P-Glycoprotein Mediates the Efflux of Quinidine across the Blood-Brain Barrier

HIROYUKI KUSUHARA, HIROSHI SUZUKI, TETSUYA TERASAKI, ATSUYUKI KAKEE, MICHEL LEMAIRE and YUICHI SUGIYAMA

Faculty of Pharmaceutical Sciences, The University of Tokyo, 7-3-1, Hongo, Bunkyo-ku, Tokyo, 113, Japan (H.K., H.S., A.K. Y.S.), Faculty of Pharmaceutical Sciences, Tohoku University, Aramaki aza-aoba, Aoba-ku, Sendai 980–77 Miyagi, Japan (T.T.), and Department of Drug Metabolism and Pharmacokinetics, Novartis Pharma AG, Basel, Switzerland (M.L.)

Accepted for publication July 8, 1997

ABSTRACT

Recent studies suggest that P-glycoprotein located on the blood-brain barrier restricts the brain uptake of its substrates. We examined the role of P-glycoprotein on the restricted entry of quinidine to the brain. Quinidine is a well known inhibitor of P-glycoprotein, although it is not yet clarified whether quinidine is the substrate for P-glycoprotein. Kinetic analysis of the uptake of quinidine into the rat brain after intravenous bolus administration revealed that the net uptake clearance is 25.5 μL/min/g brain. Intravenous administration of SDZ PSC 833, a multidrug resistance modifier, enhanced the net uptake clearance of quinidine by 15.7-fold. In contrast, no enhancement by SDZ PSC 833 was observed for the brain uptake of mannitol, a marker for the passive diffusion across the blood-brain barrier. The elimination of [3H]quinidine from the rat brain after microinjection into the cerebral cortex was inhibited by preadministered unlabeled quinidine and verapamil. In addition, the brain-to-plasma concentration ratio of quinidine at 10 min after intravenous administration was 27.6-fold higher in mdr1a knock-out mice than in control mice. These results suggest that P-glycoprotein mediates the efflux of quinidine across the blood-brain barrier, resulting in its restricted entry to the brain.

The BBB is formed by the tight junction that connects the brain endothelial cells, thus restricting the entry of compounds from the circulating blood to the brain via paracellular route. For hydrophilic compounds, therefore, the entry into the brain is restricted by the BBB. In addition, the brain uptake of some lipophilic compounds has been reported to be restricted (Levin, 1980), prompting many investigators to examine the mechanism. Recent studies have shown that P-gp, which confers MDR to tumor cells, is located on the luminal membrane of the brain capillary endothelial cells and mediates active drug efflux into the systemic circulation across the BBB. The brain uptake of substrates of P-gp (e.g., doxorubicin, vinblastine and cyclosporin A), therefore, is restricted by P-gp-mediated active efflux in both in vivo (Ohnishi et al., 1995; Sakata et al., 1994; Schinkel et al., 1995) and in vitro (Tatsuta et al., 1992; Tsuji et al., 1992, 1993) experiments.

We already reported that the entry to the brain of a basic drug, quinidine, is restricted (Harashima et al., 1985). Because quinidine has a low molecular weight (324) and high lipophilicity, the most likely mechanism would be active efflux into the systemic circulation across the BBB rather than low BBB permeability or low tissue binding in the brain. The facts that quinidine shares common characteristics (high lipophilicity, positive charge and planar structure) as a substrate of P-gp and inhibits the action of P-gp not only in tumor cells but also in brain capillary endothelial cells (Tsuji et al., 1993) support the hypothesis that P-gp mediates the efflux of quinidine across the BBB, resulting in the restricted entry to the brain. In the present study, we examined this hypothesis in vivo using an MDR modifier and mdr1a knock-out mice.

As an MDR modifier, we used SDZ PSC 833, the most potent inhibitor of P-gp (Boesch and Loor, 1994) that is reported to enhance the brain uptake of radiolabeled SDZ PSC 833 and vincristine after intravenous administration to rats (Lemaire et al., 1996). The effect of SDZ PSC 833 on the uptake clearance of quinidine across the BBB was kinetically examined in vivo. In addition, the effects of unlabeled quinidine, SDZ PSC 833 and verapamil on the elimination of [3H]quinidine from the rat brain were examined. The net brain uptake of quinidine was also investigated in mdr1a knock-out mice to evaluate the contribution of P-gp to the efflux of quinidine across the BBB because mdr1a is the dominant subtype of P-gp on the BBB (Jette et al., 1995).

Received for publication December 30, 1996.

ABBREVIATIONS: BBB, blood-brain barrier; P-gp, P-glycoprotein; BEI, brain efflux index; MDR, multidrug resistance; 3-OMG, 3-O-methyl-d-glucose; Par2, parietal cortex area 2; TLC, thin-layer chromatography; HEPES, 4-(2-hydroxyethyl)-1-piperazinethanesulfonic acid.
Materials and Methods

Chemicals. [3H]Quinidine was purchased from American Radiolabeled Chemicals (St. Louis, MO). [14C]Carboxyl-inulin and [3H]mannitol were purchased from New England Nuclear (Boston, MA). [3H]Quinidine and other radioisotopes were stored in a mixture of polyethylene castor oil and ethanol (65:35, w/w), which was kindly supplied by Novartis Pharma AG (Basel, Switzerland) and stored at 4°C until use. SDZ PSC 833 and KZI, which is a mixture of polyethoxylated castor oil and ethanol (65:35, w/w), were kindly supplied by KZI (KZI/saline 50:50, v/v), [3H]quinidine (10 mCi/ml), 0.5% NaHCO3, 1 mM D-glucose, 3 mM KCl, 1.4 mM CaCl2, 1.2 mM MgSO4, 0.4 mM K2HPO4, and 10 mM HEPES, pH 7.4, were injected via a 5-μl microsyringe (Hamilton, Reno, NV) fitted with a needle (330-μm diameter; Seiseido Medical Industry, Tokyo, Japan) into the Par2 region (at 5.5 mm lateral and 4.5 mm deep from bregma as origin). After microinjection of drug into the cerebral cortex, an aliquot of CSF was taken from the cisterna magna at an appropriate time. Immediately after CSF sampling, rats were decapitated, and the left and right cerebrum and cerebellum were removed. Rats were maintained under the anesthesia throughout the experiment with an intramuscular injection of ketamine and xylazine (ketamine 125 mg/kg and xylazine 1.22 mg/kg). The excised cerebrum or cerebellum was weighed. The BEI was defined as the percentage of drug eliminated from the brain relative to the amount of ligand microinjected into the brain. The 100 – BEI (%) that represents the remaining percentage of drug in the ipsilateral cerebrum is described by the following:

\[ \text{BEI} = \left( \frac{\text{Amount of Test Drug Injected}}{\text{Amount of Reference in the Brain}} \right) \times 100 \]  

(5)

where \( V_p(0) \) (ml/g brain) represents the capillary space and rapid adsorption/binding to the vascular surface in the brain. Equation 3 can be rewritten as

\[ K_{p_brain}(t) = \frac{C_p(t)}{C_i(t)} = \frac{AUC_{0-∞}}{C_{i0}} + V_p(0) \]  

(4)

where \( K_{p_brain}(t) \) is the brain-to-plasma concentration ratio at the time of decapitation and has the dimensions of distribution volume (ml/g brain). The slope and y-intercept of \( K_{p_brain}(t) \) against \( AUC_{0-∞} \) at time \( t \) represent \( CL_{br} \) and \( V_p(0) \), respectively. For the present analysis, \( AUC_{0-∞} \) was calculated for each animal by the trapezoidal rule.

To examine the specificity of the effect of SDZ PSC 833, the brain uptake of [3H]mannitol at 5 min after intravenous administration (1 μCi/rat) was determined according to the method described above.

Throughout, the brain contents of mannitol and quinidine are given as the amount of ligands associated with the brain tissue specimen (the sum of the amount of ligand remaining in the cerebral vascular space and that transferred into the brain parenchyma across the BBB). To determine the amount of ligand transported into the brain parenchyma across the BBB, we subtract the amount of ligand remaining in the cerebral vascular space. The reported value for the cerebral vascular space is ~10 μl/g brain (Triguero et al., 1990).

Efflux of [3H]Quinidine from the rat brain after microinjection into the cerebral cortex. Efflux of [3H]quinidine from the rat brain after microinjection into the cerebral cortex was examined using the method reported previously (Kakee et al., 1996). In brief, 0.02 μCi of [3H]quinidine and 0.0005 μCi of [14C]carboxyl-inulin dissolved in 0.5 μl of buffer containing 122 mM NaCl, 25 mM NaHCO3, 10 mM d-glucose, 3 mM KCl, 1.4 mM CaCl2, 1.2 mM MgSO4, 0.4 mM K2HPO4, and 10 mM HEPES, pH 7.4, were injected via a 5-μl microsyringe (Hamilton, Reno, NV) fitted with a needle (330-μm diameter; Seiseido Medical Industry, Tokyo, Japan) into the Par2 region (at 5.5 mm lateral and 4.5 mm deep from bregma as origin). After microinjection of drug into the cerebral cortex, an aliquot of CSF was taken from the cisterna magna at an appropriate time. Immediately after CSF sampling, rats were decapitated, and the left and right cerebrum and cerebellum were removed. Rats were maintained under the anesthesia throughout the experiment with an intramuscular injection of ketamine and xylazine (ketamine 125 mg/kg and xylazine 1.22 mg/kg). The excised cerebrum or cerebellum was solubilized in 2.5 ml of 2 N NaOH at 50°C for 1 hr. The net brain uptake clearance was determined by integration plot analysis (Yamazaki et al., 1993). With this procedure, if the uptake by tissues is measured within a short period after administration during which the efflux of parent drug and metabolites from the tissue compartment is negligible, the amount of ligand in the brain parenchyma at time \( t \) [\( X(t); \text{dpm/g brain} \)] is described by the following differential equation (Patlak et al., 1993; Yamazaki et al., 1993):

\[ \frac{dX(t)}{dt} = CL_{br} \cdot C_p(t) \]  

(1)

where \( CL_{br} (\text{ml/min/g brain}) \) is the uptake clearance defined for the plasma concentration, and \( C_p(t) \) (dpm/ml) is the plasma concentration of ligand. Integration of equation 1 from time zero to time \( t \) yields the following:

\[ X(t) = CL_{br} \cdot AUC_{0-∞} \]  

(2)

where \( AUC_{0-∞} \) (dpm/ml × time) represents the area under the plasma concentration-time curve from time 0 to time \( t \). Because the amount of ligand associated with the brain tissue in vivo [\( Am(t) \); dpm/g brain] is given by the sum of \( X(t) \) and the amount of ligand remaining in the vascular space of the brain, \( Am(t) \) is described by equation 3:

\[ Am(t) = X(t) + V_p(0) \cdot C_p(t) \]  

(3)
inserted to the femoral vein (instead of intracerebral administration due to its low solubility in the buffer) immediately before the microinjection. The residual percentage of [3H]quinidine was determined at 5 and 30 min to determine the $k_p$ values. Inhibition was evaluated by comparing the elimination rate constant with respect to control value.

**Determination of the brain-to-plasma partition coefficient ($K_{p, \text{brain}}$) of quinidine in mdrla knock-out mice.** [3H]Quinidine (10 µCi/mice) and SDZ PSC 833 (10 mg/kg) were dissolved in 200 µl of saline and a mixture of PEG-200, saline and ethanol (PEG-200/saline/ethanol 40:40:20, v/v/v), respectively. One minute after the injection of SDZ PSC 833 via the tail vein, [3H]quinidine was administered to mice via the tail vein. Blood samples (0.6 ml) were collected from the heart at appropriate time after administration. Plasma was separated by centrifugation of blood for 3 min in a refrigerated centrifugation (MRX-152; Tomy, Tokyo, Japan) and stored at −80°C until assayed. Mice was decapitated at 10 min, and the brain was quickly excised, rinsed with saline, weighed and stored at −80°C until assayed. The radioactivity of [3H]quinidine in both plasma and brain was measured as follows.

**Determination of [3H]quinidine concentration.** The separation of [3H]quinidine from its metabolites was carried out as described previously (Harashima et al., 1985) with minor modifications. Fifty microliters of 10 N NaOH and 100 µl of 95% ethanol were added to 200 µl of plasma with gentle swirling. The mixture was extracted with 5 ml of benzene by shaking for 1 hr at room temperature. After centrifugation, a 4-ml aliquot of the benzene extract was used for the assay.

To determine the [3H]quinidine content in the brain, whole brain was homogenized with 1.2 volumes of saline in an Ultra-Turrax T25 (IKA Japan, Kanagawa, Japan). The homogenate was extracted with 5 volumes of benzene by shaking for 1 hr at room temperature. After centrifugation, an aliquot of benzene extract was transferred to a test-tube containing a equal volume of 0.1 N H$_2$SO$_4$ and then shaken for 30 min. After centrifugation, the H$_2$SO$_4$ layer was transferred to another test-tube containing 0.1 volume of 95% ethanol, 0.17 volume of 10 N NaOH and 1.7 volume of benzene and then shaken for 30 min. After centrifugation, an aliquot of the benzene extract was used for the further assay.

The samples were evaporated to dryness under reduced pressure, and then 1 ml of chloroform containing 2 µg of unlabeled quinidine was added to each specimen, and further evaporation was performed. The residue was mixed with 100 µl or 70 µl of chloroform for plasma and brain specimens, respectively. The total radioactivity in 35 and 10 µl of the chloroform solutions for the plasma and brain specimens, respectively, was determined. Then, 35 and 50 µl of the chloroform solutions for plasma and brain specimens, respectively, was spotted onto a TLC plate. The TLC plate was developed at room temperature with methanol/acetone (80:20, v/v) for 40 min and then air-dried. The quinidine spots were confirmed by the blue fluorescence of unlabeled quinidine excited at 365 nm by a UV lamp, then scraped into scintillation vials and solubilized by 2 ml of Soluen-350 at 50°C for 1 hr.

**Radiochemical assay.** To determine the radioactivity, 14 ml of liquid scintillation cocktail (Hionic-fluor; Packard Instruments, Meriden, CT) was added to the sample at room temperature. Radioactive counting was performed using a double-channel system for [3H], and another channel for [14C] samples or a single-channel system for [3H] samples by LC-6000 liquid scintillation counter (Beckman Instruments, Fullerton, CA).

**Results**

**Effect of SDZ PSC 833 on the net brain uptake of [3H]quinidine in vivo.** Figure 1 shows the effect of SDZ PSC 833 (10 mg/kg i.v.) on the time profiles of plasma and brain concentrations of [3H]quinidine after intravenous administration. The total radioactivity in the brain increased 10-fold in the SDZ PSC 833-treated rats compared with the control rats, whereas no statistically significant difference was observed in the plasma-concentration time profiles of both rats. It was confirmed by TLC that the increase in radioactivity was due mainly to an increase in the [3H]quinidine itself and that the metabolism of [3H]quinidine in the brain was minimal; 76% to the applied radioactivity of [3H]quinidine was obtained as intact form after 15-min incubation at 37°C in the 40% brain homogenate. Sixty-four percent was obtained as intact form in the plasma at 10 min after intravenous administration.

The CL$_{br}$ of [3H]quinidine was evaluated by in vivo integration plot analysis in both control and SDZ PSC 833-treated rats (fig. 2). The CL$_{br}$ values were 25.5 ± 5.6 µl/min/g brain in control rats and 400 ± 102 µl/min/g brain (mean ± S.D.) in SDZ PSC 833-treated rats. The administration of SDZ PSC 833 thus enhanced the CL$_{br}$ value of [3H]quinidine.
by 15.7-fold compared with control. In contrast, no enhancement effect of SDZ PSC 833 was observed on the Kp, brain value of [3H]mannitol at 5 min after intravenous administration (table 1).

**Effect of MDR modifiers on the elimination of [3H]quinidine from the brain.** A kinetic analysis of the elimination of quinidine from the brain was performed using the BEI method. Figure 3 shows the elimination curve of [3H]quinidine after microinjection into the cerebral cortex. The k\textsubscript{el} value was 0.023 ± 0.006 min\textsuperscript{-1}. The effects of unlabeled quinidine, SDZ PSC 833 and verapamil on k\textsubscript{el} were examined to investigate whether the elimination process was carrier mediated and P-gp was involved. In the presence of unlabeled quinidine and verapamil, the elimination of [3H]quinidine from the brain was inhibited by 90% and 67%, respectively (table 2). SDZ PSC 833 (10 mg/kg i.v.) reduced the mean k\textsubscript{el} value of [3H]quinidine to 38% of the control, although this difference from control was not statistically significant.

**Determination of the Kp, brain value of quinidine in the brain of FVB mice and mdr1a knock-out mice.** More than 78% and 82% of the radioactivity in the plasma and brain specimens, respectively, were associated with intact [3H]quinidine at 10 min after intravenous administration in both FVB and mdr1a knock-out mice. Figure 4 shows the Kp, brain values of [3H]quinidine at 10 min after administration via the tail vein to control and mdr1a knock-out mice. The enhancement effect of SDZ PSC 833 on the Kp, brain value of [3H]quinidine was observed in control mice, suggesting that the net brain uptake of [3H]quinidine is restricted in FVB mice. The net brain uptake of [3H]quinidine in SDZ PSC 833-treated mice and mdr1a knock-out mice was 10.2- and 27.6-fold greater than that of control, respectively. In contrast, no significant effect of SDZ PSC 833 on Kp, brain value of [3H]quinidine was observed in mdr1a knock-out mice.

**Discussion**

We already reported that although a good correlation was observed in lung, liver, kidney, heart, muscle and adipose tissue (Harashima et al., 1985) between the tissue-to-blood concentration ratios (Kp, values) of quinidine and those of propranolol [a basic, lipophilic drug with a similar molecular weight (259)] after i.v. administration, the brain-to-blood concentration ratio (Kp, brain) of quinidine was 10-fold smaller than that predicted from the correlation. In the present study, we examined the role of P-gp on the BBB in restricting the entry to the brain of quinidine.

An MDR modifier, SDZ PSC 833, enhanced the CL\textsubscript{br} of [3H]quinidine (fig. 2), which is consistent with the hypothesis that P-gp significantly contributes to the efflux of quinidine across the BBB and with the recent study using another MDR modifier, LY-335979 (Wang et al., 1996). In analysis of the data shown in figure 2, we cannot exclude the possibility that the cerebral blood flow rate was affected by the withdrawal of blood from the experimental animals. Using the blood-to-plasma partition coefficient of quinidine (~1.5), the clearance for the uptake into the brain defined for the blood concentration (CL\textsubscript{br, blood}) can be calculated from CL\textsubscript{br}. Because the value for CL\textsubscript{br, blood} was found to be 25 μl/min/g brain in control rats, which is much lower than the cerebral blood flow rate (0.92 ml/min/g brain; Harashima et al., 1985), the net brain uptake of quinidine under control conditions may not be affected by the cerebral blood flow rate. In PSC 833-treated rats, the value of CL\textsubscript{br, blood} was about half of cerebral blood flow rate, suggesting that the brain uptake of quinidine by the treated rats is sensitive to changes in cerebral blood flow rate. Although the absolute value of CL\textsubscript{br} for...
In this method (table 2), although SDZ PSC 833 is a more much potent inhibitor of P-gp than verapamil or quinidine in vitro (Boesch and Loor, 1994). It is possible that this discrepancy can be ascribed to the different route of administration of the modifiers (intravenous for SDZ PSC 833 vs. microinjection into the cerebral cortex for verapamil and quinidine). In fact, the effect of these modifiers should really be compared using the concentration in the cerebral endothelial cells. In contrast to the lack of a significant effect of SDZ PSC 833 on the elimination of quinidine after administration into the cerebral cortex, a remarkable enhancement in the C_{Lp_{e}} of quinidine by SDZ PSC 833 was observed (fig. 1). This discrepancy may be accounted for by the following hypothesis: k_{d}, determined by the BEI method is a parameter governed by the permeability surface area products across the antiluminal and luminal membranes. If we assume (1) that SDZ PSC 833 inhibits the transport across the luminal but not that across the antiluminal membrane and (2) that the transport across the antiluminal membrane is a rate-limiting process, SDZ PSC 833 may not significantly affect the k_{d} value evaluated by the BEI method.

To confirm the contribution of P-gp to the efflux of quinidine across the BBB, a comparison of the K_{p,brain} of quinidine in control (FVB) and mdrla knock-out mice was performed.
Although the presence of two P-gp subclasses, mdr1a (mdr2) and mdr1b (mdr1), which confer MDR to tumor cells, are reported in mice, previous studies that involved the use of a immunohistochemical method and Western immunoblot prepared from the mice brain capillary (Jette et al., 1995; Schinkel et al., 1994) revealed that the dominant subclass located on the BBB is mdr1a. In addition, unlike liver or kidney, the induction of mdr1b on the BBB, which has overlapping substrate specificity with mdr1a, was not observed in mdr1a knock-out mice. Consequently, the \( K_p, \text{brain} \) values of the substrates of P-gp, vinblastine and digoxin are increased considerably in mdr1a knock-out mice (Schinkel et al., 1994, 1995). The mdr1a knock-out mice, therefore, should be a useful tool to study the contribution of P-gp to the ligand efflux across the BBB.

In control mice (FVB), SDZ PSC 833 enhanced the \( K_p, \text{brain} \) value of \(^{3}H\)quinidine (fig. 4). This result suggests that the brain uptake of \(^{3}H\)quinidine in mice is restricted, as already observed in rats. In addition, the \( K_p, \text{brain} \) value of \(^{3}H\)quinidine at 10 min was 27.6-fold higher in mdr1a knock-out mice than in control mice (FVB) (fig. 4). Furthermore, no enhancement effect of SDZ PSC 833 on the net uptake of \(^{3}H\)quinidine was observed in mdr1a knock-out mice (fig. 4). It was thus confirmed that P-gp is the dominant factor responsible for the limited entry of quinidine into the brain in mice. A difference in the brain uptake of quinidine between the SDZ PSC 833-treated control mice and mdr1a knockout-out mice was observed (fig. 4), which may result from the incomplete inhibition of P-gp function.

The results of the present study may have clinical implications; quinidine inhibits membrane potential-dependent \( Na^+ \) channel, resulting in the inhibition of conduction. It also has antimuscarinic and \( \alpha \) adrenergic receptor-blocking properties. In humans, an overdosage of quinidine gives rise to cinchonism such as tinnitus, impaired hearing, visual disturbance, headache, confusion, vertigo and vomiting (Kim and Benowitz, 1990), resulting from the adverse central nervous system effects of quinidine. Because brain concentration of quinidine is increased by SDZ PSC 833, it is possible that such a toxic effect of quinidine is observed by SDZ PSC 833 treatment. In addition, it is possible that MDR modulators affect the brain distribution of P-gp substrates (Lemaire et al., 1996; Wang et al., 1996). In clinical studies, a central nervous system side effect (ataxia) was observed in a patient who was given etoposide (75–100 mg/m\(^2\)/day) and SDZ PSC 833 (12–15 mg/kg/day, continuous infusion) (Boote et al., 1996). It is possible that the increase in the brain concentration of etoposide produced by the administration of SDZ PSC 833 may be related to the appearance of this side effect. In future clinical trials, a drug/drug interaction like this to increase the cerebral concentrations of antitumor drugs (P-gp substrates) by MDR modulators should be considered.

In addition to P-gp, cumulative kinetic evidence suggests the presence of other transporters responsible for the efflux of organic anions across the BBB (Suzuki et al., 1997). Moreover, we found the expression of MDR-associated protein (MRP), a primary active transporter for organic anions, in the immortalized cultured mouse brain endothelial cell line (M spindle cells) by Northern and Western blot analyses. Functional analysis with isolated membrane vesicles from MBE4 cells also suggests the presence of MRP activity (Sugiyama and Suzuki, 1997). Taken together, it is possible that MRP is expressed on the luminal membrane of the cerebral endothelial cells and acts as part of the detoxification system. Another protein overexpressed in P-gp negative MDR cells (lung resistance-related protein) has been cloned and identified as the human major vault protein (Scheffer et al., 1995). Immunohistochemical staining with an antibody revealed the expression of lung resistance-related protein in brain endothelial cells (Izquierdo et al., 1996). Although its function remains to be clarified, it is possible that lung resistance-related protein acts as a detoxification system, because reduced nuclear accumulation of daunorubicin has been reported in the lung resistance-related protein-overexpressing MDR cell line (SW-1573/2R120) (Schuurhuis et al., 1991). Therefore, it is possible that MRP and/or lung resistance-related protein is also expressed on the BBB and acts as part of the detoxification system.

In conclusion, P-gp-mediated active efflux across the BBB restricts the entry of quinidine to the brain, resulting in the lower \( K_p, \text{brain} \) value of quinidine than predicted from the correlation. The active efflux at the BBB takes part in the function of BBB in addition to its anatomic feature (tight junction) and works as the detoxification system in the brain by restricting the entry of exogenous compounds in the circulating blood to the brain.

References


Rushing, D. A., Raber, S. R., Rossow, K. A., Piscitelli, S. C., Plank, G. S. and...


Send reprint requests to: Yuichi Sugiyama, Ph.D., Faculty of Pharmaceutical Sciences, The University of Tokyo, 7–3–1, Hongo, Bunkyo-ku, Tokyo 113, Japan.