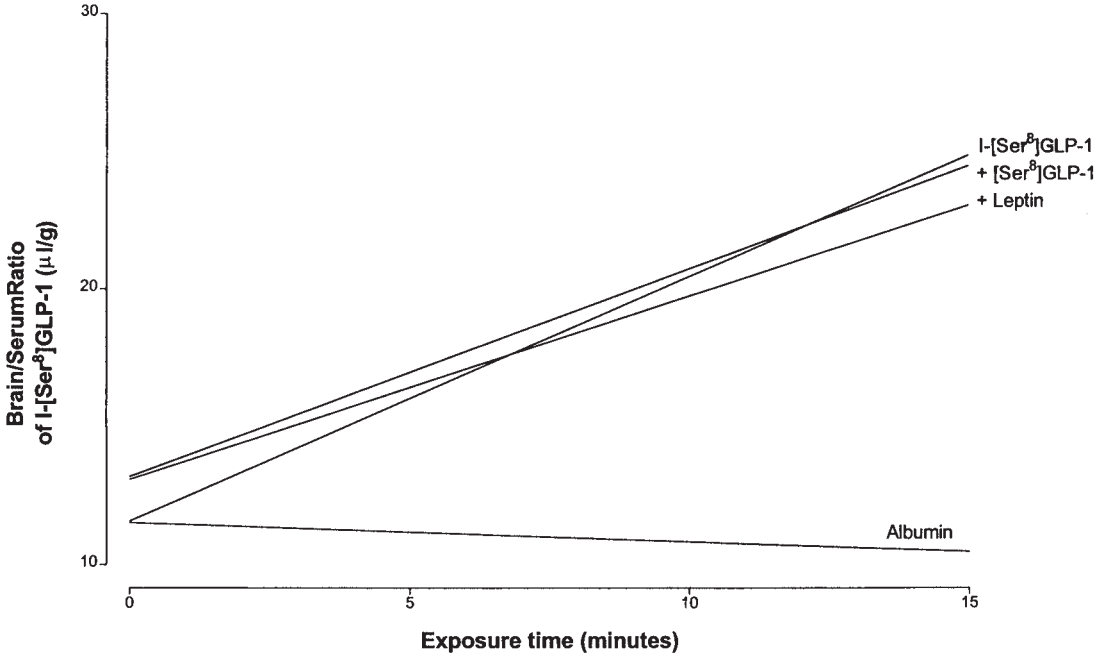

Fig. 1. The blood-to-brain influx of ^{125}I-[Ser^8]GLP-1 was rapid, but was not significantly inhibited by coadministration of an excess (5 μg/mouse) of unlabeled [Ser^8]GLP-1 or leptin.

tition coefficient expressed as the ratio of cpm in the octanol phase to cpm in the PBS phase.

Statistics

Groups were compared by ANOVA followed by Duncan's multiple comparisons test. Regression lines were determined by the least squares method and the differences between slopes compared by GraphPad Prism statistical software (GraphPad Software Inc., San Diego, CA).

Results

Test of Stability of Radiolabeled Peptide in Blood and Brain by HPLC

The stock solution of ^{125}I-[Ser^8]GLP-1 had a negligible peak representing free ^{125}I and a major peak representing the intact peptide. In both blood and brain samples, the retention time of the peaks correlated with that of the stock solution. In the supernatant of homogenized brain tissue from mice decapitated 5 min after iv injection of ^{125}I-[Ser^8]GLP-1, intact ^{125}I-[Ser^8]GLP-1 constituted 65% of the total radioactivity eluted, after correction for decay in the processing control. At 10 min after iv injection, which is the endpoint of the multiple-time regression study, 60% of the total radioactivity correlated with that of intact ^{125}I-[Ser^8]GLP-1. In serum, 85% of total radioactivity eluted at the retention time of intact ^{125}I-[Ser^8]GLP-1 at 5 min, whereas 68% intact peptide was seen at 10 min. The time needed for intact ^{125}I-[Ser^8]GLP-1, as determined from the chromatographs of samples at 0, 5, and 10 min, to decrease by 50%, was 21 min in serum and 6 min in brain.

Influx of ^{125}I-[Ser^8]GLP-1 into Brain Determined by Multiple-Time Regression Analysis

The K_i of ^{125}I-[Ser^8]GLP-1 was $8.867 \pm 0.798 \times 10^{-4}$ mL/g-min. The study was replicated five times, and the value presented is the mean of all data points included in the regression analysis. Addition of unlabeled [Ser^8]GLP-1 at 1, 2, or 5 μg/mouse did not significantly decrease the K_i of ^{125}I-[Ser^8]GLP-1 compared with the control group. In the group with 5 μg/mouse of excess unlabeled [Ser^8]GLP-1, the K_i was $7.537 \pm 1.154 \times 10^{-4}$ mL/g-min.

Coadministered leptin at 5 μg/mouse revealed a K_i of $6.646 \pm 0.929 \times 10^{-4}$ mL/g-min for ^{125}I-[Ser^8]GLP-1. This did not represent a statistically significant inhibition of the influx. Similarly, exendin(9–39), insulin, glucagon, NPY, or MCH at the same dose (5 μg/mouse) also failed to significantly decrease the K_i for ^{125}I-[Ser^8]GLP-1, as did food deprivation for 18 h.

In all of the above studies, the K_i for ^{125}I-[Ser^8]GLP-1 was significantly higher than that of ^{99m}Tc-albumin ($K_i = 0.695 \pm 0.860 \times 10^{-4}$ mL/g-min). Figure 1 shows the combined results of all experiments involving iv administration of ^{125}I-[Ser^8]GLP-1 with or without unlabeled [Ser^8]GLP-1 or leptin.

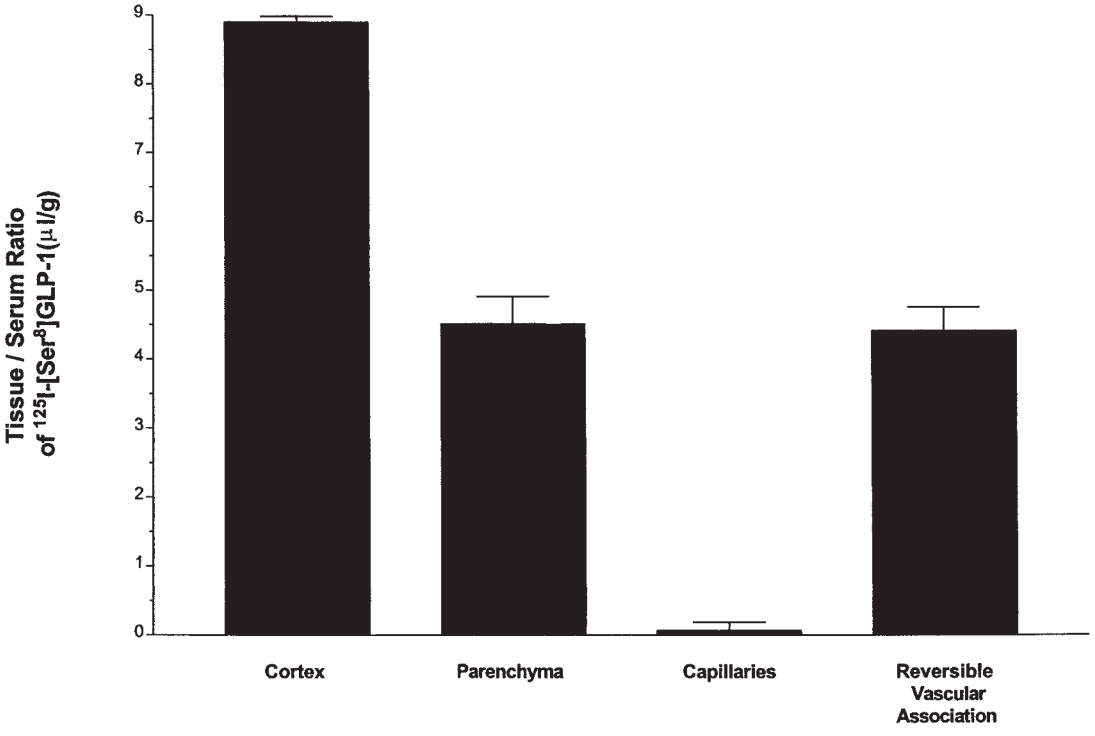

Fig. 2. Capillary depletion: After iv injection of ^{125}I-[Ser^{8}]-GLP-1, more radioactivity reached the brain parenchyma than was bound to the capillary endothelial cells that compose the BBB. A large amount of injected ^{125}I-[Ser^{8}]-GLP-1 was reversibly associated with the vasculature, as shown by the value after washout.

Identification of ^{125}I-[Ser^{8}]GLP-1 in Brain Parenchyma by Capillary Depletion and Washout

Figure 2 shows the uptake of ^{125}I-[Ser^{8}]GLP-1 by the brain at 5 min after iv injection. The radioactivity of ^{125}I-[Ser^{8}]GLP-1 in the nonperfused cerebral cortex, after correction of vascular contamination by subtraction of ^{99m}Tc-albumin, is representative of three major compartments: the brain parenchyma, which is the radioactivity in the supernatant of the perfused brain; the capillary internalization, which is the radioactivity in the pellet of the perfused brain; and the reversible binding before penetrating the BBB, which is the difference between the nonperfused and perfused cortex. ^{125}I-[Ser^{8}]GLP-1 in the parenchyma was 50.6% of the total brain uptake at this time, about 65 times higher than that endocytosed in the capillary endothelial cells composing the BBB, and the difference was significant ($p < 0.0001$). The rest of the radioactivity, nearly 49.4% of total uptake, was reversibly associated with the vasculature.

Perfusion in a Blood-Free Solution

The influx rate of ^{125}I-[Ser^{8}]GLP-1 by *in situ* brain perfusion was $1.52 \pm 0.26 \times 10^{-4}$ mL/g-min, whereas that of ^{125}I-GLP-1 was $12.03 \pm 0.49 \times 10^{-4}$ mL/g-min, almost eight times faster. However, there was no significant inhibition of the influx of either peptide by nonradiolabeled excess [Ser^{8}]GLP-1 or GLP-1, respectively. In addition to the lack of self-inhibition, there was no cross-inhibition between the two peptides, and there was no inhibition of the influx by the receptor antagonist exendin(9–39).

Efflux from Brain

The half-time disappearance from brain was 14.1 min for ^{125}I-[Ser^{8}]GLP-1 and 14.5 min for ^{99m}Tc-albumin. This indicates the absence of a brain-to-blood transport system for ^{125}I-[Ser^{8}]GLP-1. These results are shown in Fig. 3.

Octanol/PBS Partition Coefficient

The lipid solubility was determined by octanol/PBS partition coefficient, and calculated as the radioactivity in the octanol phase divided by that in the PBS phase. The octanol/PBS partition coefficient for ^{125}I-[Ser^{8}]GLP-1 was 0.0238 ± 0.0032.

Discussion

As GLP-1 has great potential in the treatment of diabetes mellitus by inducing satiety as well as

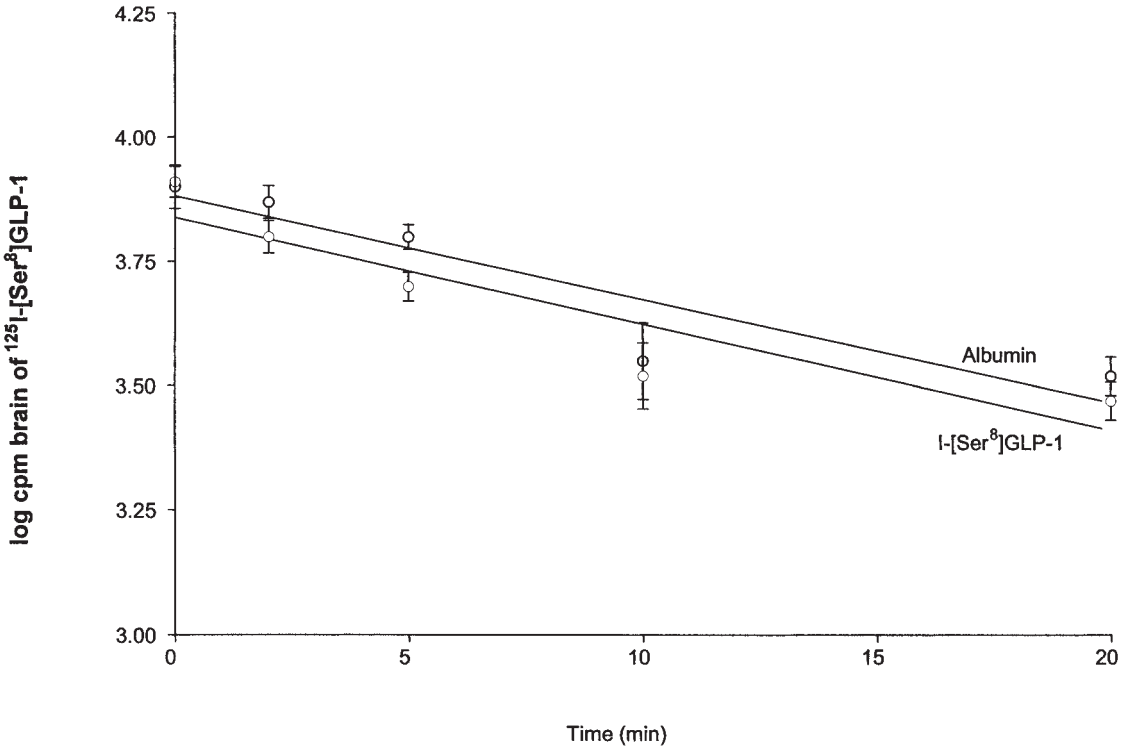

Fig. 3. Efflux: ^{125}I-[Ser^{8}]GLP-1 and ^{99m}Tc-albumin exited the brain at similar rates, indicating the lack of interference of efflux with quantification of the influx rate of ^{125}I-[Ser^{8}]GLP-1.

decreasing insulin requirements, and as GLP-1 affects the CNS after peripheral administration by a possible action in the arcuate nucleus inside the BBB, it is important to identify how the BBB interacts with GLP-1. A stable analog, [Ser^{8}]GLP-1, was used in this study. [Ser^{8}]GLP-1 retains the insulinotropic bioactivity of GLP-1, and this biological activity is inhibited by the GLP-1 receptor antagonist exendin(9–39). This indicates that [Ser^{8}]GLP-1 binds to the same receptor despite the single amino acid substitution (Ritzel et al., 1998). ^{125}I-[Ser^{8}]GLP-1 had a rapid transfer rate of nearly 9×10^{-4} mL/g-min into brain, as compared with several other peptides we have studied by the same method. Furthermore, the lipid solubility of ^{125}I-[Ser^{8}]GLP-1 was low. For instance, the octanol/PBS partition coefficient of orexin A is ten times higher, yet orexin A enters the brain only by passive diffusion (Kastin and Akerstrom, 1999b). The fast entry rate with relatively low lipophilicity could suggest the presence of a transport system for [Ser^{8}]GLP-1.

Nevertheless, studies both in vivo and by *in situ* brain perfusion failed to show a saturable transport system for [Ser^{8}]GLP-1 at the BBB. In the multiple-time regression analysis, the influx of radiolabeled [Ser^{8}]GLP-1 was not inhibited by excess [Ser^{8}]GLP-1, GLP-1 or the receptor antagonist exendin(9–39). Other ingestive peptides tested also failed to modulate the rate of influx. The lack of inhibition was not due to metabolic degradation, interaction with blood components, or self-aggregation, as *in situ* brain perfusion studies, using blood-free perfusion buffer, also did not show saturation. The lack of inhibition also was not due to a rapid efflux transport system, as [Ser^{8}]GLP-1 exited the brain at the same rate as albumin, indicating that it follows reabsorption of the CSF by bulk flow. Therefore, the blood-to-brain influx of [Ser^{8}]GLP-1 probably occurs by passive diffusion affected by physicochemical properties other than lipophilicity.

We did observe a high degree of reversible vascular association of ^{125}I-[Ser^{8}]GLP-1 with endothelial cells composing the BBB. This high degree of vascular association seen by capillary depletion would contribute to the volume of distribution of [Ser^{8}]GLP-1 in brain and therefore the relatively low influx rate by *in situ* brain perfusion. This high reversible binding indicates that the actual influx may have been much lower, although

still greater than that of ^{99m}Tc-albumin or some, but not all (Kastin and Akerstrom, 1999), ingestive peptides entering by passive diffusion, including NPY (Kastin and Akerstrom, 1999a), orexin A (Kastin and Akerstrom, 1999b), MCH (Kastin et al., 2000), and AgRP (Kastin et al., 2000).

The large proportion of reversible interaction with the vasculature suggests a unique role of the BBB in controlling the duration of action of a peptide. The capillary endothelium interacts with GLP-1 predominantly by way of dipeptidyl peptidase IV (DPP IV), which rapidly inactivates GLP-1 by amino terminal cleavage (Hansen et al., 1999). This indicates that the amount of [Ser^8]GLP-1, as well as GLP-1, would be rapidly degraded by DPP IV in the cerebral vasculature, possibly to different degrees in different regions (Dauch et al., 1993; Kastin et al., 2001), thus effectively terminating its biological activity. In this case, the nature of simple diffusion rather than saturable transport for GLP-1 would be advantageous when GLP-1 is delivered into blood in large doses, as more GLP-1 could escape enzymatic degradation and reach the brain in the intact form.

By *in situ* brain perfusion, the influx of GLP-1 was almost eight times greater than that of [Ser^8]GLP-1. This may reflect physicochemical differences in the molecules, although the intrinsic nature of the perfusion procedure may effectively washout any peptide loosely associated with the vasculature. It raises the possibility that even though the analog is much more stable in blood, its high degree of loose association within brain capillaries after iv injection may not necessarily make it more effective in reaching brain parenchyma.

In summary, [Ser^8]GLP-1 in brain capillaries not only had a relatively high amount of reversible binding with the endothelial cells, but also crossed the BBB completely and rapidly to reach brain parenchyma by passive diffusion. Although the degree of permeation across the BBB may not be the rate-limiting factor for biological activity, sufficient amounts of peptide may be required to saturate the endothelial enzymes that rapidly degrade the peptide. This suggests that inhibition of the degrading enzyme DPP IV or high doses of GLP-1 to saturate the enzyme should enhance the penetration of GLP-1 across the BBB.

Acknowledgments

Supported by the Department of Veterans Affairs and NIH (DK54880).

References

Banks W. A., Fasold M. B., and Kastin A. J. (1997) Measurement of efflux rates from brain to blood, in *Methods of Molecular Biology, Neuropeptide Protocols*, Vol. 73 (Irvine G. B. and Williams C. H., eds.), Humana Press Inc., Totowa, NJ, pp. 353–360.

Banks W. A. and Kastin A. J. (1989) Quantifying carrier-mediated transport of peptides from the brain to the blood, in *Methods in Enzymology*, Vol. 168, Conn, P. M. (ed.), Academic Press, San Diego, pp. 652–660.

Creutzeldt W. O. C., Kleine N., Willms B., Orskov C., Holst J. J., and Nauck M. A. (1996) Glucagonostatic actions and reduction of fasting hyperglycemia by exogenous glucagon-like peptide 1 (7–36) amide type 1 diabetic patients. *Diabetes Care* **19,** 580–586.

Dauch P., Masuo Y., Vincent J. P., and Checler F. (1993) A survey of the cerebral regionalization and ontogeny of eight exo- and endopeptidases in murines. *Peptides* **14,** 593–599.

Elias C. F., Kelly J. F., Lee C. E., et al. (2000) Chemical characterization of leptin-activated neurons in the rat brain. *J. Comp. Neurol.* **423,** 261–281.

Flint A., Raben A., Astrup A., and Holst J. J. (1998) Glucagon-like peptide 1 promotes satiety and suppresses energy intake in humans. *J. Clin. Invest.* **101,** 515–520.

Goldstone A. P., Mercer J. G., Gunn I., et al. (1997) Leptin interacts with glucagon-like peptide-1 neurons to reduce food intake and body weight in rodents. *FEBS Lett.* **415,** 134–138.

Gutzwiller J.-P., Drewe J., Goke B., et al. (1999) Glucagon-like peptide-1 promotes satiety and reduces food intake in patients with diabetes mellitus type 2. *Am. J. Physiol.* **276,** R1541–R1544.

Hansen L., Deacon C. F., Orskov C., and Holst J. J. (1999) Glucagon-like peptide-1-(7-36)amide is transformed to glucagon-like peptide-1-(9-36)amide by dipeptidyl peptidase IV in the capillaries supplying the L cells of the porcine intestine. *Endocrinology* **140,** 5356–5363.

Jin S. L., Han V. K., Simmons J. G., Towle A. C., Lauder J. M., and Lund P. K. (1988) Distribution of glucagon-like peptide I (GLP-I), glucagon, and glicentin in the rat brain: an immunocytochemical study. *J. Comp. Neurol.* **271,** 519–532.

Kanse S. M., Kreymann B., Ghatei M. A., and Bloom S. R. (1988) Identification and characterization of glucagon-like peptide-1 7-36 amide-binding sites in the rat brain and lung. *FEBS Lett.* **241,** 209–212.

Kastin A. J. and Akerstrom V. (1999a) Nonsaturable entry of neuropeptide Y into the brain. *Am. J. Physiol.* **276,** E479–E482.

Kastin A. J. and Akerstrom V. (1999b) Orexin A but not orexin B rapidly enters brain from blood by simple diffusion. *J. Pharmacol. Exp. Ther.* **289,** 219–223.

Kastin A. J., Akerstrom V., Hackler L., and Zadina J. E. (2000) Phe^{13},Tyr^{19}-Melanin-concentrating hormone and the blood-brain barrier: role of protein binding. *J. Neurochem.* **74,** 385–391.

Kastin A. J. and Akerstrom V. (1999) Entry of CART into brain is rapid but not inhibited by excess CART or leptin. *Am. J. Physiol.* **277,** E901–E904.

Kastin A. J., Akerstrom V., and Hackler L. (2000) Agouti-related protein (83-132) aggregates and crosses the blood-brain barrier slowly. *Metabolism* **49,** 1444–1448.

Kastin A. J., Hahn K., and Zadina J. E. (2001) Regional differences in peptide degradation by rat cerebral microvessels: a novel regulatory mechanism for communication between blood and brain. *Life Sci.* **69,** 1305–1312.

Kieffer T. J., McIntosh C. H. S., and Pederson R. A. (1995) Degradation of glucose-dependent insulinotropic polypeptide and truncated glucagon-like peptide 1 in vitro and in vivo by dipeptidyl peptidase IV. *Endocrinology* **136,** 3585–3596.

Kreymann B., Ghatei M. A., Burnet P., et al. (1989) Characterization of glucagon-like peptide-1-(7–36) amide in the hypothalamus. *Brain Res.* **502,** 325–331.

Larsen P. J., Tang-Christensen M., Holst J. J., and Orskov C. (1997) Distribution of glucagon-like peptide-1 and other preproglucagon-derived peptides in the rat hypothalamus and brainstem. *Neuroscience* **77,** 259–270.

Mercer J. G., Moar K. M., Findlay P. A., Hoggard N., and Adam C. L. (1998) Association of leptin receptor (OB-Rb), NPY and GLP-1 gene expression in the ovine and murine brainstem. *Regul. Pept.* **75–76,** 271–278.

Naslund E., Barkeling B., King N., et al. (1999) Energy intake and appetite are suppressed by glucagon-like peptide-1 (GLP-1) in obese men. *Int. J. Obesity* **23,** 304–311.

Ritzel U., Leonhardt U., Ottleben M., et al. (1998) A synthetic glucagon-like peptide-1 analog with improved plasma stability. *J. Endocrinol.* **159,** 93–102.

Shimizu I., Hirota M., Ohboshi C., and Shima K. (1987) Identification and localization of glucagon-like peptide-1 and its receptor in rat brain. *Endocrinology* **121,** 1076–1082.

Tang-Christensen M., Larsen P. J., Goke R., et al. (1996) Central administration of GLP-1-(7-36) amide inhibits food and water intake in rats. *Am. J. Physiol.* **271,** R848–R856.

Toft-Nielsen M. B., Madsbad S., and Holst J. J. (1999) Continuous subcutaneous infusion of glucagon-like peptide 1 lowers plasma glucose and reduces appetite in type 2 diabetic patients. *Diabetes Care* **22,** 1137–1143.

Tritos N. A., Vincent D., Gillette J., Ludwig D. S., Flier E. S., and Maratos-Flier E. (1998) Functional interactions between melanin-concentrating hormone, neuropeptide Y, and anorectic neuropeptides in the rat hypothalamus. *Diabetes* **47,** 1687–1692.

Turton M. D., O'Shea D., Gunn I., et al. (1996) A role for glucagon-like peptide-1 in the central regulation of feeding. *Nature* **379,** 69–72.