Biochemical Pharmacology, Vol. 36, No. 20, pp. 3425–3431, 1987.
Printed in Great Britain.

0006-2952/87 $3.00 + 0.00

INHIBITION OF BLOOD–BRAIN BARRIER PERMEABILITY TO DL-PROPRANOLOL BY SERUM FROM ACUTE RENAL FAILURE RATS*

TSU-HAN LIN, YASUFUMI SAWADA, YUICHI SUGIYAMA, TATSUJI IGA† and MANABU HANANO

Faculty of Pharmaceutical Sciences, University of Tokyo, Tokyo 113, Japan

(*Received* 23 *September* 1986; *accepted* 19 *February* 1987)

Abstract—The effect of uranyl nitrate-induced acute renal failure on the brain uptake of DL-propranolol was investigated in rats with a series of tissue-sampling single-carotid injection techniques. When the buffer solution was used as an injection solution, the brain uptake index (*BUI*), the extraction ratio (E_T), and the blood–brain barrier (BBB) permeability-surface area product (PS_{app}) and PS_u (corrected PS_{app} for the unbound fraction) in uremic rats were significantly lower than those in control rats. These parameters for DL-propranolol were decreased significantly in both control and uremic rats receiving injection of the uremic serum. The PS_u values in both of the control and uremic rats injected with either control or uremic rat serum were significantly higher than those in rats injected with the buffer solution, suggesting the presence of a protein-mediated transport mechanism; that is, the conventional assumption that the fraction of the drug which is available for the uptake *in vivo* is equal to the unbound fraction as measured *in vitro* may not hold. In contrast, the brain extraction of D-[^{14}C]glucose, [^{3}H]inulin and [^{3}H]water, which show no binding to serum protein, was not affected by the coinjection of either control or uremic rat serum. On the other hand, using either the ultrafiltrate from serum (control and uremic) or supernatant fraction from heat-treated serum (control and uremic) as the injection solution, no significant difference in the PS_u value for DL-propranolol was observed between control and uremic serum. These results suggest that (1) the decrease in the PS_u value for DL-propranolol in uremic rats may be attributed mainly to the presence of an endogenous inhibitory substance(s) for the brain uptake or to the decrease in the exchangeable fraction *in vivo* in the uremic serum; (2) the decrease in the PS_u value for DL-propranolol may also be partly attributed to the change in the BBB permeability and/or surface area; (3) the inhibitor for the brain uptake may be characterized as a temperature-sensitive and nonfiltrable substance(s) at physiological pH; and (4) the ability of protein-mediated transport for DL-propranolol into brain was decreased in uremic rats.

Evidence has accumulated that pharmacokinetic and pharmacodynamic parameters of many drugs such as phenytoin, salicylate and propranolol may be changed in patients suffering from renal disease [1–3]. Recent *in vivo* and *in vitro* studies using isolated perfused liver from rats in acute renal failure describe a decreased presystemic biotransformation or hepatic extraction of propranolol due to the presence of an inhibitory factor(s) in the blood of rats [4, 5]. In another study, the uptake rate of propranolol by isolated liver decreased in uremic rats [6]. Kidney dysfunction results in an array of symptoms that may reflect pathophysiological alterations in every organ system [7]. Renal failure affects the uptake and metabolism by the brain of substances such as amino acids [8], inulin and sucrose [9, 10]. There is, however, little other information on drug uptake by the brain during renal failure.

In the present studies, we used DL-propranolol as a model drug to investigate the effect of acute renal failure on the permeability of the blood–brain barrier (BBB) by means of a tissue-sampling single-injection technique via the common carotid artery according to the method of Oldendorf [11].

* This study was supported by a grant-in-aid for Scientific Research provided by the Ministry of Education, Science and Culture of Japan.

† All correspondence should be addressed to: Dr. Tatsuji Iga, Faculty of Pharmaceutical Sciences, University of Tokyo, Hongo, Bunkyo-ku, Tokyo 113, Japan.

MATERIALS AND METHODS

Chemicals

DL-[4-^{3}H]Propranolol hydrochloride (20 Ci/mmol) was purchased from Amersham International plc; other isotopes, [G-^{3}H]inulin (430.6 mCi/g), D-[U-^{14}C]glucose (14.4 mCi/mmol), [^{3}H]water (18 Ci/mmol), and *N*-[1-^{14}C]butanol (1.0 mCi/mmol), Protosol (tissue solubilizer) and Biofluor (scintillation mixture) were purchased from New England Nuclear (Boston, MA). All other reagents were commercially available and of analytical grade.

Induction of acute renal failure.

Male Wistar albino rats (200–300 g) were used. Following the procedure reported by Giacomini *et al.* [12], experimental acute renal failure was induced by a single i.v. administration of uranyl nitrate (5 mg/kg); control rats were given only saline. The rats had free access to tap water and standard laboratory chow except before experiments when they were fasted for 24 hr. Five days after uranyl nitrate treatment, the rats were used for studies.

Preparation of injection solution.

4-(2-Hydroxyethyl)-1-piperazine-ethanosulfonic acid (HEPES)-buffered Ringer's solution, rat serum, and variously treated rat sera were used as the injection solution. HEPES-buffered Ringer's solution (5 mM HEPES) adjusted to pH 7.55 was equilibrated with 95% O_2–5% CO_2 gas to maintain a pH of 7.4. The control serum (CS) and uremic serum (US) were collected from the control (N = 6) and uremic rats (N = 7) respectively. Aliquots of CS and US (both of which were adjusted to pH 7.4) were centrifugally filtered through a semipermeable membrane (MPS 1, Amicon) at 1000 rev/min for 20 min at room temperature (20°), and the ultrafiltrates were collected to use as the injection solutions, CS(F) and US(F), where (F) denotes the ultrafiltrate. Other aliquots of CS and US were boiled for 15 min. These heat-treated sera were allowed to stand to cool and then were stirred slightly with a glass rod before centrifugation (3000 rev/min for 20 min). The supernatant fractions were collected to use as the injection solutions, CS(H) and US(H), where (H) denotes the supernatant. Serum and variously treated rat sera were stored at −40° until use. The injection solution contained 5–10 μCi/ml ^{3}H-test compound (DL-[^{3}H]propranolol, [^{3}H]water or [^{3}H]inulin) and 1–2 μCi/ml [^{14}C]butanol reference in the transport studies of DL-[^{3}H]propranolol, [^{3}H]inulin or [^{3}H]water. The measurement of D-[^{14}C]glucose transport involved an injection solution containing 1–2 μCi/ml D-[^{14}C]glucose and 5–10 μCi/ml [^{3}H]water reference. The pH values of all the injection solutions were adjusted to pH 7.4 immediately before the experiments.

Brain transport studies

The brain transport of DL-[^{3}H]propranolol and other test compounds was measured by the tissue-sampling single-injection technique (brain uptake index method) developed by Oldendorf [11]. The animal was anesthetized with intraperitoneal pentobarbital (62 mg/kg for control rats, 57 mg/kg for uremic rats) 15 min prior to carotid injection of the test substances. In two groups of animals, these doses produced unresponsiveness to painful stimuli with preservation of spontaneous respiration (*ca.* 55–60 breaths/min). The animal was placed in a supine position, and the left common carotid artery was isolated. The test compound and the internal reference compound, a freely diffusible substance, were rapidly injected (< 0.5 sec) via a sharp 27 gauge needle into the common carotid artery as an approximately 200 μl bolus of injection solution. Because the rate of injection (< 0.5 sec) exceeded the rate of carotid blood flow, the injection solution should have traversed the brain microcirculation as a bolus without mixing significantly with the circulating blood [13–15]. The animals were decapitated 15 sec after injection, except in the experiment measuring the extraction time course of DL-[^{3}H]propranolol and [^{14}C]butanol in order to determine their efflux rates. This period was sufficient for a single pass of the bolus through the brain but short enough to minimize the efflux of labeled compound from the brain or the recirculation of labeled compound [16].

Sample analyses

The cerebral hemisphere ipsilateral to the injection was removed from the cranium, solubilized in triplicate in 1.5 ml Protosol (New England Nuclear) at 50° overnight in an incubator, decolorized with 33% H_2O_2 and mixed with 10 ml of Biofluor before double-isotope liquid scintillation counting. An aliquot of injection solution added to control brain tissue was treated similarly. Blood urea nitrogen (BUN) was determined with the urease-indolphenol method using a commercial kit (UN set "Daiichi", Daiichi Pure Chemical Co., Ltd., Tokyo). β-Lipoprotein was determined with an enzyme method using a commercial kit (β-Lipoprotein C-Test, Wako Pure Chemical Ind., Ltd., Osaka). Serum albumin was determined with the BCG method using a commercial kit (Albumin B Test, Wako Pure Chemical Co., Ltd.). The serum concentration of α_1-acid glycoprotein (α_1-AGP) was determined by radial immunodiffusion [17] using rabbit anti-rat α_1-AGP antiserum. Rat serum α_1-AGP was purified basically according to the method of Hao and Wickerhauser [18]. The purity was checked by sodium dodecyl sulfate polyacrylamide gel electrophoresis. The rat α_1-AGP thus purified was de-sialyzed by mild hydrolysis at pH 1.6 and 80° for 1 hr [19]. Antibodies to rat α_1-AGP were raised in New Zealand White rabbits by injecting 0.5 mg of de-sialyzed rat α_1-AGP in Freund's complete adjuvant once every two weeks for 4 weeks and a subsequent booster (0.5 mg α_1-AGP).

Equilibrium dialysis method

The unbound fraction (*fu*) of DL-[^{3}H]propranolol in the various injection solutions was measured by equilibrium dialysis at 37° using HEPES-buffered Ringer's solution (pH 7.4) in semimicrocells (Kokugo-Gomu Co., Tokyo) with semipermeable membranes (Spectrum Medical Industries Inc., Los Angeles, CA). After equilibration was attained at 6 hr, the drug concentrations on the protein side and the buffer side were measured in a liquid-scintillation spectrometer. Correction of drug binding for the volume shift was done according to the method reported by Lima *et al.* [20].

Data analysis

Cerebral blood flow (Q). Cerebral blood flow was calculated as [21]:

$$Q = K_B \cdot V / E_B \tag{1}$$

where K_B is the rate constant (min^{-1}) of [^{14}C]butanol efflux from brain during a 2-min circulation period after carotid injection, V is the ratio (0.88 ml/g) of distribution volume of [^{14}C]butanol in the brain to that in blood [22], and E_B is the extraction fraction of [^{14}C]butanol, which is assumed to be 1.0 [23].

Brain transport parameters. The brain uptake index (*BUI*) was calculated as follows [24]:

$$BUI = \frac{^3\text{H}/^{14}\text{C dpm (in brain)}}{^3\text{H}/^{14}\text{C dpm (in injection solution)}} = \frac{E_T}{E_R} \tag{2}$$

where E_T and E_R are the extraction ratios of the test compound and the reference compound, respectively, 15 sec after injection. The E_T or E_R represents the maximal extraction of unidirectional influx into the brain minus the efflux of the test or reference compound during the period between the bolus flow through the brain (~2–5 sec after injection) and the decapitation (15 sec after injection). With regard to the reference compound, [^{14}C]butanol, the maximal extraction ($E_{R,max}$) was reported to be 100% [24, 25]. The relationship between $E_{R,max}$ and the extraction at 15 sec $E_{R(15\,sec)}$ is defined as [25]:

$$E_{R(15\,sec)} = E_{R,max} \cdot e^{-k_{efflux} \cdot t'} \quad (3)$$

where k_{efflux} is the efflux rate constant for [^{14}C]butanol in rat brain, and $t' = 15$ sec $- 5$ sec (the time between bolus entry into the brain and decapitation). The test drug was retained by brain and returned to blood very slowly as shown later, probably due to extensive binding to the cellular components. Therefore, the drug extraction ratio measured in the present studies represents the maximal extraction of the unidirectional influx into the brain.

The BBB permeability-surface area product (PS_{app}) was calculated by the Kety–Renkin–Crone equation [26]:

$$PS_{app} = -Q \cdot \ln(1 - E_T) \quad (4)$$

where Q is the brain blood flow.

The BBB permeability-surface area product for the unbound drug (PS_u) in the brain capillary was calculated by the following equation based on a conventional assumption that the fraction of propranolol which is exchangeable *in vivo* in the brain capillaries is equal to the fraction that is free as measured *in vitro*.

$$PS_u = PS_{app}/fu \quad (5)$$

where fu is the unbound fraction of drug determined by equilibrium dialysis. Therefore, based on this conventional assumption, the PS_u value should be constant irrespective of the presence of serum protein(s) in the injectate. If this conventional assumption holds, the theoretical extraction ratio ($E_{T,cal}$) with rat serum in the injectate can be calculated by the following equation:

$$E_{T,cal} = 1 - e^{-\frac{PS_{u,buffer} \cdot fu}{Q}} \quad (6)$$

where $PS_{u,buffer}$ represents the PS_u value determined with buffer alone in the injectate.

Statistical analysis

All means are presented with their standard errors (mean ± SE). Student's t-test was utilized to estimate the significant difference with $P = 0.05$ as the minimal level of significance.

RESULTS

The biochemical measurements for serum are listed in Table 1. The serum concentration of α_1-AGP was significantly ($P < 0.05$) lower in the uremic serum, whereas β-lipoprotein and albumin were unchanged. However, the fu (unbound fraction) of DL-propranolol was not changed significantly in the uremic serum. The BUN (blood urea nitrogen) of the uremic serum was *ca.* 6-fold higher than that of the control serum. The efflux of [^{14}C]butanol from rat brain is depicted in Fig. 1. The efflux rate constants of [^{14}C]butanol for the control and uremic rats were 0.665 min^{-1} and 0.628 min^{-1} respectively. No marked difference between the efflux rate constants in the two groups of rats was observed. Substituting these values into equation 1, the values of Q (cerebral blood flow) were calculated to be 0.585 and 0.553 [ml·min^{-1}·(g brain)$^{-1}$] for the control and uremic rats respectively. The brain levels of DL-propranolol at various time points (5, 15, 30, 60 and 120 sec) after carotid injection of the buffer solution were measured. The efflux rate constants (k_{efflux}) calculated from the slope of the semilogarithmic plot for the control and uremic rats were 0.145 min^{-1} and 0.135 min^{-1}, respectively. The drug thus slowly

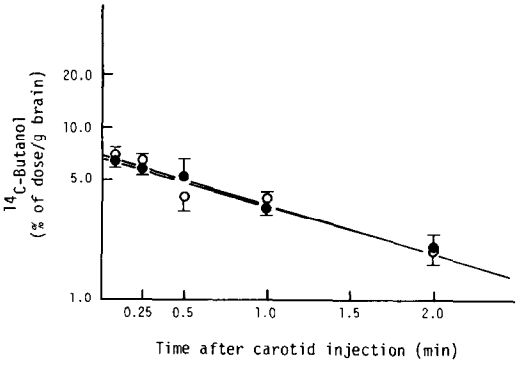

Fig. 1. Rate constant of [^{14}C]butanol efflux from brain in control (○) and uremic rats (●). The rate constant was derived from the slope of the plot of the natural log of brain [^{14}C]butanol radioactivity (dpm), expressed as percent of the injected dose/g brain, versus decapitation time. Each point represents the mean ± SE from four rats.

Table 1. Biochemical data for the control serum (CS) and uremic serum (US)*

Serum	β-Lipoprotein (mg/ml)	Albumin (g/dl)	α_1-AGP (μg/ml)	BUN (mg/dl)	fu
CS (6)	0.23 ± 0.01	3.38 ± 0.06	139.7 ± 16.2	17.72 ± 0.60	0.160
US (7)	0.28 ± 0.05	3.25 ± 0.04	101.1 ± 4.60†	105.4 ± 6.73‡	0.185

* Data are expressed as mean ± SE. The number of rats used is given in parentheses. Abbreviations: α_1-AGP, α_1-acid glycoprotein; BUN, blood urea nitrogen; and fu, serum unbound fraction of DL-propranolol.

† $P < 0.05$, compared to the control serum.

‡ $P < 0.001$, compared to the control serum.

effluxed from the brain to blood; it may be reasonable, therefore, to assume that the extraction ratio of the drug at 15 sec was essentially identical to the maximal extraction ratio of the unidirectional influx. The results of transport studies with DL-propranolol are summarized in Table 2. When the buffer solution alone was injected, the values of E_T, PS_{app} and PS_u were significantly ($P < 0.05$) lower in the uremic rate than in the control rats. When rat serum (CS, US) was used as the injection solution, the PS_u (corrected for the *fu*) increased, although the *BUI*, E_T, and PS_{app} values in both groups of rats (especially in the control rats) decreased. These findings indicate that the conventional assumption that the fraction of propranolol that is available for uptake *in vivo* by the brain capillaries is equal to the fraction that is free as measured *in vitro* may not hold. If this conventional assumption holds, the E_T of propranolol with rat serum in the injectate can be calculated by equation (6). The $E_{T,cal}$ values in control rats were 0.295 and 0.335 with control and uremic sera in the injectate, respectively, and for uremic rats were 0.206 and 0.237 with the control and uremic sera in the injectate, respectively. Thus, the observed E_T values were approximately 2–3 times as large as the corresponding $E_{T,cal}$ values calculated based on the conventional assumption. We hereafter designate this assumption as the "protein-mediated" brain uptake.

In the homologous and heterologous injection studies (Table 2, Expt. No. 3–6), the uptake of DL-propranolol by the brain decreased in both groups of rats receiving the injection of the uremic serum. This phenomenon may have been due to the decrease in the α_1-AGP concentration in the uremic serum (see Table 1), considering that the protein-mediated transport exists. However, the uptake of DL-propranolol by the brain still decreased in the control rats injected with the uremic serum to which α_1-AGP purified from control rats was added so that the final α_1-AGP concentration was the same as that in the control serum. In other homologous and heterologous injection studies (Table 3), using the ultrafiltrate from serum [CS(F), US(F)] and the supernatant fractions from the heat-treated serum [CS(H), US(H)] as the injection solutions, the uptake of DL-propranolol by the brain in the control rats was the same in the two groups [CS(F) vs US(F) and CS(H) vs US(H)]. Moreover, the PS_u value of the BBB for DL-propranolol was higher in the rats injected with the supernatant fraction obtained from the heat-treated serum than in rats injected with the ultrafiltrate from the serum (Table 3).

Protein-mediated transport of DL-propranolol was still present with heat-treated serum, because α_1-AGP was temperature resistant and remained in the supernatant. The α_1-AGP concentrations in CS(H) and US(H) were 94.4 and 89.4 μg/ml respectively. The effects of rat serum on the apparent extraction and uptake of [^{3}H]inulin, [^{3}H]water and D-[^{14}C]glucose are shown in Tables 4 and 5. Since no effect of rat serum on the extraction of these substances was found, the protein-mediated transport of DL-propranolol may not have been due to the nonspecific perturbation of BBB caused by serum proteins.

Table 2. Comparison of the transport parameters by brain capillary of DL-propranolol in control (C) and uremic (U) rats for different injection vehicles used as the carotid injection solution*

Expt. No.	Rat	Injectate	*BUI* (%)	E_T	*fu*	PS_{app} (ml·min^{-1}·g^{-1})	PS_u (ml·min^{-1}·g^{-1})
1	C (8)	B	95.52 ± 3.84	0.876 ± 0.023	1	1.292 ± 0.112	1.292 ± 0.112
2	U (5)	B	81.72 ± 7.45	0.735 ± 0.067†	1	0.809 ± 0.144†	0.809 ± 0.144†
3	C (7)	CS	74.21 ± 3.42‡	0.664 ± 0.031‡	0.158	0.656 ± 0.061‡	4.150 ± 0.384‡
4	U (7)	CS	70.60 ± 3.40	0.636 ± 0.031	0.158	0.572 ± 0.052	3.620 ± 0.331§
5	C (8)	US	65.47 ± 1.21‖,¶	0.586 ± 0.011‖,¶	0.185	0.517 ± 0.015‖,¶	2.800 ± 0.083
6	U (5)	US	64.42 ± 2.00	0.580 ± 0.020	0.185	0.482 ± 0.024¶	2.609 ± 0.132§, **,††

* Parameters are expressed as the mean ± SE. The number of rats used is given in parentheses. Abbreviations: *BUI*, brain uptake index; E_T, unidirectional extraction ratio; *fu*, serum unbound fraction; PS_{app}, BBB permeability-surface area products; PS_u, PS_{app} corrected for *fu*; B, buffer solution; CS, control serum; and US, uremic serum. The cerebral blood flow (Q) values of the control and uremic rats were 0.585 and 0.553 [ml·min^{-1}·(g brain)$^{-1}$] respectively.

† $P < 0.05$, compared to Expt. No. 1.
‡ $P < 0.001$, compared to Expt. No. 1.
§ $P < 0.05$, compared to Expt. No. 3.
‖ $P < 0.001$, compared to Expt. No. 1.
¶ $P < 0.001$, compared to Expt. No. 2.
** $P < 0.01$, compared to Expt. No. 3.
†† $P < 0.05$, compared to Expt. No. 4.

Table 3. Parameters of transport by the BBB of DL-propranolol in variously treated sera used as solutions for injection into control rats

Rat	Injectate	*BUI* (%)	E_T	*fu*	PS_{app} ($ml \cdot min^{-1} \cdot g^{-1}$)	PS_u ($ml \cdot min^{-1} \cdot g^{-1}$)
C (3)	CS(H)	83.14 ± 3.54	0.744 ± 0.032	0.410	0.806 ± 0.071	1.965 ± 0.173
C (3)	US(H)	92.72 ± 5.35	0.834 ± 0.048	0.424	1.103 ± 0.175	2.599 ± 0.412
C (4)	CS(F)	91.27 ± 8.95	0.812 ± 0.087	0.941	1.119 ± 0.222	1.189 ± 0.236
C (4)	US(F)	90.76 ± 3.98	0.812 ± 0.036	0.943	1.011 ± 0.113	1.073 ± 0.120

* Parameters are expressed as the mean ± SE. The number of rats is given in parentheses. Abbreviations: *BUI*, E_T, *fu*, PS_{app} and PS_u are defined in the legend of Table 2; CS(H), supernatant fractions obtained from heat-treated control serum; US(H), supernatant fraction obtained from heat-treated uremic serum; CS(F), ultrafiltrate obtained from control serum; and US(F), ultrafiltrate obtained from uremic serum.

Table 4. Brain extraction ratio of [^{3}H]inulin in control (C) and uremic (U) rats with the buffer solution (B) and the control serum (CS) used as the carotid injection solution*

Rat	Injectate	*BUI* (%)	E_T
C (4)	B	1.191 ± 0.142	0.0107 ± 0.0013
C (4)	CS	1.411 ± 0.122	0.0126 ± 0.0011
U (3)	B	1.489 ± 0.170	0.0141 ± 0.0015
U (3)	CS	1.703 ± 0.093	0.0153 ± 0.0008

* Parameters are expressed as the mean ± SE. The number of rats used is given in parentheses. The abbreviations *BUI* and E_T are defined in the legend of Table 2.

DISCUSSION

Cerebral blood flow (Q) was measured according to the method of Pardridge and Oldendorf [16] using [^{14}C]butanol and the anesthesic conditions used in the present studies; blood-flow-limited brain uptake was observed [27]. The value of Q [0.585 $ml \cdot min^{-1} \cdot (g\ brain)^{-1}$] for the control rats observed in the present study is comparable to that [0.6 $ml \cdot min^{-1} \cdot (g\ brain)^{-1}$] reported by Pardridge and Oldendorf [16] and that [0.5 $ml \cdot min^{-1} \cdot (g\ brain)^{-1}$] determined by us with the ^{51}Cr microsphere method*. No difference between the values for Q in the control and uremic rats was observed. Since the rate constants of the efflux of butanol from the brain in both groups of rats were similar (Fig. 1), butanol may be suitable as a reference substance in carotid injection studies.

Pardridge *et al.* [13, 28] showed that serum protein-bound ligands, such as steroids and *l*-propranolol, are also transported into brain. We have also found that PS_u, the BBB permeability-surface area product (PS_{app}) of DL-propranolol corrected for the *fu*, was higher after serum injection than after buffer injection (Table 2), suggesting the presence of a "protein-mediated" brain uptake mechanism, that could not be accounted for by the conventional assumption (see "Results" and "Data Analysis" in Materials and Methods). Since the apparent extraction ratios of D-[^{14}C]glucose, [^{3}H]inulin and [^{3}H]water which exhibited no binding to serum proteins, were not affected by the presence of rat serum in the injectate (Tables 4 and 5), the protein-mediated transport phenomenon seen for DL-propranolol is probably not the result of a nonspecific perturbation of BBB permeability caused by rat serum. Our previous studies showed that the phenomenon of protein-mediated transport was also present for imipramine and desipramine when serum proteins like albumin and α_1-AGP were used as the injectate [29]. The mechanism of the protein-mediated transport is not known yet, but the following two mechanisms may be possible [28, 30]. First is the possibility that the serum (plasma) protein(s) may increase the BBB permeability to a drug when the drug is presented to the endothelial cell surface in a serum-protein-bound

* T. Itoh, Y. Sawada, T-H. Lin, T. Iga and M. Hanano, manuscript submitted for publication.

Table 5. Parameters of the transport of [^{3}H]water and D[^{14}C]glucose by the brain capillary in control (C) and uremic (U) rats with the buffer solution (B) and the control serum (CS) used as the carotid injection solution*

		[^{3}H]Water		D-[^{14}C]Glucose	
Rat	Injectate	*BUI* (%)	E_T	*BUI* (%)	E_T
C (4)	B	80.49 ± 5.02	0.720 ± 0.045	24.32 ± 2.43	0.175 ± 0.018
C (4)	CS	71.46 ± 3.03	0.642 ± 0.027	26.72 ± 1.48	0.172 ± 0.010
U (4)	B	74.10 ± 6.36	0.642 ± 0.027	22.79 ± 1.60	0.152 ± 0.011
U (4)	CS	78.88 ± 2.63	0.710 ± 0.024	25.72 ± 1.43	0.183 ± 0.010

* Parameters are expressed as the mean ± SE. The number of rats used is given in parentheses. The abbreviations *BUI*, E_T, B and CS are defined in the legend of Table 2.

form. Second is the possibility that the exchangeable fraction of the drug *in vivo* in the brain capillaries is much greater than the unbound fraction as measured *in vitro*, due to the endothelial-cell-mediated enhanced dissociation of the drug from the serum protein(s) within the brain capillary. In the latter mechanism, the binding of the drug to serum proteins may be competitively inhibited by the rapid nonspecific interactions of serum proteins with the glycocalyx of the capillary endothelial cell surface. In fact, electrostatic interaction of serum proteins with the endothelial glycocalyx has been suggested [31, 32].

The brain extraction and the PS_u value of DL-propranolol in uremic rats was reduced significantly when the buffer solution was used as the injection solution (Table 2). This result suggested that the transport function of the BBB for DL-propranolol was reduced due to the renal failure induced by uranyl nitrate. In the homologous and heterologous injection studies, brain extraction and the PS_u values of DL-propranolol were decreased in both the control and uremic rats receiving the uremic-serum injection (Table 2). This phenomenon is similar to that observed in the liver [5]. Terao and Shen [5] investigated the extraction of *l*-propranolol by perfused rat liver and suggested that the presence of endogenous substance(s) in uremic blood could inhibit the hepatic extraction of *l*-propranolol. Recently, Pardridge *et al.* [33] demonstrated that acidosis could result in the inhibition of BBB transport of *l*-propranolol and lidocain. In the present studies, however, all of the injection solutions were adjusted to pH 7.4 immediately before the uptake experiment. Therefore, the possibility of a reduction in a brain uptake due to the pH change can be excluded. The results in Table 2 (Expt. No. 3–6) indicate that the decrease in the PS_u of DL-propranolol with uremic serum in the injectate may have been partly due to the reduction in protein-mediated transport caused by the decrease in the α_1-AGP concentration (Tables 1 and 2). In fact, Pardridge *et al.* [28] investigated the brain extraction of *l*-propranolol with the BUI method and showed that the protein-mediated transport phenomenon for *l*-propranolol could occur in the presence of α_1-AGP in the injectate. However, the reduction of the PS_u value of DL-propranolol still occurred in the uremic serum supplemented with α_1-AGP, despite the fact that the α_1-AGP concentration was the same as in the control serum. Therefore, these results suggest that the presence of the inhibitor(s) in uremic serum is one of the plausible explanations for the reduced extraction or permeability clearance (PS_u) by the BBB of DL-propranolol. Alternatively, considering the above-mentioned second possibility of explaining the protein-mediated transport, the difference in the extraction ratios of propranolol when injected with normal serum vs uremic serum may not have been due to differences in BBB permeability (PS product) but, instead, to differences in the exchangeable fractions *in vivo* ($f_{in\ vivo}$) [28, 34]. The $f_{in\ vivo}$ may be calculated by the following equation.

$$f_{in\ vivo} = \frac{\ln (1 - E_{T,\text{serum}})}{\ln (1 - E_{T,\text{buffer}})} \tag{7}$$

where $E_{T,\text{buffer}}$ and $E_{T,\text{serum}}$ represent the E_T of propranolol with buffer and serum in the injectate respectively. The calculated $f_{in\ vivo}$ values of control rats with control and uremic serum in the injectate were 0.523 and 0.423, respectively, and those of uremic rats with control and uremic serum were 0.761 and 0.653, respectively, indicating that the $f_{in\ vivo}$ values were smaller in control rats and with uremic serum. The smaller $f_{in\ vivo}$ value with uremic serum in the injectate may be explained by the increase in the affinity of the drug for the uremic serum protein(s) *in vivo* compared to that for the control serum protein(s), although the *in vitro* bound fraction in the uremic serum rather lower than that in the normal serum (Tables 1 and 2).

Recently, Hori *et al.* [6] investigated the uptake of DL-propranolol in both control and acute renal failure rats using the perfused rat liver and suggested that the decrease in hepatic clearance of DL-propranolol in renal failure rats is due to a decreased uptake of the drug from the blood into liver cells. There is some evidence to show that uremic serum can inhibit several different transport systems such as the uptake of *p*-aminohippuric acid by rabbit kidney slices [35] and rat liver [36], and the transepithelial transport of sodium by the isolated frog skin [37]. According to the studies by Pardridge *et al.* [33] using the *BUI* method, the transport of *l*-propranolol across the BBB of rats is mediated by a saturable transport system with a low affinity and high capacity. In addition, studies in our laboratory* using isolated bovine brain microvessels showed that the transport of DL-propranolol into the brain microvessels was saturable and was inhibited by other basic drugs (imipramine and quinidin) but not by acidic drugs (salicylic acid and phenobarbital). Furthermore, the transport was not inhibited by metabolic inhibitors (2,4-dinitrophenol and KCN). These results suggest that DL-propranolol is taken up by brain microvessels via a facilitated diffusion system which is specific for basic drugs. It is possible, therefore, that the extraction of DL-propranolol by the brain is reduced via the blockade of carrier-mediated transport by the uremic substance(s).

As shown in Table 3, an inhibitory effect on the transport of DL-propranolol did not occur in either the ultrafiltrate from the uremic serum or the supernatant from the heat-treated uremic serum. This indicates that the uremic inhibitor(s) is temperature-sensitive and not ultrafilterable at physiological pH (7.4). These characteristics may be accounted for by strong binding of the inhibitor(s) to high molecular weight substance(s) in serum. In a report by Sjoholm *et al.* [38], evidence is given for the presence of nondialyzable, strongly bound inhibitors of serum protein binding of acidic drugs in uremic plasma. Studies by Lichtenwalner and Suh [39] also demonstrate the presence of nondialyzable inhibitor(s) of serum protein binding of some drugs, such as 5,5-diphenylhydantoin, digitoxin and diazepam, in uremic serum. On the other hand, clinical studies

* A. Kurihara, Y. Sawada, Y. Sugiyama, T. Iga and M. Hanano, manuscript submitted for publication.

by Bianchetti *et al.* [40] show that dialysis treatment can reduce the inhibitory effect of uremia on propranolol clearance. Further study is necessary to clarify the characteristics of the inhibitor(s).

REFERENCES

1. M. M. Reidenberg, *Clin. Pharmacokinet.* **1**, 121 (1976).
2. F. D. Thompson, A. M. Joekes and D. M. Foulkes, *Br. med. J.* **2**, 434 (1972).
3. D. T. Lowenthal, W. A. Briggs, T. P. Gibson, H. Nelson and W. J. Cirksena, *Clin. Pharmac. Ther.* **16**, 761 (1974).
4. N. Terao and D. D. Shen, *J. Pharmac. exp. Ther.* **227**, 295 (1983).
5. N. Terao and D. D. Shen, *J. Pharmac. exp. Ther.* **233**, 277 (1985).
6. R. Hori, K. Okumura, M. Yasuhara and H. Katayama, *Biochem. Pharmac.* **34**, 2679 (1985).
7. J. P. Knochel and D. W. Seldin, in *The Kidney* (Eds. B. M. Brenner and F. C. Rector), 2nd edn, p. 2137. W. B. Saunders, Philadelphia (1981).
8. B. Jeppsson, H. R. Freund, Z. Gimmon, J. H. James, M. F. von Meyenfeldt and J. E. Fischer, *Surgery, St. Louis* **92**, 30 (1982).
9. E. Preston, N. Haas and M. Allen, *Brain Res. Bull.* **12**, 133 (1984).
10. R. A. Fishman and N. H. Raskin, *Archs Neurol. Chicago* **17**, 10 (1967).
11. W. H. Oldendorf, *Brain Res.* **24**, 372 (1970).
12. K. M. Giacomini, S. M. Roberts and G. Levy, *J. pharm. Sci.* **70**, 117 (1981).
13. W. M. Pardridge and L. J. Mietus, *J. clin. Invest.* **64**, 145 (1979).
14. W. M. Pardridge, E. M. Landaw, L. P. Miller, L. D. Braun and W. H. Oldendorf, *J. Cerebr. Blood Flow Metab.* **5**, 576 (1985).
15. W. M. Pardridge and L. J. Mietus, *J. clin. Invest.* **66**, 367 (1980).
16. W. M. Pardridge and W. H. Oldendorf, *Biochim. biophys. Acta* **382**, 377 (1975).
17. G. Mancini, A. O. Carbonara and J. H. Heremans, *Immunochemistry* **2**, 235 (1965).
18. Y-L. Hao and M. Wickerhauser, *Biochim. biophys. Acta* **322**, 99 (1973).
19. K. Schmid, A. Plis, K. Hunziker, R. Fricke and M. Yayoshi, *Biochem. J.* **104**, 361 (1967).
20. J. J. Lima, J. J. Mackichan, N. Libertin and J. Sabino, *J. Pharmacokinetics Biopharm.* **11**, 483 (1983).
21. M. W. B. Bradbury, C. S. Patlak and W. H. Oldendorf, *Am. J. Physiol.* **229**, 1110 (1975).
22. W. M. Pardridge, T. L. Moeller, L. J. Mietus and W. H. Oldendorf, *Am. J. Physiol.* **239**, E96 (1980).
23. A. Gjedde, A. J. Hansen and E. Siemkiowicz, *Acta physiol. scand.* **108**, 321 (1980).
24. W. H. Oldendorf and L. D. Braun, *Brain Res.* **113**, 219 (1976).
25. W. H. Oldendorf, W. M. Pardridge, L. D. Braun and P. D. Crane, *J. Neurochem.* **38**, 1413 (1982).
26. C. Crone, *Acta physiol. scand.* **58**, 292 (1963).
27. R. van Uitert, J. I. Sage, D. E. Levy and T. Duffy, *Brain Res.* **222**, 365 (1981).
28. W. M. Pardridge, R. Sakiyama and G. Fierer, *J. clin. Invest.* **71**, 900 (1983).
29. T-H. Lin, Y. Sawada, Y. Sugiyama, T. Iga and M. Hanano, *Chem. pharm. Bull, Tokyo* **35**, 294 (1987).
30. W. M. Pardridge and E. M. Landaw, *J. clin. Invest.* **74**, 745 (1984).
31. C. C. Michel, M. E. Phillips and M. R. Turner, *J. Physiol., Lond.* **332**, 111 (1982).
32. E. E. Schneeberger and M. Hamelin, *Am. J. Physiol.* **247**, H206 (1984).
33. W. M. Pardridge, R. Sakiyama and G. Fierer, *Am. J. Physiol.* **247**, R582 (1984).
34. W. M. Pardridge, *Endocr. Rev.* **2**, 103 (1981).
35. J. B. Hook and J. R. Munro, *Proc. Soc. exp. Biol. Med.* **127**, 289 (1968).
36. J. R. Ciccone, A. I. Keller, S. R. Braun, H. V. Murdaugh and H. G. Preuss, *Biochim. biophys. Acta* **163**, 108 (1968).
37. J. Bourgoignie, S. Klahr and N. S. Bricher, *J. clin. Invest.* **50**, 303 (1971).
38. I. Sjoholm, A. Kober, I. Odar-Cederlof and O. Borga, *Biochem. Pharmac.* **25**, 1205 (1976).
39. D. M. Lichtenwalner and B. Suh, *J. clin. Invest.* **71**, 1289 (1983).
40. G. Bianchetti, G. Graziani, D. Braincaccio, A. Morganti, G. Leonetti, M. Manfrin, R. Sega, R. Gomeni, C. Ponticelli and P. L. Morselli, *Clin. Pharmacokinet.* **1**, 373 (1976).