Application of Microdialysis for Study of Caffeine Distribution into Brain and Cerebrospinal Fluid in Rats

Takenori NAKAZONO, Teruo MURAKAMI, Sachiko SAKAI, Yutaka HIGASHI, and Noboru YATA*

*Institute of Pharmaceutical Sciences, Hiroshima University School of Medicine, 1-2-3 Kasumi, Minami-ku, Hiroshima 734, Japan.
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The usefulness of microdialysis was examined for the chronological determination of caffeine concentration in the brain and cerebrospinal fluids (CSF) following intravenous administration of caffeine in rats. The recovery percent of caffeine by microdialysis, the concentration ratio of caffeine in the dialysate against that in the brain tissue or CSF was determined. The recovery percent was proved to be constant at 5 different steady-state plasma concentrations of caffeine (0.1—280 nmol/ml) and in different collecting periods of dialysate ranging from 30 s to 10 min. The mean recovery percent in the brain and CSF were 10.9 and 13.1%, respectively. Thus, microdialysis was proved useful for determination of drug concentration in the tissue and biological fluids with time resolution of more than 30 s.

The microdialysis method was then applied for the chronological determination of caffeine concentration in the brain and CSF following intravenous bolus administration. The estimated caffeine concentration in the brain and CSF was the same as those obtained by direct determination in isolated brain and CSF, respectively. Transfer of caffeine from plasma to brain and CSF were further pharmacokinetically analyzed using a modified 2-compartment model. In this kinetic model, the transfer of caffeine between the CSF and brain was neglected, since the mutual transfer of caffeine was not detected in *in vivo* experiments. Calculated curves were well fitted on observed caffeine concentrations in the plasma, brain and CSF.

Keywords microdialysis; brain; cerebrospinal fluid; caffeine; recovery percent; brain distribution; cerebrospinal fluids distribution; kinetic analysis; rat

In vivo microdialysis has recently been widely used as a new technique for determining the unbound concentration of substances in the extracellular space of organs and/or biological fluids.[1-6] This technique involves the implantation of a microdialysis probe into the tissues and single perfusion or recirculation of a dialyzing fluid inside a semi-permeable membrane located at the tip of the probe. Microdialysis has been used to monitor chemical events in the brain in neurochemical study. Much interesting information concerning the composition of the brain interstitial fluids such as cathecholamines,[7,8] amino acid,[9,10] endogenous ions[11,12] and neuropeptides[13-15] has been obtained under various conditions in discrete brain areas.

There are few applications of microdialysis for the quantitative determination of drug concentration in the brain and/or cerebrospinal fluids (CSF). However, for this, the recovery ratio of a drug in the dialysate must be precisely determined. The recovery of a drug in the dialysate from external fluid is known to be affected by many factors such as flow rate of the perfusate,[16] diffusion coefficient of drugs,[17] interaction between drug molecules and the dialysis membrane,[18] membrane area[19] and composition of perfusate and drainage.[20,21] Several mathematical approaches for estimation of the brain interstitial concentration from microdialysis data have been reported by Jacobson *et al.*,[16] Lindefors *et al.*,[22] and Ungerstedt *et al.*[23] However, Benveniste concluded that there is no doubt that most of the results obtained with microdialysis are only a reflection of the brain interstitial concentration of the substance and are not correct values.[20] Thus, the theoretical analysis of microdialysis data is still under investigation.

In the present study, we examined the usefulness of microdialysis for chronological determination of drug concentration in the brain tissue and CSF of rats *in vivo*. Caffeine was employed as a model compound, since caffeine is known to be distributed from plasma into the brain and CSF. Further, the concentration of caffeine in the brain and CSF following intravenous bolus administration was determined using microdialysis and was pharmacokinetically analysed.

Materials and Methods

Materials Caffeine, theophylline and theobromine were purchased from Wako Pure Chemical Ind., Ltd. (Osaka, Japan). 1-Methyl-[^{14}C]caffeine (55 mCi/mmol) and [H^3]inulin (1.7 Ci/mmol) were purchased from American Radiolabeled Chemicals Inc. (St. Louis, MO., U.S.A.) and Amersham International Ltd. (Buckinghamshire, U.K.), respectively, through Japan Isotope Society (Tokyo, Japan). Clear-sol I as a scintillation cocktail was obtained from Nacalai Tesque, Ltd., Kyoto, Japan. All other reagents were of reagent grade and used without further purification.

Animal Study Implantation of the Microdialysis Probe: Male Wistar rats weighing 200—250 g were anesthetized by an intraperitoneal injection of sodium pentobarbital (Nembutal, 50 mg/kg) and then were mounted on a stereotaxic frame in a prone position. After exposing and drilling the skull, two or three commercially available guide cannulas (length, 12.3 mm; outer diameter, 1.05 mm, Carnegie Medicine, Stockholm, Sweden) were inserted 2.0 mm above targeting sites (frontal cortex, cisterna magna and/or right lateral ventricle) with a micromanipulator according to the brain atlas reported by Paxinos and Watson[24] and were fixed with dental cement: (coordinates: anterior (A) = −0.2 mm, lateral (L) = 5.7 mm, ventral (V) = 6.7 mm for frontal cortex; A = −16.0 mm, L = 0 mm, V = 8.0 mm with an anterial inclination of 10° for cisterna magna; and A = 5.2 mm, L = 5.0 mm, V = 7.5 mm for right lateral ventricle, respectively). Dummy probes were inserted into the guide cannula to prevent the leakage of extracellular or cerebrospinal fluids. The cannula implanted rats were housed in a separate cage with free access to food and water. The cages were placed in a 12 h dark/light cycle room kept at 25 °C. After at least 6 d, rats were anesthetized and mounted on a stereotaxic frame in a small booth kept at 37 °C, and dummy probes were substituted for microdialysis probes (CMA/10, Carnegie Medicine, Stockholm, Sweden) by a micromanipulator so that the semi-permeable membrane portion (length, 2.0 mm; outer diameter, 0.64 mm) of the probe was exposed to the targeting sites (frontal cortex, cisterna magma, and/or right lateral ventricle). The semi-permeable membrane of the microdialysis probe is made from a polycarbonate–ether polymer and has a surface area of 3.14 mm²; compounds having

molecular weight of more than 20 kilodaltons will be cut-off by the membrane.

The inlet tubing of the microdialysis probe was connected to a microinfusion pump (CMA/100, Carnegie Medicine) and an artificial CSF solution (pH 7.30) was perfused at a flow rate of $2 \mu l/min$ in a single perfusing manner. The artificial CSF solution consists of 122 mM NaCl, 15 mM NaHCO$_3$, 10 mM glucose, 3 mM KCl, 1.4 mM CaCl$_2$, 1.2 mM MgSO$_4$, 0.4 mM K$_2$HPO$_4$ and 10 mM N-2-hydroxyethylpiperazine-N'-2-ethanesulfonic acid.[25]

Recovery of Caffeine in the Brain and CSF by Microdialysis *in Vivo*: To estimate the concentration of caffeine in the brain and CSF, the recovery percent, concentration ratio of [^{14}C]caffeine in the dialysate against in the brain tissue or CSF, was determined. A rat implanted with guide cannulas was anesthetized and was cannulated with polyethylene tubing (PE 10, Clay Adams, U.S.A.) in a femoral vein and an artery for the administration of drug solution and collection of blood, respectively. After implanting the microdialysis probes at the sites of frontal cortex and cisterna magna, an artificial CSF solution was perfused for 30 min before the drug administration. Steady state plasma concentration of caffeine was settled by an intravenous bolus administration followed by a constant rate infusion of caffeine containing a trace amount of [^{14}C]caffeine *via* the cannula in the femoral vein. The dose of [^{14}C]caffeine was constant at 23.61 μCi/kg for the intravenous bolus administration and 118.1 nCi/min/kg for the constant rate infusion and the dose of unlabelled caffeine was varied to settle 5 different steady state plasma concentrations ranging from 0.1 to 280 nmol/ml. After achievement of steady state plasma concentration, 2 h after initiation of drug infusion, dialysates were collected five times from the outlet tubing of a probe for each successive 0.5, 1, 2, 4, and 10 min. At the end of 10 min-collection, microdialysis probes were removed and 50 μl of CSF was directly and quickly collected by inserting PE 10 polyethylene tubing into the cisternal magna *via* the guide cannula. Then, the rat was immediately killed by decapitation to remove the brain. On this occasion, it was confirmed that the probe had been inserted into the precise targeting site, otherwise, the data were not adopted. The dialysate in each collecting duration was spotted on silica gel thin layer chromatography (TLC) (60F$_{254}$, 20 × 20 cm, E. Merck, Darmstadt, West Germany) to separate [^{14}C]caffeine and its metabolites. The isolated brain and CSF samples were used for the determination of caffeine concentration and were stored at $-30 °C$ until analysis. For calculation of the recovery percent of caffeine by the microdialysis, the radioactive concentration of [^{14}C]caffeine recovered in the dialysate in each collecting duration was divided by the radioactive caffeine concentration determined in the isolated brain or CSF. The concentration of caffeine in the isolated brain tissue involves caffeine both in the extracellular space and in the brain cells. Thus, caffeine concentration in the brain tissue can be estimated by such a recovery percent, irrespective of determining caffeine concentration in brain interstitial fluids by microdialysis technique.

Brain/CSF Uptake of Caffeine after Intravenous Bolus Administration: After implantation of microdialysis probes into frontal cortex and cisterna magna under anesthesia, an artificial CSF solution was perfused for 30 min before drug administration at a flow rate of $2 \mu l/min$. Then, caffeine was given from left femoral vein at a dose of $30 \mu mol/kg$ containing a trace amount of [^{14}C]caffeine (50 μCi/kg). Sampling of each dialysate was performed at appropriate time intervals. The collecting period was changed according to the concentration of caffeine in the brain or CSF to secure the total radioactivity in the dialysate above the assay limit in the following successive way: 30 sec-period 4 times, 2 min-period once, 5 min-period 3 times, 6 min-period once, 10 min-period 5 times. For the first sampling, the time lag of 3.5 min which is necessary for the perfusate to come out in the sampling tube due to the void volume of 7 μl of the outlet tubing was also taken into consideration. Blood samples were also chronologically collected *via* polyethylene tubing cannulated in the right femoral artery. In a separate experiment, brain and CSF were isolated 15, 30, 60 or 90 min after the intravenous bolus administration of caffeine at the same dose, and the concentrations of [^{14}C]caffeine in the brain and CSF were determined to validate the results in the microdialysis study.

Transfer of Caffeine between CSF and Brain: The transfer of caffeine from CSF into the brain was examined *in vitro* and *in vivo*. The *in vitro* study was carried out as follows: A freshly isolated brain (about 1.5 g) from a control rat was preincubated in 1.5 ml of an artificial CSF solution for about 5 min at 37 °C, then an aliquot of artificial CSF solution containing tracer amounts of [^{14}C]caffeine and [^{3}H]inulin or toluidine blue O was added to the incubating solution. Final concentra-

tion of [^{14}C]caffeine, [^{3}H]inulin and toluidine blue O was 25, 25 nCi/ml and 1.0 μmol/ml, respectively. The incubation was carried out under slow shaking (20 time strokes/min). Inulin and toluidine blue O were used as extracellular space marker and brain permeable marker, respectively. Sampling of the incubating solution was made at appropriate time intervals for 3 h to determine the remaining concentrations of [^{14}C]-caffeine, [^{3}H]inulin or toluidine blue O.

In vivo transfer of caffeine from CSF to the brain was examined as follows: Rats implanted with three guide cannulas in advance at frontal cortex, cisterna magna and right lateral ventricle were cannulated with polyethylene tubing in a femoral artery for blood collection. Microdialysis probes were implanted into the frontal cortex and cisterna magna through two of the guide cannulas. An artificial CSF solution was perfused for 30 min before drug administration. Then, 5 μl of the artificial CSF solution containing [^{14}C]caffeine (1 μCi) and [H^3]inulin (5 μCi) was injected into the right lateral ventricle in 1 min through the guide cannula followed by sealing of the cannula with a dummy probe to prevent the leakage of drug solution. Dialysate from the frontal cortex and cisterna magna was chronologically collected at appropriate collecting periods and was spotted on a silica gel TLC to separate [^{14}C]caffeine, its metabolites and [^{3}H]inulin. In this study, the recovery percent of [^{3}H]inulin by microdialysis was also determined from the concentration of [^{3}H]inulin in the dialysate at the final sampling, and by the concentration of inulin in the CSF in cisterna magna obtained by direct sampling immediately after the end of this experiment.

Transfer of caffeine from the brain to CSF *in vivo* was examined as follows: A rat implanted with three guide cannulas in advance at frontal cortex, cisternal magna and striatum (coordinate: A = 0.7 mm, L = 3.0 mm and V = 6.7 mm) was cannulated with polyethylene tubing in a femoral artery for blood sample collection. After setting microdialysis probes at frontal cortex and cisterna magna, an artificial CSF solution was perfused for 30 min before drug administration. Then, an artificial CSF solution (5 μl) containing [^{14}C]caffeine (1 μCi) and [^{3}H]inulin (5 μCi) was injected into the striatum, followed by plugging with a dummy probe, and dialysates from frontal cortex and cisterna magna were chronologically collected at appropriate periods.

Analysis Blood was centrifuged at 3000 rpm for 10 min to obtain plasma sample. Isolated brain was homogenized in distilled water to make a 33% homogenate. Plasma, brain homogenates and directly obtained CSF were deproteinized with an equal volume of acetonitrile. Supernatants (40 μl) of the mixture obtained by centrifusing at 10000 rpm were spotted on a silicagel TLC film. All dialysates in microdialysis were spotted on a TLC film without any pretreatment. The TLC film was developed with a developing solution consisting of chloroform, acetone, n-butanol and NH$_4$OH (30 : 30 : 40 : 10, v/v) to separate caffeine, its metabolites such as paraxanthine and theophylline and/or inulin.[26] A part corresponding to the Rf value of each authentic compound (Rf value: caffeine, 0.70; paraxanthine, 0.21; theophylline, 0.34; inulin, 0) was cut off by scissors. The piece of TLC film was put into a vial containing 4 ml of scintilation cocktail. Radioactivity of these samples was counted in a liquid scintillation counter (LSC-903, Aloka, Tokyo, Japan).

Data Analysis A pharmacokinetic analysis was made using MULTI or MULTI-RUNGE, a nonlinear least squares analysis program for microcomputer developed by Yamaoka and Nakagawa,[27] with minor modifications.

Results

Evaluation of Microdialysis to Determine Caffeine Concentration in the Brain and Cerebrospinal Fluid
Feasibility of the microdialysis method for the simultaneous and chronological determination of caffeine concentration in the brain and CSF was examined under different steady-state plasma concentrations of caffeine. The plasma concentration–time profiles of caffeine, theophylline and paraxanthine following the intravenous bolus administration of caffeine (30 μmol/kg) containing tracer amount of [^{14}C]caffeine (50 μCi/kg) are shown in Fig. 1. Determination of the concentration of caffeine and its metabolites is based on the radioactivity of labelled carbon atom in 1-methyl group of caffeine molecular structure. Therefore, the concentration of theobromine, one metabolite of
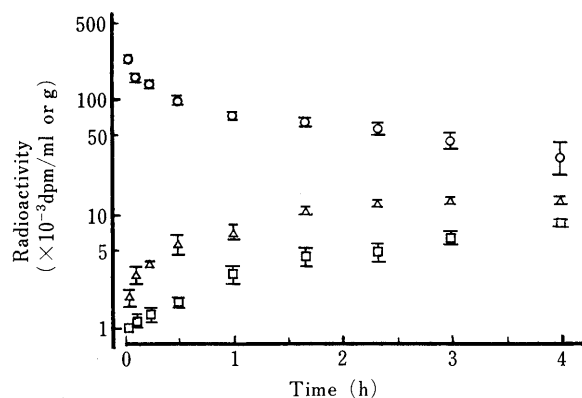
Fig. 1. Radioactivity of [^{14}C]Caffeine and Its Metabolites in Plasma Following Intravenous Administration of [^{14}C]Caffeine (50 μCi/kg) with Cold Caffeine (30 μmol/kg) in Rats

○, caffeine; △, paraxanthine; □, theophylline. Each point represents the mean ± S.E.M. of 4 trials.

TABLE I. CSF/Plasma and Brain/Plasma Values of Caffeine under Different Steady-State Plasma Concentrations in Rats

C_{ss} concn. (nmol/ml)	CSF/plasma	Brain/plasma
0.096 ± 0.006	0.848 ± 0.040	1.051 ± 0.166
0.402 ± 0.013	0.950 ± 0.034	0.965 ± 0.063
11.43 ± 1.44	0.821 ± 0.053	0.745 ± 0.064
60.87 ± 2.05	0.931 ± 0.038	0.748 ± 0.034
281.7 ± 6.2	0.905 ± 0.033	0.836 ± 0.046

Each value represents the mean ± S.E.M. ($n = 3$—8).

caffeine in rats having no methyl group at a position C_1, was not determined. The disappearance of caffeine from plasma was pharmacokinetically analyzed using a 2-compartment model. The calculated values of the distribution volume of central compartment (V_1) and total body plasma clearance (CL_{total}) of caffeine were 337.6 ± 28.0 ml/kg, and 4.53 ± 0.80 ml/min/kg, respectively.

The CSF-to-plasma and brain-to-plasma concentration ratios of caffeine determined in the isolated CSF and brain under 5 different steady state plasma concentrations (C_{ss}) are listed in Table I. In this case, observed C_{ss} values of caffeine were markedly different from the predicted values calculated with the pharmacokinetic data following intravenous bolus administration, because the CL_{total} of caffeine, calculated by dividing the infusion rate (I_0) by C_{ss}, decreased with an increase of C_{ss} value as follows: 20.8 ml/min/kg at C_{ss} of 0.097 nmol/ml; 17.9 at 0.402; 6.65 at 11.4; 3.58 at 60.9; 2.55 at 281.7. This dose–dependent plasma kinetics of caffeine may be due to the saturation of enzymic metabolism to theophylline, theobromine and paraxanthine.[4] However, the distribution of caffeine into the CSF and brain was almost the same degree within the C_{ss} (Table I). Mean CSF-to-plasma and brain-to-plasma ratios were 0.887 ± 0.022 and 0.858 ± 0.045, respectively.

Recovery percent of caffeine in CSF and brain in microdialysis in 5 different collecting periods (0.5, 1.0, 2.0, 4.0, 10.0 min) and at 5 different C_{ss} values (0.1, 0.4, 10, 60, 280 nmol/ml) is shown in Figs. 2 and 3, respectively (total trial number = 54). As shown in these figures, almost constant recovery percent in brain and CSF dialysates was obtained at different C_{ss} of caffeine and different dialysate
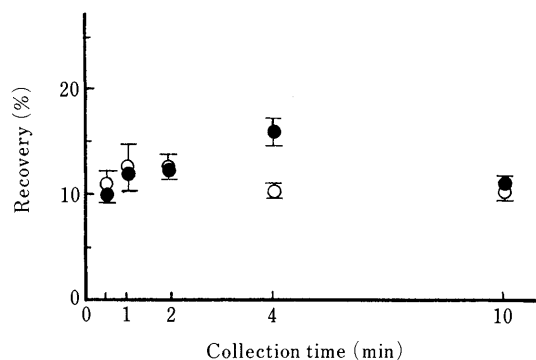

Fig. 2. Recovery Percents of [^{14}C]Caffeine by Microdialysis at 5 Different Dialysate Collecting Periods in Rats

Recovery in the brain (○) and CSF (●) dialysate, determined at a steady-state plasma concentration of caffeine ranging from 0.1 to 280 nmol/ml. Each point represents the mean ± S.E.M. of 4 to 17 trials. No significant difference was detected in recovery percents among different conditions.

Fig. 3. Recovery Percents of [^{14}C]Caffeine by Microdialysis at 5 Different Steady-State Plasma Concentrations of Caffeine in Rats

Recovery in the brain (○) and CSF (●) dialysate, determined at different dialysate collecting periods ranged from 30 s to 10 min. Each point represents the mean ± S.E.M. of 4 to 10 trials. No significant difference was detected in recovery percents among different conditions.

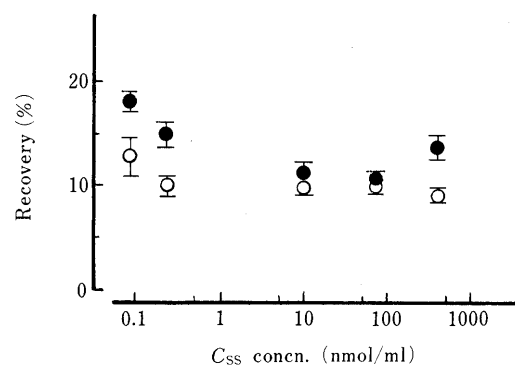collecting periods. The recovery percent of caffeine in brain and CSF dialysates was 10.9 ± 0.7 and 13.1 ± 0.7%, respectively.

In microdialysis, only the unbound substances in the extracellular spaces of organs and/or biological fluids[1—6] can be dialyzed. Supposing that the concentration of unbound caffeine in the brain extracellular space is in equilibrium with the total caffeine concentration in the brain tissue and defining recovery percent as the concentration ratio between brain tissue and dialysate, the caffeine concentration in the brain tissue could be estimated by microdialysis.

Application of Microdialysis for the Determination of Caffeine Concentrations in the Brain and CSF Following Intravenous Administration The brain and CSF caffeine concentrations following the intravenous bolus administration of caffeine (30 μmol/kg) containing tracer amount of [^{14}C]caffeine (50 μCi/kg) were determined by microdialysis. Plasma level of caffeine was also determined at appropriate time intervals. In this experiment, the frontal cortex was used to evaluate the caffeine concentration in the brain, since caffeine was found to be almost equally distributed to all brain regions in a preliminary experiment as follows: cortex, 1; striatum, 0.96; hippocampus, 0.88; hypothalamus, 0.94; mid brain, 0.94; cerebellum, 1.08;
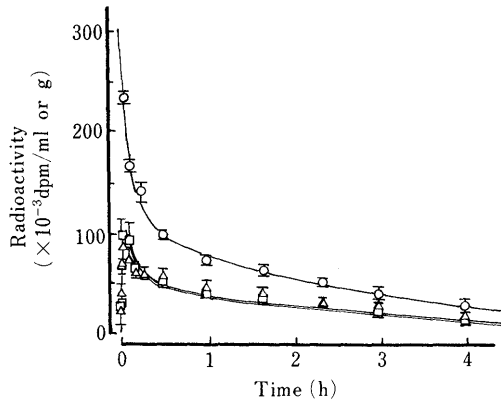
Fig. 4. Brain and CSF Distributions of Caffeine Following Intravenous Administration in Rats

Dose of caffeine: 30 μmol/kg containing tracer amount of [^{14}C]caffeine (50 μCi/kg). △, brain; □, CSF; ○, plasma. Each point represents the mean ± S.E.M. of 4 trials. Solid line represents the fitting curves calculated using parameters listed in Fig. 7.
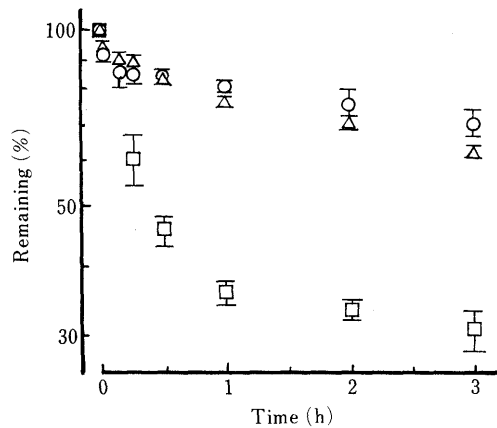

Fig. 6. Radioactivity of [^{14}C]Caffeine (A) and [^{3}H]Inulin (B) in Plasma and CSF after Intralateralventricular Injection of [^{14}C]Caffeine (1 μCi) and [^{3}H]Inulin (5 μCi) in Rats

○, □, plasma; △, CSF. Each point represents the mean ± S.E.M. of 6 trials.

Fig. 5. Disappearance of Caffeine, Inulin and Toluidine Blue O from Artificial CSF in Vitro

△, [^{14}C]caffeine; ○, [^{3}H]inulin; □, toluidine blue O. Each point represents the mean ± S.E.M. of 4 trials.

medullaoblongata, 1.04 by relative value as unity in the cortex. The caffeine concentration–time profile in the brain, CSF and plasma is shown in Fig. 4. The concentration of caffeine in the brain and CSF which were estimated by the results of microdialysis using the recovery percent was coincident with the results in a separate experiment in which the caffeine concentrations in the isolated brain and CSF were determined at each designated time without using microdialysis.

To analyze the distribution of caffeine from plasma into the brain and CSF, transfer of caffeine between brain and CSF also should be taken into consideration. As shown in Fig. 5, in an in vitro study, caffeine disappeared slowly from the medium at an almost identical rate as inulin, which was used as an extracellular space marker. On the other hand, the disappearance of toluidine blue O, a brain cell permeable marker, was far faster and more complete than inulin and caffeine. This finding means caffeine can slowly diffuse into extracellular space of the brain but not into brain cells as inulin.

The possible transfer of caffeine from the CSF into the brain was also examined in vivo. The time course of the caffeine concentration in the frontal cortex and the cisterna
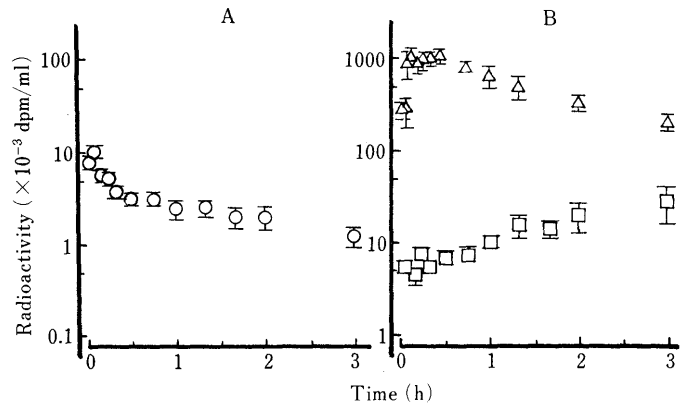
magna was determined using microdialysis after injection of tracer amounts of [^{14}C]caffeine (1 μCi) and [^{3}H]inulin (5 μCi) into the right lateral ventricle at a dosage of 5 μl/rat. The recovery percent of inulin in the dialysate from CSF in cisterna magna was 1.87 ± 0.07%. As shown in Fig. 6B, inulin injected at the right lateral ventricle appeared in cisterna magna at a maximal level after 30 min, and this level then decreased gradually whereas plasma level increased. No inulin was recovered from frontal cortex dialysate. Caffeine was not detected in either cisterna magna or frontal cortex dialysates, whereas a rapid transfer of caffeine into the blood circulation was observed (Fig. 6A). From the in vitro results, inulin and caffeine should be found in frontal cortex dialysate; however, in vivo study showed no such an evidence. These in vivo data probably indicate that inulin administered at the lateral ventricle mainly diffuses into the cisterna magna through the third and fourth ventricles by the bulk flow of CSF without reaching the frontal cortex, and is relatively slowly eliminated to the plasma via arachinoid villus. Also, caffeine which is permeable through the blood-brain barrier and blood-CSF barrier does not seem to diffuse into the brain tissue from the lateral ventricle to a great extent, but seems to be quickly eliminated into the blood circulation via choroid plexus, surrounding brain capillary, and/or circumventricular organs without reaching the cisterna magna. Caffeine and inulin injected in the striatum were not detected in the dialysate from the frontal cortex and cisterna magna in spite of the quick appearance of caffeine in the plasma. Thus, the above in vitro and in vivo studies suggest that the transfer of caffeine between CSF and the brain is almost negligible.

Based on these findings, a pharmacokinetic model (Fig. 7) was constructed to predict the brain and CSF distributions of caffeine from plasma following the intravenous bolus administration. In this model, the transfer of caffeine from plasma to the brain and CSF and vice versa were assumed not to affect the plasma concentration of caffeine, since the amount of caffeine uptaken by the central nervous system was fairly small compared to the amount in plasma. Also, the transfer between CSF and brain was neglected in this model, since the mutual transfer in vivo was not detected as demonstrated in Fig. 6. Among some

--------- equations ---------

$$\frac{dC(1)}{dt} = -(k_{10}+k_{14}) \times C(1) + k_{41} \times C(4)$$

$$\frac{dC(2)}{dt} = k_{12} \times C(1) - k_{21} \times C(2)$$

$$\frac{dC(3)}{dt} = k_{13} \times C(1) - k_{31} \times C(3)$$

$$\frac{dC(4)}{dt} = k_{14} \times C(1) - k_{41} \times C(4)$$

brain (2)

k_{12} k_{21}

k_{10}

central (1)

k_{13}

k_{31}

CSF (3)

k_{14} k_{41}

peripheral (4)

modified 2-compartment model

$C(1) = \text{dose}/V_1$

$C(2), C(3), C(4) = 0$ at time 0

parameters: (min^{-1})

k_{12}: 0.1859

k_{21}: 0.4148

k_{13}: 0.3018

k_{31}: 0.6562

k_{14}: 0.06563

k_{41}: 0.05227

k_{10}: 0.01286
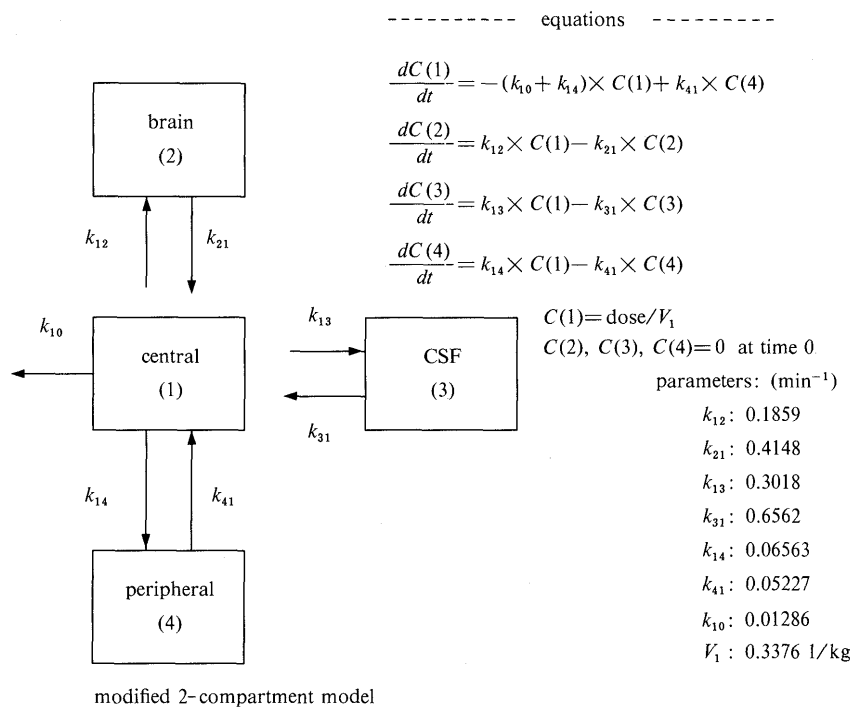
V_1 : 0.3376 1/kg

Fig. 7. Pharmacokinetic Compartment Model for Brain and CSF Uptake of Caffeine

possible pharmacokinetic models constructed to analyze the present results, the model shown in Fig. 7 showed a best fitting on the observed caffeine concentrations in the brain, CSF and plasma.

In the pharmacokinetic analysis using this model, parameters between the central and peripheral compartments such as elimination rate constant from the central compartment (k_{10}), transfer rate constant from the central to peripheral compartments (k_{14}), transfer rate constant from the peripheral to central compartment (k_{41}), and distribution volume of central compartment (V_1) were supposed to be the same as those analyzed in plasma disposition in Fig. 1. The transfer rate constant between plasma and the brain, $(k_{12}$ and $k_{21})$, and between plasma and CSF, $(k_{13}$ and $k_{31})$ were calculated. Estimated parameters are summarized in Fig. 7. Also, fitting curves shown in Fig. 4 as solid lines were in good agreement with the measured concentrations in plasma, brain and CSF.

Discussion

Many techniques have been developed to study the metabolism and function of putative neurotransmitters in the brain. Gaddum[28] developed the push–pull technique and Bito et al.[29] introduced a small dialysis bag into brain tissue. Also, Ungerstedt and Pycock[30] used a hollow dialysis fiber for intracerebral microdialysis. Recently, commercially available microdialysis equipment has been in broad use mainly in neurochemistry, since this technique has many advantages over other methods as reviewed by Benveniste et al.[31] In the present study, microdialysis was used to determine the concentration of caffeine in the brain and CSF. For this purpose, recovery percent of caffeine was examined in advance from the viewpoints of two main influencing factors. One was the dialysate collecting periods and the other was caffeine concentrations in the outside fluid of the probe at a fixed flow rate of perfusing

solution. As demonstrated in Figs. 2 and 3, it was found that recovery percent of caffeine in the brain and CSF dialysates was almost constant under different conditions. Regarding the brain distribution of caffeine, McCall et al. reported the existence of saturable, carrier-mediated transport of caffeine using the brain uptake index (BUI) method and employing caffeine concentrations from 10 to 100 μmol/ml.[32] In contrast, a linear relationship in caffeine distribution into the brain was observed in the present study. This may be due to the extremely low caffeine concentrations in the plasma (0.1—280 nmol/ml) compared to those used by McCall et al.[32] Recently, Ståhle et al.[33] also reported a linear relationship between the dose of theophylline and brain dialysate concentration. The concentration of caffeine in the brain and CSF calculated by the dialysis concentration and the recovery percent was identical with the value determined in isolated brain and CSF at several points in separate experiments. These findings suggest that microdialysis is an useful technique for the chronological determination of drug concentrations in the tissue and biological fluid with more than a 30 s collecting period of dialysate, if recovery percent of the drug in the dialysate of microdialysis against that in the tissue can be determined. However, it was difficult to reduce the collecting period to less than 30 s, since the absolute volume of dialysate collected for 30 s was only 1 μl at a perfusate flow rate of 2 μl/min. Increasing the flow rate of the perfusate would make it possible to reduce the collecting period.

To analyze the distribution characteristics of a drug into the brain, transfer of the drug between blood and brain, blood and CSF, and also between brain and CSF should be determined separately. Microdialysis is considered to be one of the feasible techniques for such determinations in a very small restricted brain area, since this means can determine drug concentrations simultaneously at separate

portions *in vivo*. Regarding the CSF sampling, a simple technique has been reported for repeated direct sampling of CSF in freely moving rats by Sarna *et al.*[34] However, this microdialysis method for CSF sampling still has some advantages over the direct sampling of CSF, for example, it does not change the CSF volume and, consequently, the concentration gradient of drug between the brain tissue and CSF.

The possible transfer of caffeine between CSF and the brain was examined *in vitro* and *in vivo* (Figs. 5 and 6). These studies implied that caffeine may diffuse slowly only in the brain extracellular space from CSF *in vitro*, but is undetectable *in vivo* because of the existence of CSF bulk flow, and the diffusibility of caffeine in the brain tissue *in vivo* is almost negligible. It is generally accepted that there is no tight barrier for drug permeability in ependyma between the CSF and brain tissue. However, it is also known that the diffusion rate of drugs such as sucrose,[35] inulin[36] and blue dextran[37] in the brain extracellular space is quite slow. The *in vivo* results after injection of caffeine into the lateral ventricle or striatum indicated that the diffusion of caffeine in the brain and between CSF and brain is also quite slow, if any. In the present study, caffeine was found to transfer very rapidly from CSF or brain into the central circulation. However, the detailed transfer route by which caffeine is eliminated from CSF was not investigated. Further investigation will be necessary to determine the transfer route of caffeine from CSF to central blood circulation as suggested by Fenstermacher *et al.*[35]

In this study, we used caffeine as a model compound, since caffeine is known to distribute into the brain and CSF in mice,[38] rats[39] and humans.[37] Also, no presence of a special binding site of caffeine in the brain has been reported. In fact, almost the same concentration of caffeine was observed in various brain regions and CSF, indicating that no appreciable accumulation takes place in the brain. On the other hand, however, some compounds such as scopolamine,[40] zolpidem[41] and imipramine[2,42,43] are known to have special binding sites in the brain, resulting in regional variation in the distribution. Such compounds may show different concentration ratios of the drug between the interstitial fluids and the brain tissues at different drug concentrations. In such a case, it may be difficult to obtain a constant recovery percent in microdialysis.

In conclusion, we demonstrated the usefulness of microdialysis for the estimation of the time dependent change of caffeine concentration in the brain and CSF for pharmacokinetic study. The brain and CSF uptake of caffeine were well analyzed pharmacokinetically using a modified 2-compartment model.

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