J. Pharm. Pharmacol. 1992, 44: 583–588
Received September 3, 1991

In-vivo Blood-brain Barrier Transport of a Novel Adrenocorticotropic Hormone Analogue, Ebiratide, Demonstrated by Brain Microdialysis and Capillary Depletion Methods

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Abstract—The transport of ebiratide, a novel adrenocorticotropic hormone (ACTH) analogue, [H-Met-(O_2)-Glu-His-Phe-D-Lys-Phe-NH(CH_2)$_8$-NH_2], through the blood-brain barrier was directly demonstrated in-vivo. [^{125}I]Ebiratide (16·9 MBq mL^{-1}) or [^{14}C]sucrose (29·2 MBq mL^{-1}) known to be restrictively transported through the blood-brain barrier was infused into the rat internal carotid artery at a flow rate of 50 μL min^{-1} for 10 min, and after 15 min infusion the distribution volume of each compound in the brain parenchyma was determined by the capillary depletion method. The distribution volume of [^{125}I]ebiratide was 167·8 $\pm$ 62·2 μL (g brain)$^{-1}$, which was about seven times higher than that of [^{14}C]sucrose (24·9 $\pm$ 4·0 μL g brain)$^{-1}$, indicating the uptake of ebiratide into brain parenchymal cells. During the infusion into the internal carotid artery, brain microdialysis was simultaneously performed to directly collect the brain interstitial fluid as the dialysate. Radioactivity was detected in the dialysate during the [^{125}I]ebiratide infusion and HPLC analysis of the dialysate revealed that the intact ebiratide accounted for $\geqslant$80% total radioactivity. The concentrations of [^{125}I]ebiratide and [^{14}C]sucrose in the brain interstitial fluid were estimated based on the relative recovery obtained in the in-vitro recovery study. The brain interstitial fluid/internal carotid arterial blood concentration ratio for [^{125}I]ebiratide was determined to be $1{\cdot}47 \times 10^{-2} \pm 0{\cdot}17 \times 10^{-2}$ and was about eight times higher than that for [^{14}C]sucrose ($1{\cdot}92 \times 10^{-3} \pm 0{\cdot}36 \times 10^{-3}$), indicating significant transport of ebiratide to the brain interstitial fluid. Accordingly, it was demonstrated that ebiratide is taken up into the brain in the intact form possibly via an absorptive-mediated transport through the blood-brain barrier.

Evaluation of the transport of physiologically active peptides into the brain is essential for elucidating the mechanism of onset of their pharmacological effects. However, such evaluation is difficult because of in-vivo instability and poor penetrability through the blood-brain barrier (BBB) (Banks et al 1991).

Ebiratide [H-Met(O_2)-Glu-His-Phe-D-Lys-Phe-NH(CH_2)$_8$-NH_2], newly synthesized by Hoechst AG (Frankfurt, Germany) is a CNS-acting peptide related to the adrenocorticotropic hormone (ACTH) with reduced in-vivo metabolism/degradation and enhanced pharmacological activity. Ebiratide has been reported to have potent neurotrophic effects in rats and mice following peripheral administration (Hock et al 1988), and superior biological stability compared with the native $ACTH_{4-10}$ fragment and the $ACTH_{4-9}$ analogue [H-Met(O_2)-Glu-His-Phe-D-Lys-Phe-NH_2] (Shimura et al 1990), and is under development as an agent for treatment of Alzheimer's disease.

Ebiratide is thought to act directly on the CNS, so it is important to evaluate its transport mechanism into the brain. We have already clarified that ebiratide is taken up into the brain capillary via the basicity-based absorptive-mediated endocytosis in in-vitro experiments using the bovine isolated brain capillary (Shimura et al 1991b) and the primary cultured bovine capillary endothelial cell system (Terasaki et al 1992a). However, investigations using a combination of the brain microdialysis method and the brain perfusion method have succeeded in testing the in-vivo transport of a dynorphin-like analgesic peptide (E-2078) (Terasaki et al 1991). Thus we have confirmed the brain microdialysis method to be a useful strategy to test the in-vivo transport of substrates with relatively low BBB penetrability, such as neuropeptides.

The purpose of the present study is to demonstrate the in-vivo transport of ebiratide into the brain through the BBB by simultaneous application of the capillary depletion method and brain microdialysis method using a trans-cranial type cannula.

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Materials and Methods

Animals

Male Wistar rats (Sankyo Laboratory Co., Toyama, Japan), 190–260 g, were used in the study. The animals had free access to food and water.

Chemicals

Ebiratide (Fig. 1) was synthesized at Hoechst AG (Germany). Na^{125}I, [U-^{14}C]sucrose (185 MBq mmol^{-1}) and Protosol (tissue solubilizer) were purchased from New England Nuclear Corp, Boston, MA, USA. Clear-sol (liquid scintillation fluid) and Ketaral 50 (ketamine HCl) were supplied by Nacalai Tesque Inc., Kyoto, Japan and Sankyo Co. Ltd, Tokyo, Japan, respectively. Dextran (industrial

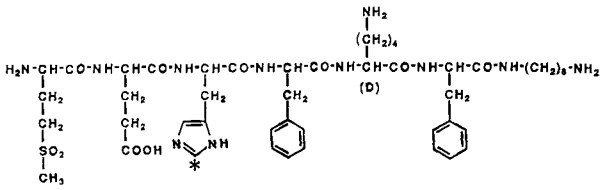

FIG. 1. Structure of ebiratide. *Represents ^{125}I-labelled position.

grade, mol wt. 71 500), bovine serum albumin (Fraction V) and xylazine HCl were obtained from Sigma Chemical Co. (St Louis, MO, USA). All other chemicals were of reagent grade.

Radioiodination of ebiratide

Ebiratide was labelled with ^{125}I by a previously reported method (Shimura et al 1991b) adopting the chloramine T method (Hunter & Greenwood 1962) except for the purification of [^{125}I]ebiratide which was obtained from the reaction mixture by HPLC. The HPLC conditions were: column, VYDAC 218TP5415 (The Separations Group Co. Ltd, Hasperia, CA, USA); eluent, water: acetonitrile: trifluoroacetic acid (80:15:0·1); flow rate, 2·0 mL min^{-1}; radiodetector (Model-170, Beckman Co. Ltd, Palo Alto, CA, USA). The obtained [^{125}I]ebiratide had a sp. act. of about 37 TBq g^{-1} and a chemical purity of $\geq$95%.

Infusion into rat internal carotid artery

The brain perfusion method of Takasato et al (1984) was used with slight modification. The right common carotid artery of male rats was exposed under ketamine-xylasine anaesthesia (235 and 2·3 mg kg^{-1}, respectively). A polyethylene tube (SP-10, Natsume Seisakusho Ltd, Tokyo, Japan) filled with heparin (100 int. units mL^{-1}) was canulated into the external carotid artery and used as a catheter for infusion into the internal carotid artery. The pterygopalatine, occipital and superior arteries were cauterized.

[^{125}I]Ebiratide or [^{14}C]sucrose was added to a buffer (comprising (mM): NaCl 141, KCl 4·0, $CaCl_2$ 2·8, $MgSO_4$ 1·0, HEPES 10, D-glucose 10 and 0·1% BSA, pH 7·4) oxygenated with 95% O_2–5% CO_2 beforehand, to a concentration of 16·9 MBq mL^{-1}, respectively, and infused at a rate of 50 μL min^{-1} for 10 min. The common carotid artery was not ligated so that the drug solution could be delivered into the brain in the systemic blood flow. After the termination of the infusion and brain microdialysis, blood was collected from the abdominal aorta using a heparinized disposable syringe (Terumo Co. Ltd, Tokyo, Japan), and the brain was isolated. The right hemisphere of the brain was used in the capillary depletion study after removal of the choroid plexus.

Capillary depletion study

After the infusion and brain microdialysis of [^{125}I]ebiratide or [^{14}C]sucrose, the brain was isolated and treated by the capillary depletion technique (Triguero et al 1990; Terasaki et al 1991). [^{125}I]Ebiratide in treated capillary and parenchyma fractions and plasma were determined with a γ-counter (Aloka Co. Ltd, Tokyo, Japan) and subjected to HPLC to separate unchanged ebiratide and metabolites. The (apparent) distribution volume of [^{125}I]ebiratide in each fraction was calculated in terms of the concentration of unchanged ebiratide. In the case of [^{14}C]sucrose, the capillary and parenchyma fractions solubilized with Protosol, and the plasma were mixed with scintillation fluid and subjected to radioactivity determination.

Brain microdialysis study

The brain microdialysis was conducted by a similar procedure to that reported by Kang et al (1990) and Terasaki et al (1991). A brain microdialysis canula of the transcranial type was constructed from a dialysis fibre with a mol. wt cut-off of 12 500 Da and an inner diameter of 200 μm (Lento H.F., Organon Technica Corp., OK, USA) and a stainless steel tubing (i.d. 0·2 mm; MT Giken, Tokyo, Japan). The fibre of the constructed cannula was 4 mm in length. Ringer–HEPES buffer (comprising (mM): NaCl 141, KCl 4·0, $CaCl_2$ 2·8 and HEPES 10, pH 7·4) containing 0·1% BSA and 20 mM K_2 EDTA was perfused through the microdialysis canula at a constant flow rate of 10 μL min^{-1} with a 5 mL Luer-Lok tip 2141 syringe (Beckton-Dickinson, Rutherford, USA) and a syringe infusion pump (Model 22, Harvard Apparatus, South Natick, USA). Here, 0·1%BSA and 20 mM K_2 EDTA were added to Ringer–HEPES buffer in order to prevent the adsorption of ebiratide to the dialysis fibre and to inhibit the metabolism of ebiratide in the dialysate, respectively.

After determination of the in-vitro relative recovery (see in-vitro recovery study), the microdialysis fibre was implanted in the hippocampus of rats anaesthetized with ketamine and xylazine (235 and 2·3 mg kg^{-1}, respectively) as follows. A 1·0 mm hole was made on both sides of the skull at 3·4 mm posterior to the bregma and 3·5 mm below the dura using a dental drill (JP-30, Takamiya Dental MFG Ltd, Tokyo, Japan). The microdialysis canula was passed through the holes and fixed with dental cement. The rats with the microdialysis fibre were allowed free access to food and water for 48 h and then used in the microdialysis study.

During perfusion of the dialysate through the microdialysis canula, the oxygenated buffer containing [^{125}I]ebiratide (16·9 MBq mL^{-1}) or [^{14}C]sucrose (29·2 MBq mL^{-1}) was infused through the infusion catheter fixed to the external carotid artery at a flow rate of 50 μL min^{-1} (see Infusion into rat internal carotid artery). The perfused dialysate was collected at 5 min intervals up to 25 min after the start of the infusion. From the collected volume of the dialysate, the flow rate was estimated and the radioactivity was determined with a γ-counter for [^{125}I]ebiratide and with a liquid scintillation counter for [^{14}C]sucrose.

In-vitro recovery study

Ringer–HEPES buffer containing 20 mM K_2 EDTA, 0·1% BSA and [^{125}I]ebiratide (0·29 MBq) or [^{14}C]sucrose (0·28 MBq) was used as the reservoir solution. The dialysis fibre was placed into 20 mL of reservoir solution kept at 37°C, and the dialysis was carried out with the same reservoir solution without the labelled compound at a constant flow rate of 10 μL min^{-1}. The dialysate was collected every 10 min and its radioactivity was determined with a γ-counter for [^{125}I]ebiratide and with a liquid scintillation counter for [^{14}C]sucrose. The in-vitro relative recovery of each fibre was estimated from the dialysate-to-reservoir solution concentration ratio.

HPLC analysis

Separation of the unchanged compound and metabolites of [^{125}I]ebiratide was performed by HPLC. The dialysate (pooled 0 to 10 min) for each rat and the methanol extract of the sample obtained in the capillary depletion study were injected into an HPLC column in a volume of about 60 μL per sample. The HPLC conditions were column, VYDAC 214TP104 (The Separations Group Co. Ltd, Hasperia, CA, USA); eluent, an 8:1 mixture of 1% phosphoric acid and acetonitrile adjusted to pH 3·0 with triethylamine; and flow rate, 1·0 mL min^{-1}. The eluate was obtained in 0·5 mL fractions using a fraction collector (FRAC-100, Pharmacia LKB Biotechnology Inc, Uppsala, Sweden) and the radioactivity of each fraction was counted with a γ-counter.

Data calculation

The apparent volumes of distribution (Vd_{app}) in the capillary and parenchyma fractions were calculated by the following equation:

$$Vd_{app} = \frac{\text{d min}^{-1} \text{ in capillary fraction or parenchyma fraction (g organ)}^{-1}}{\text{d min}^{-1}\ \mu\text{L}^{-1} \text{ plasma}}$$

The radioactivities of [^{125}I]ebiratide and [^{14}C]sucrose in the dialysate were converted into the concentrations in the brain interstitial fluid (ISF) using the following methods. Each method is described in detail in our previous reports (Deguchi et al 1991; Terasaki et al (1992b).

The recovery method. Assuming that the in-vivo microdialysis recovery is the same as that in-vitro, the concentration of a drug in the brain ISF (C_{isf}) can be obtained as follows.

$$C_{isf} = C_{d,vivo}\ (C_r/C_d) \quad (1)$$

where $C_{d,vivo}$ is the concentration in the dialysate obtained by the microdialysis, and C_d and C_r are the dialysate and reservoir concentrations in the in-vitro recovery study, respectively.

The reference method. To correct the difference between the in-vivo and in-vitro microdialysis efficiencies, antipyrine was used as the in-vivo reference compound (Terasaki et al 1992b).

The in-vitro and in-vivo permeability rate constants (PA_{vitro} and PA_{vivo}) were determined by the following equations:

$$CL_{vitro} = F \cdot C_d/C_r = F[1\text{-}\exp(-PA_{vitro}/F)] \quad (2)$$

$$CL_{vivo} = F \cdot C_d/C_{isf} = F[1\text{-}\exp(-PA_{vivo}/F)] \quad (3)$$

where CL_{vitro} and CL_{vivo} are the in-vitro and in-vivo dialysis clearances, respectively, C_{isf} is the concentration of the unbound reference compound in the ISF and F is the dialysis flow rate. The effective dialysis coefficient (R_d), which is the ratio of the in-vivo to in-vitro permeability rate constants of the dialysis fibre, was defined as follows:

$$R_d = PA_{vivo}/PA_{vitro} \quad (4)$$

Here, the R_d value was cited from the previously reported value (0·389) for antipyrine as the reference compound in the brain (Terasaki et al 1992b). Assuming that $R_{d,drug}$ is equal to $R_{d,ref}$, the equation to extrapolate the concentration of the unbound drug in the brain ISF, C_{isf}, is as follows:

$$C_{isf} = C_{d,vivo}/[1\text{-}\exp(-R_{d,ref} \cdot PA_{vitro}/F) \quad (5)$$

The concentration ratios of the brain ISF to the blood in the internal carotid artery for [^{125}I]ebiratide and [^{14}C]sucrose were calculated as follows:

$$\text{Interstitial/blood} = \frac{C_{isf}\ (\text{d min}^{-1}\ \text{mL}^{-1})}{\text{Blood concentration in internal carotid artery (d min}^{-1}\ \text{mL}^{-1})}$$

where [^{125}I]ebiratide and [^{14}C]sucrose concentrations in the internal carotid arterial blood were calculated from the blood flow rate at the carotid artery (0·6 mL min^{-1} (Takasato et al 1984)) and the infusion rate of the drug solution (50 μL min^{-1}).

Results

Capillary depletion study

Table 1 shows the Vd_{app} of [^{125}I]ebiratide and [^{14}C]sucrose in the rat brain parenchyma and capillary after the termination of the infusion. The parenchyma Vd_{app} for [^{125}I]ebiratide was about 35 times larger than the capillary Vd_{app} and about 7 times larger than the parenchyma Vd_{app} for [^{14}C]sucrose.

Brain microdialysis study

The time-courses of radioactivities in the dialysate during and after the infusion of [^{125}I]ebiratide or [^{14}C]sucrose into the rat internal carotid artery are shown in Fig. 2. The transfer of [^{125}I]ebiratide to the dialysate was faster than that of [^{14}C]sucrose, and the fractional activity of [^{125}I]ebiratide detected in the dialysate was greater than that of [^{14}C]sucrose. HPLC analysis of the [^{125}I]ebiratide in the dialysate during the infusion of [^{125}I]ebiratide revealed that $\geq$80% of the compound remained as the intact form (Fig. 3).

The ebiratide and sucrose concentrations in the brain ISF during the infusion were estimated from those in the perfusate obtained in the brain microdialysis study using the in-vitro permeability rate constant of the dialysis fibre (PA_{vitro}) in the in-vitro recovery study. As the PA_{vitro} values for [^{125}I]ebiratide and [^{14}C]sucrose were $9{\cdot}94 \times 10^{-2} \pm 0{\cdot}32 \times 10^{-2}$ and $19{\cdot}51 \times 10^{-2} \pm 0{\cdot}37 \times 10^{-2}$ μL min^{-1} (mean $\pm$ s.e., n = 4), respectively, the individual brain ISF concentrations (C_{isf}) became $1{\cdot}19 \times 10^6 \pm 0{\cdot}14 \times 10^6$ and $2{\cdot}80 \times 10^5 \pm 0{\cdot}53 \times 10^5$ d min^{-1} mL^{-1} (mean $\pm$ s.e., n = 4), respectively. Accordingly, the concentration ratios of [^{125}I]ebiratide and [^{14}C]sucrose in the ISF to those in the

Table 1. Apparent distribution volume of [^{125}I]ebiratide and [^{14}C]sucrose in the rat brain hemisphere at 15 min after infusion into internal carotid artery for 10 min at a rate of 50 μL min^{-1}.

	Vd_{app} (μL g^{-1})	
Compound	Parenchyma	Capillary
[^{125}I]Ebiratide	167·8 ± 62·2	4·8 ± 2·7
[^{14}C]Sucrose	24·9 ± 4·0	6·2 ± 1·8

The value represents the mean ± s.e. of 4 animals.

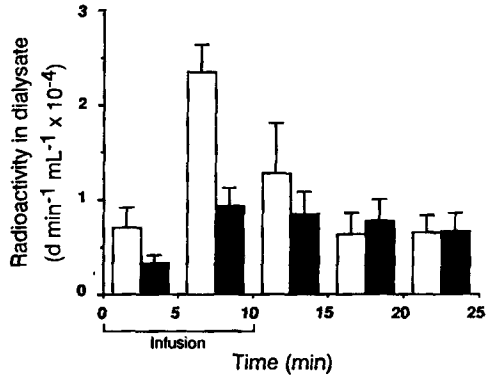

FIG. 2. Time courses of [^{125}I]ebiratide (□) and [^{14}C]sucrose (■) concentrations in the dialysate during and after the infusion of [^{125}I]ebiratide or [^{14}C]sucrose into the internal carotid artery for 10 min at a rate of 50 μL min^{-1}. The dialysate was collected every 5 min for 25 min for sampled volume measurement and radioactivity counting. The values were correlated by each dose. Each bar represents the mean ± s.e. of 4 animals.

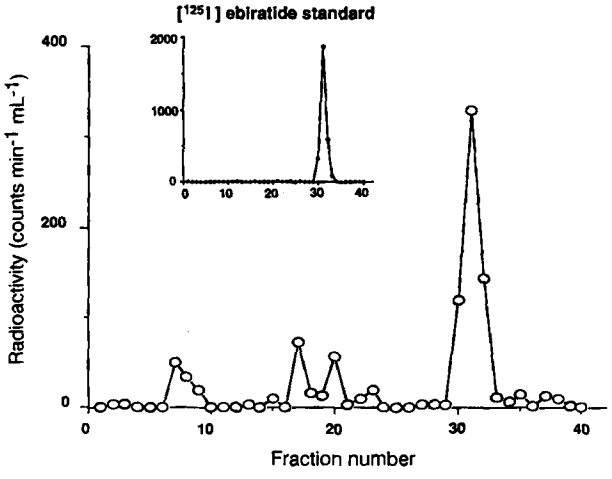

FIG. 3. HPLC of [^{125}I]ebiratide in the dialysate during the infusion of [^{125}I]ebiratide into the internal carotid artery for 10 min at a rate of 50 μL min^{-1}. HPLC analysis is described under Materials and Methods. The HPLC of the standard sample of [^{125}I]ebiratide is also shown.

internal carotid arterial blood (the blood flow at the carotid artery taken as 0·6 mL min^{-1} (Takasato et al 1984)) indicated that the brain ISF/blood concentration ratio for ebiratide was significantly ($P < 0.01$) greater, 8 times that for sucrose (Table 2).

Table 2. Comparison of the brain interstitial fluid (ISF) with internal carotid artery blood concentration ratio between [^{125}I]ebiratide and [^{14}C]sucrose in the brain microdialysis study.

	[^{125}I]Ebiratide	[^{14}C]Sucrose
ISF/blood		
A	$1.47 \times 10^{-2} \pm 0.17 \times 10^{-2}$	$1.92 \times 10^{-3} \pm 0.36 \times 10^{-3}$
B	$4.24 \times 10^{-2} \pm 0.38 \times 10^{-2}$	$7.64 \times 10^{-3} \pm 1.57 \times 10^{-3}$

The concentrations of [^{125}I]ebiratide and [^{14}C]sucrose in ISF were calculated by the recovery method and the reference method (see Materials and Methods). There are significant differences in the concentration ratio between [^{125}I]ebiratide and [^{14}C]sucrose ($P < 0.01$, Student's t-test). The value represents the mean ± s.e. of 4 animals.

Discussion

Ebiratide is a peptide with basic amino acids, His and D-Lys, and an octylamino group in its structure and shows strong basicity with an isoelectric point of 10 determined by the chromatofocusing method (Shimura et al 1991b). BBB transport mechanisms for such basic peptides include absorptive-mediated endocytosis which is internalization of peptides in the endothelial cells by electrochemical interaction between peptides and capillary endothelial cells. This has been reported for cationized albumin and β-endorphin-cationized albumin chimeric peptide (Kumagai et al 1987), dynorphin-like analgesic peptide E-2078 (Terasaki et al 1989) and histone (Pardridge et al 1989). Ebiratide has also been proved to be internalized in brain capillaries via absorptive-mediated endocytosis in in-vitro studies using isolated bovine brain capillaries (Shimura et al 1991b) and primary cultured bovine brain capillary endothelial cells (Terasaki et al 1992a). In those reports, however, it was difficult to clarify the transport of ebiratide through the BBB although the internalization mechanism of ebiratide in the brain capillary endothelial cells was demonstrated. These in-vitro experimental systems were suited to the investigation of the uptake mechanism of a compound into the BBB but restricted for the investigation of the permeation of a compound through the BBB. In the present study, therefore, we aimed to demonstrate directly the in-vivo transport of ebiratide into the brain parenchyma and ISF by the capillary depletion and the brain microdialysis techniques.

The time to conduct capillary depletion (15 min after the termination of infusion) was decided by taking into consideration the internalization velocity of ebiratide into the BBB (Shimura et al 1991b; Terasaki et al 1992a) and the wash-out of ebiratide with the blood flow.

The distribution volume obtained by the capillary depletion technique indicates the degree of transport into the brain. The Vd_{app} for [^{125}I]ebiratide was about 7 times higher than that for [^{14}C]sucrose, suggesting that [^{125}I]ebiratide was taken up into brain capillaries via absorptive-mediated endocytosis (Table 1). Here, [^{125}I]ebiratide in the parenchyma and capillary fractions was analysed by HPLC to obtain the Vd of the unchanged compound since [^{125}I]ebiratide undergoes metabolism in the body. There were large fluctuations in the Vd for the unchanged compound due to the metabolism and degradation of ebiratide caused by intracellular enzymes during the preparation of brain homogenates. It was suggested that in the case of compounds such as peptides which are unstable in the body, the application of the capillary depletion method is restricted owing to the metabolism and degradation process. However, the results obtained by the capillary depletion technique and the already reported internalization of ebiratide in brain capillaries suggested that ebiratide is taken up into brain capillaries via absorptive-mediated endocytosis and then transported into the brain parenchymal cells following exocytosis. In order to confirm this, the brain microdialysis study was conducted.

In the brain microdialysis study, a trans-cranial type canula was implanted into the hippocampus (Kang et al 1990; Terasaki et al 1991). The hippocampus, which appears to play a significant role in learning and memory function (Horel 1978) and is a capillary-rich region, is considered to be important in the onset of the antidementia effect of ebiratide. Implantation of a dialysis fibre in the hippocampus has been reported to cause no significant impairment of BBB function using α-aminoisobutyrate as a marker (Benveniste et al 1984). In our study, [^{14}C]sucrose employed as a marker of nonspecific penetration through the BBB showed a low brain ISF/blood concentration ratio ($1{\cdot}92\times10^{-3}\pm0{\cdot}36\times10^{-3}$), which was almost comparable to the value previously obtained using the combination of brain microdialysis and brain perfusion technique ($2{\cdot}7\times10^{-3}\pm1{\cdot}4\times10^{-3}$ (Terasaki et al 1991)). These results enabled us to eliminate the possibility of fibre implantation-caused damage from the evaluation of ebiratide transport into the brain. Furthermore, the administration route in the present study, infusion of [^{125}I]ebiratide and [^{14}C]sucrose into the internal carotid artery, is superior to the brain perfusion method (Takasato et al 1984; Zlokóvic et al 1988) in that physiological conditions are maintained; changes in body fluid volume can be ignored because of the slow infusion rate (50 μL min^{-1}) and because the drug solutions are delivered in the blood to brain capillaries. In the case of [^{14}C]sucrose, damage to the BBB was negligible after 10 min infusion, indicating that long term administration is possible. By this administration route, however, there is a possibility of drug circulating throughout the whole body one or more times, which may result in overestimation of the drug amount transported into the brain. In rats, the apparent volume of distribution and plasma half-life of ebiratide, the test compound in the present study, are 1·7 L kg^{-1} and 10·0 min, respectively (Shimura et al 1991a). From these values the blood ebiratide concentration after systemic circulation was estimated to be about 1/1000 of that at administration, which made it possible to disregard the probable overestimate of the uptake of ebiratide into the brain after systemic circulation.

On the other hand, sucrose is considered to be transferred into the brain after one or more systemic circulations because of its delayed metabolism. However, the radioactivity of [^{14}C]sucrose was retained in the dialysate even after the termination of the infusion (Fig. 2). The difference between ebiratide and sucrose in the metabolic rate results in underestimation of the ebiratide transfer into the brain.

[^{125}I]Ebiratide was transported to the brain ISF faster than [^{14}C]sucrose (nonspecific permeation) and disappeared more rapidly. [^{125}I]Ebiratide in the brain ISF is thought to have been transported through the BBB since [^{125}I]ebiratide appeared in the brain dialysate immediately, transfer of [^{125}I]ebiratide to the rat CSF was hardly observed even at 30 min after intravenous infusion (data not shown), and the surface area of the BBB is about 5000 times greater than that of the blood-CSF barrier (Pardridge 1983).

[^{125}I]Ebiratide and [^{14}C]sucrose concentrations in the brain ISF shown in Table 2A were calculated using the in-vitro relative recovery obtained from the concentration ratio of the dialysate to the reservoir solution in the in-vitro recovery study, on the assumption that the in-vivo microdialysis recovery is the same as the in-vitro recovery. In Table 2B, we made some corrections for parameters such as the diffusion difference between in-vitro and in-vivo clearances ascribed to the existence of cells (the reference method). We have previously reported that a definite relation (effective dialysis coefficient R_d) exists between the in-vitro and in-vivo clearances irrespective of the molecular weight of the compounds (Deguchi et al 1991). Calculation with the brain mean R_d value ($R_d=0{\cdot}389$) for antipyrine according to the report of Terasaki et al (1992b) gave a brain ISF concentration for [^{125}I]ebiratide of $3{\cdot}45\times10^{6}\pm0{\cdot}31\times10^{6}$ d min^{-1} mL^{-1} and that for [^{14}C]sucrose $1{\cdot}12\times10^{6}\pm0{\cdot}23\times10^{6}$ d min^{-1} mL^{-1}; the brain ISF/internal carotid arterial blood concentration ratios were $4{\cdot}24\times10^{-2}\pm0{\cdot}38\times10^{-2}$ and $7{\cdot}64\times10^{-2}\pm1{\cdot}57\times10^{-2}$ for [^{125}I]ebiratide and [^{14}C]sucrose, respectively (Table 2B).

In conclusion, we have provided support for the hypothesis that ebiratide is transported through the BBB and taken up into the brain in the intact form. These findings strongly suggest that the effect of ebiratide on improvement of learning and memory impairments is induced by its transfer into the CNS from the systemic circulation.

References

Banks, W. A., Kastin, A. J., Barrera, C. M. (1991) Delivery peptides to the central nervous system: dilemmas and strategies. Pharm. Res. 8: 1345–1350

Benveniste, H., Drejer, J., Shousboe, A., Diemer, N. H. (1984) Elevation of the extracellular concentration of glutamate and aspartate in rat hippocampus during transient cerebral ischemia monitored by intracerebral microdialysis. J. Neurochem. 43: 1369–1374

Deguchi, Y., Terasaki, T., Kawasaki, S., Tsuji, A. (1991) Muscle microdialysis as a model study to relate the drug concentration in tissue interstitial fluid and dialysate. J. Pharmacobio-Dyn. 14: 483–492

Hock, F. J., Gerhards, H. J., Wiemer, G., Usinger, P., Geiger, R. (1988) Learning and memory process of an $ACTH_{4-9}$ analog (Ebiratide; Hoe 427) in mice and rats. Peptides 9: 575–581

Horel, J. A. (1978) The neuroanatomy of amnesia. A critique of the hippocampal memory hypothesis. Brain 101: 403–445

Hunter, W. M., Greenwood, F. C. (1962) Preparation of iodine-131 labelled human growth hormone of high specific activity. Nature 194: 495–496

Kang, Y. S., Terasaki, T., Tsuji, A. (1990) Dysfunction of choline transport system through blood-brain barrier in stroke-prone spontaneously hypertensive rats. J. Pharmacobio-Dyn. 13: 10–19

Kumagai, A. K., Eisenberg, J. B., Pardridge, W. M. (1987) Absorptive-mediated endocytosis of cationized albumin and a β-endorphin-cationized albumin chimeric peptide by isolated brain capillaries. J. Biol. Chem. 262: 15214–15219

Pardridge, W. M. (1983) Brain metabolism: a perspective from the blood-brain barrier. Physiol. Rev. 63: 1481–1535

Pardridge, W. M., Triguero, D., Busiak, J. (1989) Transport of histone through the blood-brain barrier. J. Pharmacol. Exp. Ther. 251: 821–826

Shimura, T., Tabata, S., Hayashi, S. (1990) Characterization of an $ACTH_{4-9}$ analog (Ebiratide) in pharmacokinetic behavior. J. Pharmacobio-Dyn. 13: S-86

Shimura, T., Tabata, S., Hayashi, S. (1991a) Brain transfer of a new neuromodulating ACTH analog, ebiratide, in rats. Peptides 12: 509–512

Shimura, T., Tabata, S., Ohnishi, T., Terasaki, T., Tsuji, A. (1991b) Transport mechanism of a new behaviourally highly potent adrenocorticotropic hormone (ACTH) analog, ebiratide, through the blood-brain barrier. J. Pharmacol. Exp. Ther. 258: 459–465

Takasato, Y., Rapoport, S. I., Smith, Q. R. (1984) An in situ brain perfusion technique to study cerebrovascular transport in the rat. Am. J. Physiol. 247: H484–H493

Terasaki, T., Hirai, K., Sato, H., Kang, Y. S., Tsuji, A. (1989) Absorptive-mediated endocytosis of a dynorphin-like analgesic peptide, E-2078, into the blood brain barrier. J. Pharmacol. Exp. Ther. 251: 351–357

Terasaki, T., Deguchi, Y., Sato, H., Hirai, K., Tsuji, A. (1991) In-vivo transport of a dynorphin-like analgesic peptide, E-2078, through the blood-brain barrier: an application of brain microdialysis. Pharm. Res. 8: 815–820

Terasaki, T., Takakuwa, S., Saheki, A., Moritani, S., Shimura, T., Tabata, S., Tsuji, A. (1992a) Absorptive-mediated endocytosis of an adrenocorticotropic hormone (ACTH) analog, ebiratide, into the blood-brain barrier: studies with monolayers of primary cultured bovine brain capillary endothelial cells. Ibid. 9: 529–534

Terasaki, T., Deguchi, Y., Kasama, Y., Pardridge, W. M., Tsuji, A. (1992b) Determination of in vivo steady state unbound drug concentration in the brain interstitial fluid by microdialysis. Int. J. Pharm. in press

Triguero, D., Buciak, J., Pardridge, W. M. (1990) Capillary depletion method for quantification of blood-brain barrier transport of circulating peptides and plasma proteins. J. Neurochem. 54: 1882–1888

Zlokóvic, B. V., Lipovac, M. N., Begley, D. J., Davson, H., Rakic, L. (1988) Slow penetration of thyrotropin-releasing hormone across the blood-brain barrier of an in situ perfused guinea pig brain. Ibid. 51: 252–257