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Research Article

Assessment of Drug Disposition in the Perfused Rat Brain by Statistical Moment Analysis

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Drug disposition in the brain was investigated by statistical moment analysis using an improved *in situ* brain perfusion technique. The right cerebral hemisphere of the rat was perfused *in situ*. The drug and inulin were injected into the right internal carotid artery as a rapid bolus and the venous outflow curve at the posterior facial vein was obtained. The infusion rate was adjusted to minimize the flow of perfusion fluid into the left hemisphere. The obtained disposition parameters were characteristics and considered to reflect the physicochemical properties of each drug. Antipyrine showed a small degree of initial uptake. Therefore, its apparent distribution volume (V_i) and apparent intrinsic clearance ($CL_{int,i}$) were small. Diazepam showed large degrees of both influx and efflux and, thus, a large V_i. Water showed parameters intermediate between those of antipyrine and those of diazepam. Imipramine, desipramine, and propranolol showed a large $CL_{int,i}$ compared with those of the other drugs. The extraction ratio of propranolol significantly decreased with increasing concentrations of unlabeled propranolol in the perfusion fluid. These findings may be explained partly by the tissue binding of these drugs. In conclusion, the present method is useful for studying drug disposition in the brain.

KEY WORDS: blood–brain barrier; moment analysis; brain perfusion; indicator dilution.

INTRODUCTION

The capillary endothelium of the brain plays an important role in homeostasis within the central nervous system (1). It limits the blood-to-brain passage of some solutes while facilitating exchange of others (2). This selective permeability barrier is termed the blood–brain barrier (BBB). The BBB is produced by a combination of specific membrane transport systems, a low rate of pinocytosis, and the presence of continuous intercellular tight junctions that seal adjacent endothelial cells together (1).

Brodie *et al.*, in their first systematic study on drug transport in the brain (3,4), measured drug concentration of the cerebrospinal fluid (CSF) after intravenous drug administration. Several techniques have been developed for the study of BBB transport (5), including the indicator dilution technique (IDT) (6,7), the brain uptake index (BUI) (8), intravenous administration (9), and *in situ* brain perfusion (10). We studied the effect of a nonionic surfactant on the BBB using the *in situ* brain perfusion technique (11). However, these methods have focused only on the unidirectional solute uptake from blood to brain, and therefore, the parameter obtained is the permeability–surface area product (the clearance for membrane permeation).

The statistical moment analysis, introduced into pharmacokinetics by Yamaoka *et al.* (12) and Cutler (13), is based on the statistical concept of sum, mean, and variance. Applying this analysis to a single-pass organ perfusion system, Kakutani *et al.* evaluated the disposition characteristics of prodrugs in the rabbit muscle (14), and Nishida *et al.* examined the hepatobiliary transport of phenol red (15). This analysis permits the separate assessment of drug elimination and distribution in an organ. The purpose of the present study was to investigate the drug disposition in the brain by moment analysis using an improved *in situ* brain perfusion technique.

MATERIALS AND METHODS

Chemicals

[^{14}C]Inulin (sp act, 9.51 mCi/g), [^{3}H]inulin (212 mCi/g), [^{14}C]antipyrine (54.7 mCi/mmol), [^{3}H]imipramine (63.9 Ci/mmol), [^{3}H]desipramine (63.0 Ci/mmol), and [^{3}H]water (450 Ci/mol) were obtained from New England Nuclear (Boston, MA). [^{3}H]Diazepam (85 Ci/mmol) and [^{3}H]propranolol (20 Ci/mmol) were purchased from Amersham-Searl (Arlington Heights, IL). Radiochemical purities of these compounds were checked prior to use (>97%). All other chemicals were of analytical grade commercially available.

Animal Preparation

Arterial cannulation was performed as previously re-

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ported (10). Male Wistar rats weighing 200–250 g were anesthetized with intraperitoneal pentobarbital (40 mg/kg). The right occipital and superior thyroid arteries were ligated and cut. After ligation of the pterygopalatine artery, the right external carotid artery was catheterized for infusion with 10 cm of the polyethylene tubing (O.D., 1.0 mm; Hibiki, Tokyo) filled with heparinized saline. After the arterial cannulation, heparin (500 IU/kg) was administered. Venous cannulation procedure was as follows. The right side of the neck was excised and the posterior facial vein was exposed. The venous cannula, which was 25 cm of the vinyl tubing (I.D., 0.8 mm; O.D., 1.2 mm; Dural Plastics & Engineering, Australia) connected with the tip of a disposable 20-G needle (Terumo, Tokyo), was inserted and fixed with a surgical adhesive.

Perfusion System and Procedure of Sampling

Immediately after the venous cannulation, blood flowed through the cannula. In order to avoid loss of blood, the common carotid artery was ligated and perfusion was started as soon as possible (within 20 sec) with the infusion pump (No. 944, Harvard Apparatus, South Natick, MA). The perfusion fluid was HCO_3-buffered physiological saline (142 m*M* NaCl, 28.0 m*M* $NaHCO_3$, 4.2 m*M* KH_2PO_4, 1.7 m*M* $CaSO_4$, 1.0 m*M* $MgSO_4$, 6.0 m*M* glucose). Thirty seconds after the start of perfusion, the drug and inulin (vascular reference substance; VRS) dissolved in 0.100 ml of perfusion fluid were injected into the line of perfusion flow via a six-position-rotary valve injector (Type 50 Teflon rotary valves, Rheodyne, CA) as a rapid bolus. Venous outflow fluid (mixture of perfusion fluid and blood) was collected in weighed tubes at 1-sec intervals for 40 sec. Then the tubes were reweighed and the contents were transferred to scintillation vials. The samples were solubilized in 1 ml of Soluene 350 (Packard, IL) containing 50% isopropyl alcohol. H_2O_2 (0.4 ml) was added for decolorization, and the samples were incubated at room temperature for 15 min and then at 40°C for 15 min. After the incubation, 60 μl of 5 *N* HCl was added for both decomposition of excess H_2O_2 and neutralization of the samples. Then 8 ml of Clearsol (Nacalai Tesque, Kyoto, Japan) was added and dual-label counting was performed with a liquid scintillation counter, LSC3500 (Aloka, Tokyo). Tracer contents were calculated with an appropriate correction for counting efficiency and quenching. The concentration is expressed as percentage of injected dose per milliliter of perfusion fluid.

Percentage of Blood Weight in Venous Outflow

In the IDT, venous outflow is the mixture of the perfusion fluid from the right side of the brain and systemic blood from the left side. Since the drug is introduced into the brain and leaves the skull with the flow of perfusion fluid, mixing of perfusion fluid with systemic blood is assumed as dilution. In order to express the concentration as percentage of dose per milliliter of perfusion fluid, the volume of perfusion fluid in each fraction (i.e., the degree of mixing of perfusion fluid with blood) must be evaluated. The degree of mixing is expressed as percentage of blood weight (the percentage of blood weight in outflow fluid) in this study. For the determination of percentage of blood weight, two samples at 2-sec intervals before injection and three samples of 3-sec intervals after the sampling period were collected. Sufficient saline was added to each sample. The tubes were centrifuged and the supernatant was discarded. Then samples were air-dried overnight at about 50°C, and the dry weight of blood cells was obtained. The weight of whole blood was calculated according to the following equation:

$$\text{Weight}_{\text{whole blood}} = 5.92 \times \text{dry weight}_{\text{blood cells}}$$

This equation was determined in control experiments, using rat whole blood. The weight of whole blood was subtracted from that of the sample to obtain the weight of perfusion fluid. The percentage of blood weight of each fraction was calculated by interpolation between the percentage before and the percentage after the sampling period.

Perfusion Fluid Flow into the Left Side of the Brain on the Arterial Side

Rats were prepared as described above. Thirty seconds after the start of perfusion, [^{3}H]diazepam (0.1 μCi/0.1 ml) was injected from the carotid artery, and the rat was decapitated 15 sec after the injection. The brain was removed from the skull, divided into left and right sides, and placed separately in scintillation vials. The tissues were digested at room temperature in Soluene 350 and prepared for scintillation counting. Data are expressed as percentage of whole-brain uptake.

Data Analysis

The theoretical basis of the analysis was reported previously (14). According to the equations listed in Table I, disposition parameters were derived from the area under the outflow curve (auc_i), the mean transit time ($\bar{t}_i$), and the flow rate (Q_i, 4.6 ml/min). In the brain perfusion experiment, recirculation of VRS and the drug via systemic circulation was inevitable because of the complexity of the vascular system of the brain. Therefore, auc_i was calculated by numerical integration using the linear trapezoidal formula and extrapolation to infinite time based on monoexponential equation (see Results). $\bar{t}_i$ was corrected for lag time due to the internal volume of arterial and venous catheters. Q_i was assumed to equal the infusion pump rate (4.6 ml/min). The recovery ratio of the drug (F_i) was calculated from areas under the outflow curves of inulin and the drug as

Table I. Derivation of the Disposition Parameters from Moments

(I) Distribution	
(a) Extent	
Apparent steady-state distribution volume	$V_i = Q_i \bar{t}_i / F_i$
(II) Irreversible uptake	
(a) Extent	
Extraction ratio	$E_i = 1 - F_i$
Recovery ratio	$F_i = auc_{i,drug}/auc_{i,VRS}$
(b) Rate	
Apparent first-order uptake rate constant	$k_{up,i} = E_i/\bar{t}_i$
(III) Clearance	
Apparent intrinsic clearance	$CL_{int,i} = Q_i\bar{t}_i/E_i$

$$F_i = \text{auc}_{i,\text{drug}}/\text{auc}_{i,\text{VRS}}$$

The apparent unidirectional extraction ratio (E_u) was calculated from the following equation (7):

$$E_u = 1 - C_{\text{drug}}/C_{\text{VRS}}$$

where C_{drug} and C_{VRS} are concentrations of the drug and VRS, respectively. E_u decreased with time because of drug backflux. Therefore, the largest value was regarded as E_u. The recovery ratio of VRS was calculated from auc_i of VRS and the venous flow rate of perfusion fluid (Q) as $Q \cdot \text{auc}_i$. Q was obtained by the division of the sum of perfusion fluid in each fraction by the sampling period (40 sec).

RESULTS

Determination of the Experimental Condition

Whereas the circulatory system of the brain is complex, the simple model shown in Fig. 1 was employed. The right internal carotid artery is joined together in the skull with the left internal carotid artery, forming the circle of Willis. The arterial blood flows from the circle of Willis into anterior, middle, and posterior cerebral arteries. The venous blood from the right and left sides of the brain converges in the superior sagital sinus and separates into the right and left transverse sinuses. The blood in the transverse sinus flows out of the skull into the posterior facial vein (16). For the determination of the flow rate of the perfusion fluid, one must consider that the perfusion fluid can flow into the left side of the brain on the arterial side. Since diazepam is highly lipophilic and its tissue uptake is considered to parallel the distribution of perfusion fluid, tissue uptake of diazepam was examined in order to evaluate the perfusion fluid flow into the left side of the brain. Figure 2 represents the relation of the tissue uptake of diazepam in the left side of the brain to the infusion rate of the pump. Takasato *et al.* reported that the infusion rate was adjusted to 5.0 ml/min in order to minimize the contribution of systemic blood to perfusion fluid flow in the right side of the brain (10). In our experiment, when venous cannulation was not performed, the tissue uptake of diazepam in the left side of the brain was very small and negligible, at 5.0 ml/min. However, it showed the large value at 5.0 ml/min when venous cannulation was performed. The distribution of perfusion fluid was likely to change when venous outflow was obtained. At 4.6 ml/min, the tissue uptake of diazepam was small and perfusion fluid into the left side of the brain was considered to be negligible. Therefore, a pump infusion rate of 4.6 ml/min was chosen in the following experiments.

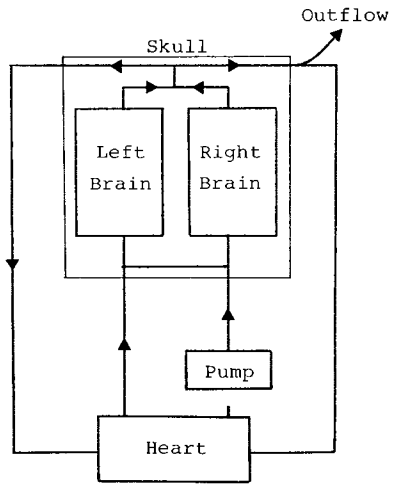

Fig. 1. Schematic representation of the circulatory system of the brain. Arrows indicate the flow direction of blood or perfusion fluid. The square drawn with fine lines represents the skull.

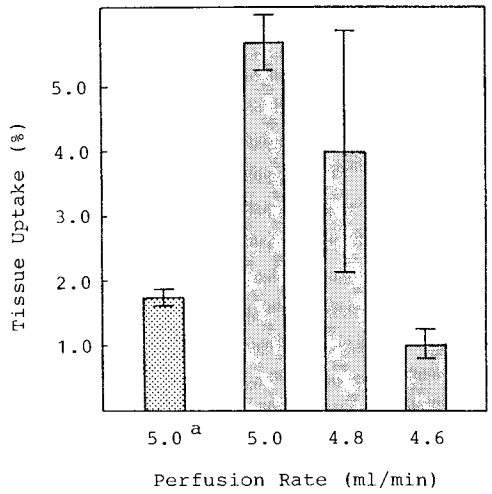

Fig. 2. The relation of tissue uptake of diazepam to infusion rate of the pump. Tissue uptake of diazepam is expressed as percentage of whole-brain uptake. Data are expressed as mean ± SD of three animals. Superscript a, the result of the experiment in which venous cannulation was not performed.

In order to determine the time when the drug and VRS are injected, the change of the venous outflow rate and percentage of blood weight vs time was examined. The venous outflow rate increased during the initial 20 sec and then became constant. On the contrary, the percentage of blood weight initially decreased and showed a constant value from 30 sec after the start of perfusion (data not shown). It was obvious that the perfusion system reached a steady state from 30 sec after the start of perfusion. Therefore, the drug and VRS were injected at 30 sec after the start of perfusion. The venous outflow was collected for 40 sec in order to obtain auc_i as accurately as possible.

The Extrapolation of the Venous Outflow Curve of Inulin

A typical venous outflow curve of inulin plotted on a semilogarithmic scale is shown in Fig. 3. The outflow curve of a single-pass system was shown to possess the monoexponential terminal phase in the case of a rapid bolus injection (17). The venous outflow curve in Fig. 3 has no monoexponential terminal phase. As can be seen from Fig. 1, this represents the influence of recirculation of VRS via the systemic circulation. Since the contribution of recirculation is considered larger in the later region of the venous outflow curve, the linear portion around 20 sec was extrapolated to obtain auc_i and the recovery ratio of inulin was calculated. Figure 4 shows the relation between the recovery ratio of inulin and that of perfusion fluid in venous outflow fluid. The recovery ratio of inulin was proportional to that of perfusion

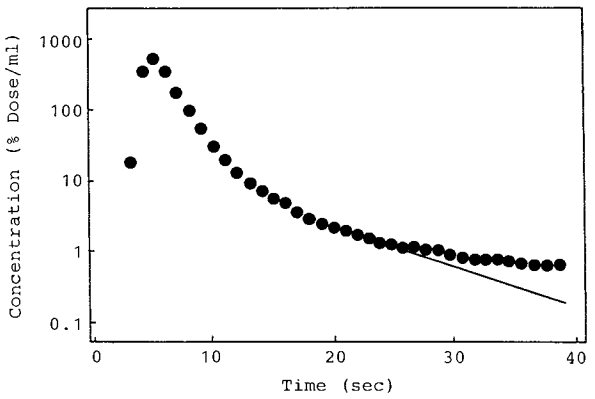

Fig. 3. The typical outflow curve of inulin plotted on a semilogarithmic scale. The line represents the extrapolation function for the estimation of auc_i.

fluid and the slope of the best-fitting line in Fig. 4 is 1.006, suggesting the validity of the extrapolation.

Disposition Characteristics of the Drugs

Figure 5 shows outflow patterns of diazepam, water, and antipyrine. Moment and disposition parameters are shown in Table II. The concentration of diazepam is low at peak and high in the terminal phase. The slope of the terminal phase of the curve of diazepam plotted on a semilogarithmic scale is small compared to those of the other drugs. These findings are consistent with the high lipophilicity of diazepam. Water and antipyrine showed similar curves. Figure 6 shows outflow patterns of imipramine, desipramine, and propranolol, and Table III lists the moment and disposition parameters. The outflow curves of these drugs were similar and indicated a large degree of uptake and a small degree of efflux. Table IV lists the moment parameters of propranolol in the presence of 0.1 and 1 m*M* unlabeled propranolol in the perfusion fluid. With increasing concentrations of unlabeled propranolol, E_i and $\bar{t}_i$ changed significantly, whereas E_u was unchanged.

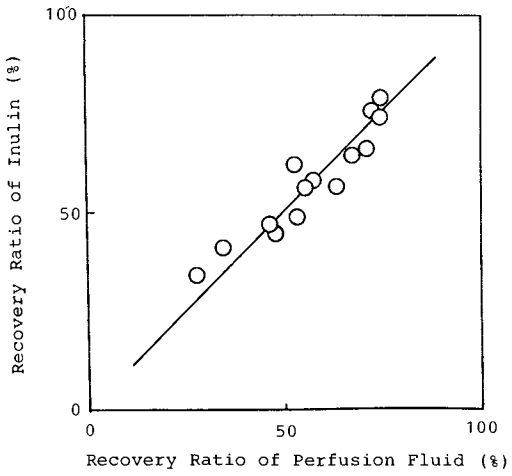

Fig. 4. The relation between the recovery ratio of inulin at infinite time and that of perfusion fluid. The line is the least-squares fit and its slope is 1.006.

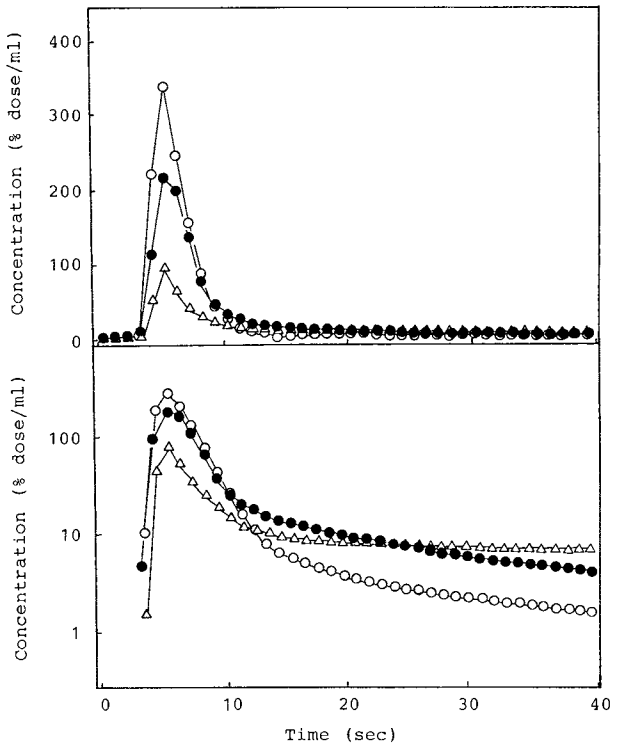

Fig. 5. Outflow curves of diazepam (△), water (●), and antipyrine (○) plotted on a normal scale (upper) and on a semilogarithmic scale (lower). Each point represents the mean of three experiments.

DISCUSSION

The combination of the indicator dilution technique with *in situ* brain perfusion provides methodological advantages. First, the cerebral perfusion rate is constant. This allows the accurate measurement of drug parameters, since the inherent variability of cerebral blood flow caused by the physiological state of the animal has direct influences on the measurements. It is possible to measure brain blood flow simultaneously (18), but this is technically difficult. Second, the contribution of recirculation is considered to be small. One of the problems of brain IDTs is recirculation of the drug. A second peak was sometimes observed (19). Our findings showed that the percentage of blood weight is 30.7 ± 9.2% (mean of nine animals), with the greater part of venous outflow being perfusion fluid. Since systemic blood contains the recirculating drug, the contribution of recirculation is expected to be less than 30%. Moreover, more than 50% of injected VRS was recovered in the venous outflow during sampling period. Although the recovery ratio of the drug or VRS had never been calculated in previous brain IDTs, the amount of recirculating drug might be much smaller in our system. Third, there are no complexities such as serum protein binding and interaction of the drug with blood cells. Mixing of perfusate with systemic blood was only 0.23% with the *in situ* brain perfusion technique (20). Fourth, the regional perfusion flow rate is six- to eightfold greater compared with regional cerebral blood flow in barbiturate-anesthetized rats (10,21). With higher flow, measurement of the extraction fraction of highly lipophilic and highly extracted drug becomes less dependent on flow, making more accurate measurements possible.

Table II. Moments and Disposition Parameters of Inulin, Diazepam, Water, and Antipyrine[a]

	Inulin	Diazepam	Water	Antipyrine
(a) Moment parameter				
auc_i (% sec/ml)	1354.3 ± 149.5	902.1 ± 135.2	1201.7 ± 41.0	1266.2 ± 32.9
$\bar{t}_i$ (sec)	2.26 ± 0.15	54.4 ± 6.3	9.37 ± 0.31	3.17 ± 0.25
σ_i^2 (sec^2)	27.5 ± 15.3	5256.4 ± 1485.3	275.6 ± 35.6	61.0 ± 21.1
(b) Disposition parameter				
(1) Distribution				
V_i (ml)	0.173 ± 0.012	5.83 ± 0.83	0.802 ± 0.028	0.255 ± 0.021
(2) Irreversible uptake				
E_i		0.282 ± 0.036	0.126 ± 0.032	0.048 ± 0.020
$k_{up,i}$ (min^{-1})		0.313 ± 0.050	0.799 ± 0.172	0.911 ± 0.373
(3) Clearance				
$CL_{int,i}$ (ml/min)		1.82 ± 0.31	0.646 ± 0.165	0.233 ± 0.101
(c) Unidirectional extraction ratio				
E_u		0.826 ± 0.044	0.461 ± 0.026	0.167 ± 0.015

[a] Data are means ± SD of three experiments.

Hertz and Bowlig have reported the importance of extracerebral contamination to brain IDTs (22). Extracerebral contamination causes overestimation of extraction ratios of Na^+, glucose, etc. Ligation of the pterygopalatine artery is necessary to avoid extracerebral contamination (23). The D-[^{14}C]glucose extraction ratio of −0.019 (observed in our study) indicated that the extracerebral contamination can be ignored.

During brain IDT in the perfusion system, O_2 delivery may be insufficient to maintain normal aerobic brain metabolism. The rate of O_2 delivery is only half the O_2 demand of barbiturate-anesthetized rats (21). However, perfusion for up to 60 sec does not significantly alter cerebrovascular permeabilities to nonelectrolytes (10). Further, 10-min perfusion of low-pH saline did not affect the ability of the BBB to exclude [^{14}C]mannitol (24). Since the total perfusion time was 70 sec, the metabolic effects of anoxic perfusion should have no influence on our results.

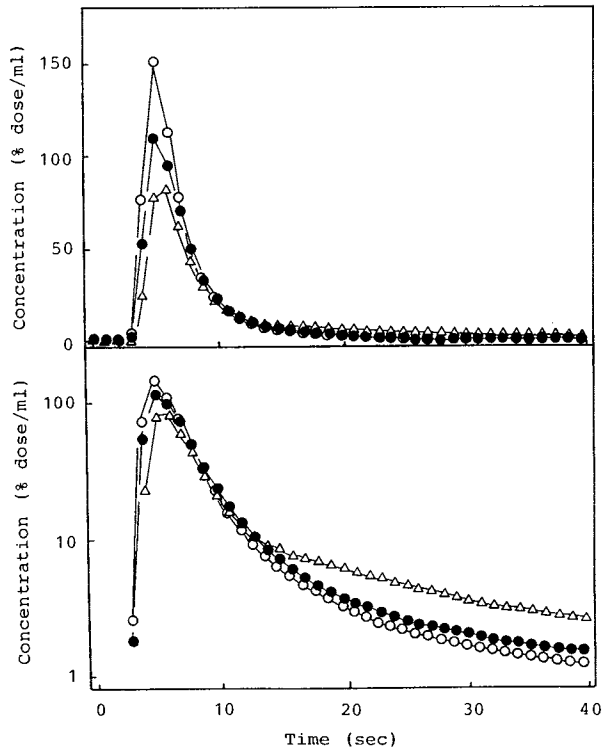

Fig. 6. Outflow curves of imipramine (○), desipramine (△), and propranolol (●) plotted on a normal scale (upper) and on a semilogarithmic scale (lower). Each point represents the mean of three experiments.

Various analytical methods have been used for the evaluation of drug disposition in an organ. Kakutani *et al.* applied moment analysis to a single-pass organ perfusion system, studying drug disposition in muscle (14), liver (15), and tumor-bearing tissue (25). Hori *et al.* also studied drug disposition in the kidney by moment analysis (26). Remarkably, the obtained parameters are free from restrictions arising from complex modeling. Therefore, these analytical methods are widely applicable. In addition, drug disposition in an organ can be separated into distribution and elimination, and the elimination process can be evaluated with respect to rate and extent. Therefore, moment analysis of the outflow curve provides a more detailed information on drug disposition in the brain.

In Table II, the V_i of inulin is given as 0.173 ml. Since inulin does not cross the BBB measurably, its V_i shows the internal volume of the vasculature through which the perfusion fluid flowed. The intravascular (intracapillary) volume of the brain was reported to be 1% (v/w) in a perfusion experiment (10). Therefore, the V_i of inulin approximates the internal volume of artery and vein. Diazepam was initially taken up by 82.6%, and 71.8% of the injected dose was recovered in the outflow, suggesting large degrees of both influx and efflux. Therefore, the V_i of diazepam was large compared with those of antipyrine and water. Water is one of the diffusible reference agents used in the BUI method. The E_u obtained by our method was 0.461, which is comparable with the value reported by Pardridge *et al.* (27). The V_i of water corrected for intravascular volume with the V_i of inulin seemed to be consistent with the volume of the perfused brain parenchyma, since its weight was approximately 0.6 g and brain specific gravity is 1.04 (9). Antipyrine was initially taken up by 16.7% and almost all of the injected dose

Table III. Moments and Disposition Parameters of Imipramine, Desipramine, and Propranolol[a]

	Imipramine	Desipramine	Propranolol
(a) Moment parameter			
auc_i (% sec/ml)	628.6 ± 61.1	555.5 ± 19.3	561.8 ± 68.6
$\bar{t}_i$ (sec)	5.39 ± 0.41	11.57 ± 0.85	6.74 ± 0.45
σ_i^2 (sec^2)	151.6 ± 20.9	371.7 ± 123.4	182.9 ± 15.9
(b) Disposition parameter			
(1) Distribution			
V_i (ml)	0.889 ± 0.123	2.14 ± 0.12	1.23 ± 0.17
(2) Irreversible uptake			
E_i	0.530 ± 0.047	0.584 ± 0.014	0.575 ± 0.032
$k_{up,i}$ (min^{-1})	5.92 ± 0.56	3.03 ± 0.27	5.13 ± 0.19
(3) Clearance			
$CL_{int,i}$ (ml/min)	5.28 ± 1.03	6.47 ± 0.39	6.28 ± 0.79
(c) Unidirectional extraction ratio			
E_u	0.545 ± 0.032	0.734 ± 0.094	0.701 ± 0.019

[a] Data are means ± SD of three experiments.

was recovered in venous outflow. Therefore, antipyrine showed small $CL_{int,i}$ and V_i, reflecting its low lipophilicity. Imipramine, desipramine and propranolol all displayed large E_i and $CL_{int,i}$ in comparison with those of the drugs described above. Pardridge *et al.* suggested that the physical basis of the sequestration of these lipophilic amines is binding to high-capacity cytoplasmic proteins (28). Our findings that the E_i and $\bar{t}_i$ of propranolol were significantly changed in the presence of unlabeled propranolol may be due to saturation of binding to these proteins. The large E_i and $CL_{int,i}$ of these drugs also may be attributed to protein binding.

Recently, Sawada *et al.* investigated the cerebrovascular transport in detail based on the model proposed by Goresky (23). However, the parameter obtained by previous brain IDTs was only the unidirectional extraction ratio, partly because the influence of recirculation was large. Therefore, the sampling period was a few seconds after drug injection without drug recirculation and the tissue uptake was truly unidirectional. Some of these problems can be solved with the use of brain IDT in the perfusion system. Further, with moment analysis, drug disposition in the brain can be divided into distribution and irreversible uptake processes. This study is the first example of evaluating drug disposition characteristics in the brain by moment analysis.

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Table IV. $\bar{t}_i$, E_i, and E_u of Propranolol at Various Concentrations of Unlabeled Propranolol in the Perfusion Fluid[a]

	$\bar{t}_i$	E_i	E_u
0 m*M*	6.74 ± 0.45	0.575 ± 0.032	0.701 ± 0.019
0.1 m*M*	7.11 ± 0.77	0.465 ± 0.045*	0.676 ± 0.031
1 m*M*	10.50 ± 0.34**	0.408 ± 0.013**	0.745 ± 0.018

[a] Values represent means ± SD of three experiments.
* $P < 0.05$, compared with 0 m*M* by Student's *t* test.
** $P < 0.01$, compared with 0 m*M* by Student's *t* test.

REFERENCES

1. S. I. Rapoport. *Blood-Brain Barrier in Physiology and Medicine*, Raven Press, New York, 1979.
2. W. M. Pardridge and W. H. Oldendorf. Transport of metabolic substrate through the blood-brain barrier. *J. Neurochem.* 28:5–12 (1977).
3. S. Mayer, R. P. Maickel, and B. B. Brodie. Kinetics of penetration of drugs and other foreign compounds into cerebrospinal fluid and brain. *J. Pharmacol. Exp. Ther.* 127:205–211 (1959).
4. B. B. Brodie, H. Kurz, and L. S. Schanker. The importance of dissociation constant and lipid-solubility in influencing the passage of drugs into the cerebrospinal fluid. *J. Pharmacol. Exp. Ther.* 130:20–25 (1960).
5. J. D. Fenstermacher, R. G. Blasberg, and C. S. Patlak. Methods for quantifying the transport of drugs across brain barrier system. *Pharmacol. Ther.* 14:217–248 (1981).
6. C. Crone. The permeability of capillaries in various organs determined by use of the indicator dilution method. *Acta Physiol. Scand.* 58:292–305 (1963).
7. C. Crone. The permeability of brain capillaries to non-electrolytes. *Acta Physiol. Scand.* 64:407–417 (1965).
8. W. H. Oldendorf. Measurement of brain uptake of radiolabeled substances using a tritiated water internal standard. *Brain Res.* 24:372–376 (1970).
9. S. I. Rapoport, K. Ohno, and K. D. Pettigrew. Drug entry into brain. *Brain Res.* 172:354–359 (1979).
10. Y. Takasato, S. I. Rapoport, and Q. R. Smith. In situ brain perfusion technique to study cerebrovascular transport in the rat. *Am. J. Physiol.* 247:H484–H493 (1984).
11. T. Sakane, C. Tanaka, A. Yamamoto, M. Hashida, H. Sezaki, H. Ueda, and H. Takagi. The effect of polysorbate 80 on brain uptake and analgesic effect of D-kyotorphin. *Int. J. Pharm.* 57:77–83 (1989).
12. K. Yamaoka, T. Nakagawa, and T. Uno. Statistical moments in pharmacokinetics. *J. Pharmacokinet. Biopharm.* 6:547–558 (1978).
13. D. J. Cutler. Theory of the mean absorption time, an adjunct to conventional bioavailability studies. *J. Pharm. Pharmacol.* 30:476–478 (1978).
14. T. Kakutani, K. Yamaoka, M. Hashida, and H. Sezaki. A new method for assessment of drug disposition in muscle: application of statistical moment theory to local perfusion systems. *J. Pharmacokinet. Biopharm.* 13:609–631 (1985).
15. K. Nishida, C. Tonegawa, T. Kakutani, M. Hashida, and H. Sezaki. Statistical moment analysis of hepatobiliary transport of phenol red in the perfused rat liver. *Pharm. Res.* 6:140–146 (1989).

16. E. C. Greene. *Anatomy of the Rat,* Hafner, 1963.
17. M. Weiss. Theorems on long-convex disposition curves in drug and tracer kinetics. *J. Theor. Biol.* **116**:355–368 (1985).
18. O. B. Paulson, M. M. Hertz, T. G. Bolwig, and N. A. Lassen. Filtration and diffusion of water across the blood-brain barrier in man. *Microvasc. Res.* **13**:113–124 (1977).
19. T. W. Furlow and N. H. Bass. Cerebral hemodynamics in the rat assessed by a non-diffusible indicator dilution technique. *Brain Res.* **110**:366–370 (1976).
20. S. Momma and Q. R. Smith. Validity of four assumptions of the in situ brain perfusion technique. *Soc. Neurosci. Abstr.* **11**:840 (1985).
21. B. K. Siesjo. *Brain Energy Metabolism,* John Wiley & Sons, Chichester, 1978.
22. M. M. Hertz and T. G. Bolig. Blood-brain barrier studies in the rat: An indicator dilution technique with tracer sodium as an internal standard for estimation of extracerebral contamination. *Brain Res.* **106**:333–343 (1976).
23. Y. Sawada, C. S. Patlak, and R. G. Blasberg. Kinetic analysis of cerebrovascular transport based on indicator dilution technique. *Am. J. Physiol.* **256**:H794–H812 (1989).
24. J. Greenwood, A. S. Hazell, and P. J. Luthert. The effect of a low pH saline perfusate upon the integrity of the energy-depleted rat blood-brain barrier. *J. Cerebr. Blood Flow Metab.* 9:234–242 (1989).
25. R. Atsumi, K. Endo, T. Kakutani, Y. Takakura, M. Hashida, and H. Sezaki. Disposition characteristics of mitomycin C-dextran conjugate in normal and tumor-bearing muscles of rabbits. *Cancer Res.* 47:5546–5551 (1987).
26. R. Hori, Y. Tanigawara, Y. Saito, Y. Hayashi, T. Aiba, K. Okumura, and A. Kamiya. Moment analysis of drug disposition in kidney: Transcellular transport kinetics of p-aminohippurate in the isolated perfused rat kidney. *J. Pharm. Sci.* 77:471–476 (1988).
27. W. M. Pardridge and G. Fierer. Blood-brain barrier transport of butanol and water relative to N-isopropyl-p-iodoamphetamine as the internal reference. *J. Cerebr. Blood Flow Metab.* **5**:275–281 (1985).
28. W. M. Pardridge, R. Sakiyama, and G. Fierer. Blood-brain barrier transport and brain sequestration of propranolol and lidocaine. *Am. J. Physiol.* 247:R582–R588 (1984).