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Effects of Albumin and α_1 -Acid Glycoprotein on the Transport of Imipramine and Desipramine through the Blood–Brain Barrier in Rats¹⁾

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The permeability of the blood–brain barrier (BBB) of rats to ^3H -imipramine (IMP) and ^3H -desipramine (DMI) was measured by a tissue-sampling single-injection technique to examine the protein mediated transport. Increased concentrations of serum proteins added to the carotid injection solution resulted in decreases in the brain extraction and PS_{app} of ^3H -IMP and ^3H -DMI. The extraction ratio of ^3H -IMP (0.92) was higher than that of ^3H -DMI (0.24) in the case of the buffer injection solution. The values of *in vitro* binding activity (n/K_d) of ^3H -IMP to bovine serum albumin (BSA), human serum albumin (HSA) and α_1 -acid glycoprotein (α_1 -AGP) were 4.52, 1.48 and 57.89 mm^{-1} , respectively, and that of ^3H -DMI to α_1 -AGP was 38.92 mm^{-1} . On the other hand, the *in vivo* n/K_d values of the binding of ^3H -IMP to BSA, HSA and α_1 -AGP estimated from the single-injection experiment were 0.597, 0.754 and 14.73 mm^{-1} , respectively, and that of ^3H -DMI to α_1 -AGP was 5.90 mm^{-1} . The *in vitro* n/K_d values of the binding of ^3H -IMP and ^3H -DMI to various serum proteins were thus larger than the corresponding *in vivo* n/K_d values. A marked difference was found between the observed extraction ratios and what would be predicted if only the unbound ^3H -IMP or ^3H -DMI is transported, although the difference was not large in the case of HSA. These results suggest that protein mediated transport, previously found for propranolol and lidocaine with α_1 -AGP by Pardridge *et al.* (*J. Clin. Invest.*, **71**, 900 (1983)), also operates for ^3H -IMP and ^3H -DMI, and this phenomenon seems to be independent of both the species and the source of serum protein, at least for ^3H -IMP. Further, the brain extraction ratio of ^3H -DMI was much lower than that of the parent drug ^3H -IMP, probably due to their different degrees of lipophilicity.

Keywords—blood–brain barrier; brain uptake; imipramine; desipramine; albumin effect; BSA; HSA; α_1 -acid glycoprotein

Basic lipophilic drugs such as imipramine, desipramine, propranolol and lidocaine are widely used in clinical practice. The pharmacological effect of these drugs is related to the total drug concentration in plasma.^{2–4)} It has been shown that many basic drugs bind not only to albumin,^{5,6)} but also to α_1 -acid glycoprotein,^{7–9)} and lipoprotein.^{7,10)} The concentrations of these proteins, particularly α_1 -acid glycoprotein, are affected in various diseases and by surgery, and this variation would affect plasma drug levels *via* drug protein binding. Although it has generally been believed that only the drug unbound to protein is available for entry into tissues,^{11,12)} recent reports have indicated that serum protein-bound ligands are also available for transport into tissues such as the liver^{13–16)} and brain.^{17–20)} Moreover, there is a paucity of data in regard to the permeability properties of the blood–brain barrier (BBB) to drugs that act on the central nervous system (CNS). In the present studies, an antidepressant drug, imipramine, and its pharmacologically active metabolite, desipramine, both of which bind extensively to serum proteins with high affinity and act on the CNS, were selected as model drugs to examine the possibility of the protein-mediated transport into the rat brain, and the permeability properties of these drugs were compared.

Experimental

Materials—All isotopes were purchased from New England Nuclear Co., Boston, MA. The specific activities given by the manufacturer were: [2,4,6,8-³H]imipramine hydrochloride (IMP) 44.2 Ci/mmol, [2,4,6,8-³H]desipramine hydrochloride (DMI) 68.0 Ci/mmol, and *n*-[1-¹⁴C]butanol 1.0 mCi/mmol. These radioactive compounds were at least 98% pure, except the ³H-desipramine which was 95% pure by thin layer chromatography (TLC). The ⁵¹Cr-labelled microspheres (16 ± 0.3 μ; 40 mCi/g) were suspended in 10% dextran solution with 0.01 v/v% Tween 80. Human serum albumin (HSA, fraction V), human α₁-acid glycoprotein (α₁-AGP) and bovine serum albumin (BSA, fraction V) were purchased from Sigma Chemical Co., St. Louis, MO. All other chemicals were commercial products of analytical grade.

Preparation of Injection Solution—HEPES-buffered Ringer's solution (5 mM HEPES) was titrated with 2 N NaOH to pH 7.55 and was equilibrated with 95% O₂-5% CO₂ gas to maintain a pH of 7.4. The injection solution contained 5–10 μCi/ml ³H-test compound (³H-IMP or ³H-DMI) and 1–2 μCi/ml ¹⁴C-butanol and either buffer alone or various concentrations of serum protein.

Animal Study—The permeability of the BBB to labelled IMP and DMI was measured by the tissue-sampling single-injection technique developed by Oldendorf.²¹ Male Wistar rats (200–250 g) were used. They had free access to tap water and standard laboratory chow (CE-2, Clea Japan Inc., Tokyo) except before experiments, when they were fasted for 24 h.

1) Hemodynamic Study: The radioactive microsphere method^{22,23} was utilized to measure the brain blood flow. The animal was anesthetized with intraperitoneal pentobarbital (Somnopenyl; 65 mg/kg) (Pitman-Moone, Inc., Washington Crossing, U.S.A.). The right carotid artery was cannulated with polyethylene tubing. The right and left femoral arteries and left femoral vein were also cannulated with polyethylene tubing. The microspheres labelled with ⁵¹Cr were injected into the left ventricle over 20 s through a cannula passed down the right carotid artery, in a total volume of 0.2 ml. Simultaneously, arterial blood from the femoral artery was withdrawn at 0.83 ml/min for 90 s with a syringe pump (Harvard Apparatus, Millis, MA). Subsequently, the animals were killed with pentobarbital (250 mg/kg) by bolus injection into the femoral vein, and the brain was weighed and counted in a gamma counter (Aloka ARC-300, Aloka Co., Tokyo, Japan). Brain blood flow was determined by using the following equation.

$$\text{brain blood flow (ml/min/g brain)} = \frac{\text{reference sample withdrawal rate (ml/min)}}{\text{radioactivity in brain (dpm/g brain)}} \times \text{radioactivity in reference blood sample (dpm)}$$

The mean arterial blood pressure and heart rate were monitored from the right femoral arterial catheter by using a transducer coupled with a chart recorder (Nihon Koden, Tokyo).

2) Brain Uptake Study: The animal was anesthetized with intraperitoneal pentobarbital (65 mg/kg) and placed in a supine position, and the left common carotid artery was isolated. The ³H-test compound (³H-IMP or ³H-DMI) and ¹⁴C-butanol, a freely diffusible internal reference, were rapidly injected (<0.5 s) into the common carotid artery as an approximately 200 μl bolus (the exact volume is immaterial) of injection solution through a sharp 27 gauge needle. Because the rate of injection (<0.5 s) exceeds the rate of carotid blood flow, the injection solution traverses the brain microcirculation as a bolus without significant mixing with the circulating blood.^{20,24,25} The animal was decapitated 15 s after injection, except in the experiment to measure the extraction time course of IMP to determine its efflux rate. This period is sufficient for a single pass of the bolus through the brain, but short enough to minimize the efflux of labelled compound from the brain or the recirculation of labelled compound.²⁶ The cerebral hemisphere ipsilateral to the injection was removed from the cranium, solubilized in triplicate in 1.5 ml of Protosol (New England Nuclear, Boston, MA) at 50 °C overnight in an incubator, and decolorized with 33% H₂O₂, then 10 ml of Biofluor (New England Nuclear, Boston, MA) was added before double-isotope liquid scintillation counting. An aliquot of the injection solution added to the control brain tissue was similarly treated. The radioactivities of ³H-IMP, ³H-DMI and ¹⁴C-butanol were determined in a liquid scintillation spectrometer (model 3255, Packard Instrument, Downers Grove, IL).

Equilibrium Dialysis Method—The unbound fraction (*f_u*) of the labelled drug in the injection solution containing various concentrations of serum protein was measured by equilibrium dialysis at 37 °C using HEPES-buffered Ringer's solution (pH 7.4) in semimicrocells (Kokugo-Gomu Co., Tokyo) with a semipermeable membrane (Spectrum Medical Industries Inc., Los Angeles, CA). After equilibration was attained (at 6 h), the drug concentration on the protein side and the buffer side were measured in a liquid scintillation spectrometer.

Data Analysis—The brain uptake index (BUI) was calculated as follows²⁷:

$$\text{BUI} = \frac{{}^3\text{H}/{}^{14}\text{C dpm (in brain)}}{{}^3\text{H}/{}^{14}\text{C dpm (in injection solution)}} = \frac{E_T}{E_R} \quad (1)$$

where *E_T* and *E_R* are the extraction ratios of the test compound and the reference compound, respectively, 15 s 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 bolus flow through the brain (2–5 s after injection) and decapitation (15 s after injection). With regard to the reference compound, ^{14}C -butanol, the maximal extraction ($E_{R,\max}$) was reported to be 100%.^{26,27)} The relationship between $E_{R,\max}$ and the extraction at 15 s $E_{R(15s)}$ is defined as²⁸⁾

$$E_{R(15s)} = E_{R,\max} \cdot e^{-k_{\text{efflux}} \cdot t'} \quad (2)$$

where k_{efflux} is the efflux rate constant for ^{14}C -butanol (0.67 min^{-1}).²⁹⁾ Substitution of the values for $E_{R,\max}$ and k_{efflux} , with $t' = 10 \text{ s}$ (the time between bolus entry into the brain and decapitation), into Eq. 2 gives $E_{R(15s)} = 90\%$ for ^{14}C -butanol. The test drugs are retained by the brain and return to the blood very slowly ($t_{1/2} \approx 4 \text{ min}$), 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 value of $(n/K_d)_{\text{in vitro}}$ was calculated by

$$f_{u,\text{in vitro}} = \frac{C_u}{C_t} = \frac{C_u}{C_u + (n \cdot (P) \cdot C_u) / (K_d + C_u)} \quad (3)$$

where C_t is the total concentration of drug, C_u is the unbound concentration of drug, n is the number of binding sites per protein molecule, (P) is the molar concentration of protein and K_d is the dissociation constant of the drug.

At the tracer dose, where $K_d \gg C_u$, Eq. 3 can be expressed as

$$f_{u,\text{in vitro}} = \frac{1}{1 + (n/K_d)_{\text{in vitro}} \cdot (P)} \quad (4)$$

Thus, rearrangement of Eq. 4 gives

$$1/f_{u,\text{in vitro}} = 1 + (n/K_d)_{\text{in vitro}} \cdot (P) \quad (5)$$

The value of $(n/K_d)_{\text{in vivo}}$ was calculated as follows. If we assume that only unbound drug could be transported across the BBB, the following Kety–Renkin–Crone equation³⁰⁾ is obtained.

$$E_T = 1 - \text{Exp} \left(-\frac{f_{u,\text{in vivo}} \cdot PS_u}{Q} \right) \quad (6)$$

where Q is the brain blood flow (the value measured by the ^{51}Cr -microsphere method was $0.50 \text{ ml/min/g brain}$) and PS_u is the BBB permeability–surface area product for the unbound drug in the brain capillaries.

Furthermore, if Eq. 7 (similar to Eq. 4) holds *in vivo*,

$$f_{u,\text{in vivo}} = \frac{1}{1 + (n/K_d)_{\text{in vivo}} \cdot (P)} \quad (7)$$

Equation 6 becomes

$$PS_{\text{app}} = -Q \cdot \ln(1 - E_T) = \frac{PS_u}{1 + (n/K_d)_{\text{in vivo}} \cdot (P)} \quad (8)$$

Rearrangement of Eq. 8 gives

$$\frac{PS_u}{PS_{\text{app}}} = 1 + (n/K_d)_{\text{in vivo}} \cdot (P) \quad (9)$$

Statistical Analysis—Student's *t*-test was utilized to estimate the significance of differences between the buffer-injected groups and protein-containing solution injected groups with $p = 0.05$ as the minimal criterion of significance. The comparison between IMP and DMI was carried out only for the case of buffer-injected groups.

Results

The E_T values for ^3H -IMP at various times after carotid injection with nonprotein buffer solution are shown in Fig. 1. The efflux rate constant (k_{efflux}) calculated from the slope was 0.0696 min^{-1} , which is equivalent to a $t_{1/2}$ of 4.32 min. The drug was thus retained by the brain and returned to the blood very slowly. Increased concentrations of serum proteins added to the carotid injection solution resulted in decreases in the brain extraction and PS_{app} of ^3H -IMP and ^3H -DMI, as summarized in Table I. However, $PS_{u,\text{app}}$ corrected for the *in vitro* binding increased as the protein concentration increased, suggesting protein-mediated brain uptake. The extraction ratio (*ca.* 0.92) of ^3H -IMP was higher than that of ^3H -DMI (*ca.* 0.24)

Fig. 1. Time Course of Rat Brain Extraction Ratio of Imipramine (IMP) after Carotid Injection

The line represents the best fit to these data by a least-squares regression.

Fig. 2. Reciprocal of *in Vitro* Unbound Fraction ($1/f_{u, \text{in vitro}}$) and PS_u/PS_{app} for Imipramine as a Function of HSA Concentration In Carotid Injection Solution

The slopes of these lines yield the $(n/K_d)_{\text{in vitro}}$ (●) of Eq. 5 and $(n/K_d)_{\text{in vivo}}$ (○) of Eq. 9.

TABLE I. Parameters of the Brain Uptake of Imipramine (IMP) and Desipramine (DMI) with Various Concentrations of Human Serum Albumin (HSA), Bovine Serum Albumin (BSA) and Human α_1 -Acid Glycoprotein (α_1 -AGP) in the Carotid Injection Solution

Compound	Exp. No.	Protein concentration in injection solution % (w/v)	BUI (%)	E_T	$f_{u, \text{in vitro}}$	PS_{app} (ml/min/g)	$PS_{u, \text{app}}$ (ml/min/g)
IMP	1	Buffer (0%)	99.09 ± 0.99	0.892 ± 0.009	1	1.100 ± 0.040	1.100 ± 0.040
		HSA 3%	98.43 ± 4.22	0.886 ± 0.038	0.610	1.146 ± 0.093	$1.880 \pm 0.153^a)$
		5%	$88.83 \pm 2.06^a)$	$0.800 \pm 0.018^a)$	0.494	$0.800 \pm 0.048^a)$	$1.621 \pm 0.098^a)$
		10%	$77.38 \pm 3.30^b)$	$0.696 \pm 0.030^b)$	0.341	$0.597 \pm 0.055^b)$	$1.748 \pm 0.160^a)$
	2	Buffer (0%)	101.7 ± 3.24	0.916 ± 0.026	1	1.254 ± 0.137	1.254 ± 0.137
		BSA 3%	97.96 ± 2.28	0.822 ± 0.020	0.402	1.070 ± 0.086	$2.664 \pm 0.215^b)$
		5%	89.82 ± 3.28	0.809 ± 0.030	0.223	0.825 ± 0.083	$3.696 \pm 0.373^b)$
		10%	$82.29 \pm 4.96^c)$	$0.742 \pm 0.044^c)$	0.142	$0.681 \pm 0.090^c)$	$4.706 \pm 0.571^b)$
		α_1 -AGP 0.1%	104.9 ± 2.42	0.945 ± 0.036	0.499	1.554 ± 0.137	$3.114 \pm 0.301^b)$
		0.2%	$79.02 \pm 3.05^a)$	$0.711 \pm 0.028^a)$	0.338	$0.616 \pm 0.045^a)$	$1.825 \pm 0.135^c)$
		0.3%	$82.12 \pm 2.62^a)$	$0.740 \pm 0.002^a)$	0.145	$0.673 \pm 0.058^d)$	$4.633 \pm 0.320^b)$
	3	Buffer (0%)	$26.29 \pm 1.66^e)$	$0.237 \pm 0.015^e)$	1	$0.133 \pm 0.010^e)$	$0.133 \pm 0.010^e)$
		α_1 -AGP 0.1%	29.21 ± 3.39	0.263 ± 0.030	0.543	0.151 ± 0.022	$0.278 \pm 0.038^d)$
		0.2%	22.50 ± 3.04	0.203 ± 0.027	0.364	0.112 ± 0.017	$0.308 \pm 0.046^a)$
		0.3%	22.56 ± 3.29	0.203 ± 0.030	0.269	0.112 ± 0.018	$0.417 \pm 0.067^a)$

The abbreviations are as follows. BUI (%), brain uptake index based on an internal reference substance (^{14}C -butanol) calculated according to Eq. 1; E_T , maximal unidirectional extraction ratio; $f_{u, \text{in vitro}}$, unbound fraction determined by equilibrium dialysis; PS_{app} , BBB permeability-surface area product for IMP and DMI calculated according to Eq. 8 [$PS_{\text{app}} = -Q \cdot \ln(1 - E_T)$, where brain blood flow (Q) = 0.50 ± 0.05 ml/min/g brain (determined by the ^{51}Cr microsphere method)]; $PS_{u, \text{app}}$, PS_{app} corrected for the *in vitro* binding calculated according to $PS_{u, \text{app}} = PS_{\text{app}}/f_{u, \text{in vitro}}$. Data are expressed as mean $\pm$ S.E. ($n=4-8$). a) Significantly different ($p < 0.01$) from the buffer injected group in the same Exp. No.; b) Significantly different ($p < 0.001$) from the buffer-injected group in the same Exp. No.; c) Significantly different ($p < 0.05$) from the buffer-injected group in the same Exp. No.; d) Significantly different ($p < 0.02$) from the buffer-injected group in the same Exp. No. e) Significantly different ($p < 0.001$) from the case of IMP.

TABLE II. *In Vitro* and *In Vivo* n/K_d Values of Imipramine (IMP) and Desipramine (DMI) for Various Serum Proteins^{a)}

Protein	<i>In vitro</i> n/K_d (mm ⁻¹)		<i>In vivo</i> n/K_d (mm ⁻¹)	
	IMP	DMI	IMP	DMI
BSA	4.52 ± 0.43	ND ^{b)}	0.597 ± 0.214	ND ^{b)}
HSA	1.48 ± 0.20	ND ^{b)}	0.754 ± 0.244	ND ^{b)}
α_1 -AGP	57.79 ± 5.92	38.92 ± 4.48	14.73 ± 4.07	5.90 ± 3.46

a) The *in vitro* n/K_d value were calculated by means of Eq. 4 based on the data determined by equilibrium dialysis. The *in vivo* n/K_d values were calculated by means of Eq. 8 based on the data obtained by the carotid injection technique. The parameters were expressed as mean ± computer calculated S.D. b) Not determined.

Fig. 3. Predicted and Observed Brain Unidirectional Extraction Ratios (E_T) for Various Concentrations of Proteins in Carotid Injection Solution

Panels a, b, and c: imipramine (IMP). Panel d: desipramine (DMI). The solid circles represent the observed values (mean ± S.E., $n=4-8$ rats, per point), and the dotted line was simulated according to Eq. 8 using the n/K_d values determined *in vitro* by equilibrium dialysis instead of $(n/K_d)_{in vivo}$.

—, observed; ----, predicted.

in the buffer injection solution (Table I). Linearized plots according to Eqs. 5 and 9 comparing the *in vitro* and *in vivo* n/K_d values of ^3H -IMP to HSA, are shown in Fig. 2. The *in vitro* n/K_d value obtained from the slope is approximately twice as large as that *in vivo*. We further calculated the *in vitro* and *in vivo* n/K_d values using a computer-aided nonlinear least-squares method according to Eqs. 4 and 8, respectively, and the results are listed in Table II. The *in vitro* n/K_d values of ^3H -IMP to BSA, HSA and α_1 -AGP were approximately 8, 2 and 4 times the corresponding *in vivo* n/K_d values, and that of ^3H -DMI to α_1 -AGP was approximately 7 times of the corresponding *in vivo* n/K_d value.

The observed extraction ratios and those predicted by Eq. 8 using the *in vitro* n/K_d values are shown in Fig. 3. A marked difference was found between the observed values and what would be predicted if only the unbound IMP is transported, although the difference was not large in the case of HSA. A similar difference between the observed and the predicted values was

found for the transport of DMI. These studies suggested that serum protein-bound IMP and DMI are available for transport into the brain.

Discussion

Figure 1 validates the assumption that the efflux of lipophilic basic drugs such as IMP can be neglected within 15 s. Therefore, it may be reasonable to assume that the extraction ratio of the drug at 15 s is essentially identical to the maximal extraction ratio of unidirectional influx. The carotid artery injection technique was used to quantitate the extraction of the drug in the present studies. Crucial to this technique is the assumption that there is minimal mixing of the injection solution bolus with the circulating plasma following rapid artery injection. Recently, Pardridge *et al.*²⁴⁾ reported that mixing does not occur to any significant extent in this technique.

Data obtained in the present studies show that serum protein-bound drugs such as IMP and DMI are readily available for transport into the brain. The transport of drugs into the brain was inhibited by serum protein, but the extent of inhibition was less than that predicted based on the "free drug hypothesis," which assumes that only the unbound drug *in vitro* is available for transport into tissues *in vivo* (Fig. 3). This result may be related to the previous report²⁾ that a linear correlation was observed between the clinical effect and the total plasma concentration of IMP or DMI and that there seems to be no need to take into account only the unbound drug concentration.

There have been several reports suggesting the serum protein-mediated transport of metabolic substances^{13,15,19)} hormone¹⁷⁾ and drugs^{14,18,31)} into the liver and brain. For instance, Weisiger *et al.*^{13,16)} presented the albumin receptor-mediated transport concept to account for the transport of fatty acid, sulfobromophthalein and bilirubin into the liver, and Forker and Luxon^{14,15)} suggested the presence of albumin-mediated transport in the liver for rose bengal and taurocholate. Subsequently, we also found an albumin-mediated hepatic uptake of warfarin using the multiple indicator dilution method.³¹⁾ Pardridge *et al.*^{18,19)} suggested a "free intermediate model" to explain the transport of many substances such as steroid hormone and *l*-propranolol (PL) into the brain. Recent *in vivo* studies showed that the transit time of albumin through the liver and brain is not delayed compared with that of an extracellular marker such as sucrose.^{32,33)} In addition, *in vitro* studies using liver plasma membranes³⁴⁾ and brain microvessels³³⁾ could not detect any specific binding to albumin and failed to support the albumin receptor-mediated transport concept in either liver or brain. However, as suggested by Pardridge *et al.*,³³⁾ this fact does not preclude the possibility of rapid nonspecific interactions of circulating serum proteins with the glycocalyx of capillary endothelial cell surface. In fact, it has been suggested that charged moieties on the plasma proteins interact electrostatically with the negatively charged moieties of the endothelial glycocalyx.^{35,36)}

The relation of the *in vitro* and *in vivo* n/K_d values for IMP and DMI is shown in Fig. 4, together with those for other substances reported by Pardridge *et al.*^{18,37,38)} It was shown that the *in vivo* n/K_d values were markedly lower than those measured *in vitro* except for two cases (PL-BSA, IMP-HSA). Our data showed that the decrease in the *in vivo* n/K_d value compared to the *in vitro* value seems to be independent of the species and the source of serum proteins, although no marked difference was observed for IMP-HSA. As discussed above, no specific binding of albumin to brain capillaries was detected either *in vivo* or *in vitro*. However, Mizuma *et al.*³⁹⁾ recently succeeded in detecting the interaction between albumin and liver cells using electron spin resonance (ESR) and fluorescence spectroscopy, although this binding also appeared to be nonspecific. Therefore, the nonspecific interaction between the protein and the cell surface might be possible mechanism to explain the discrepancy between

Fig. 4. Relationship of the *in Vitro* and *in Vivo* n/K_d Values

The solid circles represent the values obtained by the carotid injection technique in the present studies, while the open circles represent the values obtained from the reports by Pardridge *et al.*^(18,37,38) The solid line represents a positive 1:1 relationship. Abbreviations are as follows. PL, propranolol; T₃, liothyronine.

in vitro and *in vivo* n/K_d values (Fig. 2, Table II), *i.e.*, the protein-mediated brain uptake of drugs.

It seemed interesting to estimate the contributions of albumin and α_1 -AGP to the protein-mediated brain uptake of IMP. The serum concentrations of albumin and α_1 -AGP in normal humans are approximately 5% (0.075 mm) and 0.07% (0.016 mm), respectively. Therefore, the contribution of each protein to the binding of IMP to the circulating serum may be calculated as follows based on the *in vitro* n/K_d values (Table II) under the condition of linear binding.

$$(\text{binding to albumin}): (\text{binding to } \alpha_1\text{-AGP}) = 1.48 \times 0.075 : 57.8 \times 0.016 = 1.3 : 1$$

On the other hand, in order to estimate the contribution of each protein to the brain uptake of IMP, it may be reasonable to use the *in vivo* n/K_d value (Table II), since the *in vivo* values may represent the drug binding in the brain capillaries from which the drug is taken up into the brain. A similar calculation yields:

$$(\text{binding to albumin}): (\text{binding to } \alpha_1\text{-AGP}) = 0.75 \times 0.075 : 14.7 \times 0.016 = 2.6 : 1$$

Thus, in the *in vivo* brain capillaries, the binding of IMP to albumin may be a few times greater than that to α_1 -AGP.

As shown in Table I, no increase in the $PS_{u,app}$ value for IMP was observed with increase in the HSA concentration in the injection solution, although an increase in the $PS_{u,app}$ value was observed for other proteins. The reason for this finding is not known, but the lower binding affinity of IMP to HSA may be related to this phenomenon. That is, the range of f_u values of IMP associated with the change in the HSA concentrations (3–10%) was relatively small due to the lower affinity of IMP to HSA. This may explain why we could not detect any increase in the $PS_{u,app}$ value with increase in the HSA concentration.

The value of E_T for DMI was much lower than that of IMP in the case of the buffer solution injection (Table I). The smaller extraction for DMI may be attributed to its lower lipophilicity as compared with IMP, because the lipophilicity is one of the major factors which

could affect the transport of substances through the BBB. In fact, Glasser and Kriegelstein reported that the partition coefficient of DMI was approximately one-tenth of that for IMP in octanol/pH 7.4 phosphate buffer.⁴⁰⁾

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