Available online at www.sciencedirect.com
SCIENCE DIRECT®
European Journal of Pharmacology 464 (2003) 39–48
www.elsevier.com/locate/ejphar

Increased plasma concentration and brain penetration of methamphetamine in behaviorally sensitized rats

Kiyoyuki Kitaichi[a], Yuki Morishita[a], Yuko Doi[a], Jun Ueyama[a], Miyoko Matsushima[a], Ying-Lan Zhao[a,b], Kenzo Takagi[a], Takaaki Hasegawa[a,*]

[a] *Department of Medical Technology, Nagoya University School of Health Sciences, 1-1-20 Daikominami, Higashi-ku, Nagoya 461-8673, Japan*
[b] *National Safety Assessment Center of Traditional Chinese Medicine, West China Hospital, Chengdu 610041, China*

Received 11 July 2002; received in revised form 1 January 2003; accepted 14 January 2003

Abstract

Exposure to methamphetamine causes behavioral sensitization in experimental animals. However, the precise mechanism of this behavioral sensitization has not yet been fully elucidated. Accordingly, we evaluated the pharmacokinetic properties of methamphetamine in rats behaviorally sensitized to methamphetamine following its repeated administration (6 mg/kg, i.p., once a day for 5 days followed by a 21-day drug abstinence period). In the sensitized rats, methamphetamine (0.8 mg/kg)-induced locomotor activity was significantly enhanced, suggesting the successful establishment of behavioral sensitization to methamphetamine. Significant increases in the concentrations of methamphetamine in plasma and brain dialysate, as well as the delayed disappearance of methamphetamine from plasma, were observed in the sensitized rats after intravenous injection of methamphetamine (5 mg/kg). The tissue to plasma concentration ratio (K*p*) of methamphetamine in lung and heart decreased in the sensitized rats. The renal excretion of methamphetamine, which is sensitive to several cations, was also decreased in the sensitized rats. Moreover, in the sensitized rats, the expression of organic cation transporter 3 (OCT3) mRNA was decreased in kidney, brain and heart as measured by reverse transcriptase-polymerase chain reaction (RT-PCR). Taken together, these results suggest that the behavioral outcome of sensitization to methamphetamine might, in part, be due to the increased levels of methamphetamine in plasma and brain extracellular areas, as well as an altered tissue distribution of methamphetamine associated with changes in the cation transport system.

Keywords: Methamphetamine; Behavioral sensitization; Pharmacokinetics; Microdialysis, brain; Drug transporter

1. Introduction

Repeated exposure to drugs of abuse, including amphetamine and methamphetamine, results in a progressive and persistent enhancement of their psychomotor and positive reinforcing effects. This phenomenon, termed behavioral sensitization (Pierce and Kalivas, 1997; Sato, 1986; Stewart and Badiani, 1993), is thought to underlie certain aspects of the development of addiction to drugs and to drug-induced psychosis (Robinson and Becker, 1986; Robinson and Berridge, 1993).

Thus far, the expression of behavioral sensitization is believed to result from neuroplastic changes in the brain circuitry involved in motivational behavior, such as long-lasting hyperreactivity of dopaminergic and glutamatergic neurons, as well as altered activity of monoamine transporters (Ellinwood et al., 1995; Pierce and Kalivas, 1997). For example, Vanderschuren et al. (1999) have recently demonstrated that even a single injection of amphetamine causes behavioral, neuroendocrine and neurochemical sensitization to amphetamine. In addition, behavioral sensitization was also found after administration of a dopamine D1 receptor agonist (SKF-82958), a dopamine D2 receptor agonist (quinpirole) and cocaine to rats pretreated with amphetamine. These results suggest that the neuro-functional changes of the dopaminergic system associated with exposure to drugs of abuse are important for altering neuronal functions, subsequently causing behavioral sensitization.

The effects of dopamine receptor antagonists on methamphetamine/amphetamine-induced behavioral sensitization are limited; dopamine receptor antagonists do not reverse

* Corresponding author. Tel.: +81-52-719-1558/1341; fax: +81-52-719-3009.
E-mail address: hasegawa@met.nagoya-u.ac.jp (T. Hasegawa).

0014-2999/03/$ - see front matter © 2003 Elsevier Science B.V. All rights reserved.
doi:10.1016/S0014-2999(03)01321-9

the established behavioral sensitization to methamphetamine (Ellinwood et al., 1995; Hamamura et al., 1991; Sato, 1983; Ujike, 1989). Moreover, amphetamine induces behavioral sensitization even in postsynaptic dopamine receptor-knockout mice (Crawford et al., 1997; Xu et al., 2000). In the light of these discrepancies, an alternative explanation for the behavioral sensitization to psychostimulants should be considered.

There are few data available concerning drug pharmacokinetics in animals behaviorally sensitized to methamphetamine/amphetamine (Cho et al., 2001; Mizugaki et al., 1993; Numachi et al., 1992; Riviere et al., 1999, 2000). This might also to be an important factor contributing to altered behavior in sensitized animals. Regarding this, inconsistent data have been reported for psychostimulant-abusing subjects, with pharmacokinetics being either changed (Änggård et al., 1970; Beckett and Rowland, 1965a,b; also see review of Busto et al., 1989) or unchanged (Cook et al., 1992).

In the present study, we investigated the pharmacokinetics and brain penetration of methamphetamine in rats behaviorally sensitized to methamphetamine. Our results revealed that the concentration–time profiles of methamphetamine in plasma and brain dialysates are increased in rats that are behaviorally sensitized to methamphetamine. Our data also suggest that the altered tissue distribution and renal excretion of methamphetamine possibly result from decreased cation transport.

2. Materials and methods

2.1. Animals

Male Wistar rats (260–330 g) were purchased from Japan SLC (Hamamatsu, Japan). Animals were kept in a temperature (22–24 °C)-, humidity (55 ± 5%)- and light (12-h light–dark cycle, lights on at 07:00)-regulated room with food and water ad libitum for at least 3 days before experiments. The procedures involving animals and their care conformed to the international guidelines Principles of Laboratory Animal Care (NIH publication no. 85-23, revised 1985) and Guiding Principles for the Care and Use of Laboratory Animals of Nagoya University, Japan.

2.2. Reagents

Methamphetamine hydrochloride was purchased from Dainippon Pharmaceutical (Osaka, Japan). β-phenylethylamine, cimetidine and carnitine were from Sigma (St. Louis, MO). Tetraethylammonium and *p*-aminohippuric acid were from Wako (Osaka, Japan), and dansyl chloride was from Merck (Darmstadt, Germany). All other chemicals used were obtained commercially and were used without further purification. Methamphetamine, tetraethylammonium, cimetidine and *p*-aminohippuric acid were dissolved in sterilized isotonic saline for in vivo use.

2.3. Repeated administration of methamphetamine

Behavioral sensitization was established as described elsewhere (Ujike et al., 1992), with a protocol that is not associated with toxic effects in the brain (Pierce and Kalivas, 1997; Suzuki et al., 1997). Methamphetamine was administered (5 mg/kg, i.p.) once a day for five consecutive days, followed by a 21-day period of drug abstinence. This period without methamphetamine prevented any effects of methamphetamine still present in the body (less than 1% of dosage, Takayasu et al., 1994). The control group was given saline instead of methamphetamine for five consecutive days.

2.4. Experiments

2.4.1. Methamphetamine-induced locomotor activity

Development of behavioral sensitization was confirmed by measuring locomotor activity induced by methamphetamine using a photosensor (SCANET MV-10, Melquest, Toyama, Japan), as described elsewhere (Kitaichi et al., 1995). Briefly, rats were placed in plastic cages (30 × 35 × 17 cm) for 30 min as a habituation period. Then, methamphetamine (0.8 mg/kg, i.v.) was given and locomotor activity was measured for 2 h at 10-min intervals. Based on our preliminary study, the dose of methamphetamine selected was enough to induce locomotor activity but not stereotyped behaviors such as rearing and sniffing.

2.4.2. Estimation of the plasma and brain dialysate concentration–time profile of methamphetamine

One day before the start of the experiments, rats were anesthetized with sodium pentobarbital (25 mg/kg body weight), and the right jugular vein was cannulated with sterilized polyethylene tubes for drug administration and/or blood sampling. Dialysis probes (AN69 hollow fibers, molecular weight cutoff <60,000, i.d. = 220 μm, o.d. = 310 μm, Hospal-Gambro, St. Leonard, Quebec) were implanted horizontally into both sides of the striatum [anterior, +0.5 mm from bregma; ventral, −5.0 mm from the surface of the skull; dialyzed area, 3 mm in each striatum, see detailed methods of Kitaichi et al. (1999) and Day et al. (2001)] according to coordinates obtained from Paxinos and Watson (1997).

On the day of the experiment, the rats were allowed to move freely, and the brain dialysis probes were perfused at a flow rate of 5 μl/min with filtered sterile buffered Ringer solution [NaCl (140 mM), KCl (3.0 mM), NaH_2PO_4 (1.5 mM), $MgCl_2$ (1.2 mM) $CaCl_2$ (1.2 mM), pH 7.4] for 1 h prior to the experiments. After constant flow was established, rats received a bolus intravenous injection of methamphetamine (5 mg/kg of body weight). Plasma samples and brain dialysate samples were then collected at designated intervals. Plasma samples were immediately centrifuged at 6000*g* for 10 min. The

collected plasma and dialysate were stored at −30 °C until analyzed. The precise location of probes and infusion cannulas was verified by standard histological examination.

2.4.3. Estimation of tissue distribution of methamphetamine

One day before the start of the experiments, the rats were anesthetized with sodium pentobarbital (25 mg/kg body weight), and the right jugular vein was cannulated as described above. On the day of the experiment, rats were allowed to move freely, and received a bolus intravenous injection of methamphetamine (5 mg/kg of body weight). Two hours after methamphetamine administration, rats were again anesthetized with sodium pentobarbital (25 mg/kg), and plasma samples and tissue samples were collected. Plasma samples were immediately obtained by centrifugation at 6000*g* for 10 min. Tissue samples were immediately frozen on dry ice. The collected plasma and tissues were stored at −30 °C until analyzed.

2.4.4. Estimation of renal clearance of methamphetamine (CL_R)

The renal excretion of methamphetamine was estimated as described elsewhere (Kiso et al., 2000). On the day of the experiment, rats were anesthetized with sodium pentobarbital (25 mg/kg), and were cannulated with polyethylene tubes in the right jugular vein for blood sampling/drug administration, in the femoral vein for drug infusion and in the urinary bladder for urine collection. The rats received bolus intravenous injections of methamphetamine with a loading dose of 1.09 mg/kg, followed by a constant-rate infusion using a Harvard infusion pump (PHD 2000, south Natick, MA) delivering a dose of 834 μg/h/kg at a flow rate 2 ml/h, until the end of the study. The loading dose and infusion dose of methamphetamine were selected to obtain 150 ng/ml of methamphetamine in the plasma of control rats, and were calculated from the above-mentioned pharmacokinetic study. After 60 min of methamphetamine infusion associated with steady-state methamphetamine plasma concentration, urine samples were collected at 20-min intervals for three times and plasma samples (approximately 0.25 ml) were also collected at the mid-time point of each urine collection.

To elucidate the effects of several drugs (cimetidine, tetraethylammonium, carnitine, *p*-aminohippuric acid) on the plasma concentration and the renal clearance of methamphetamine, each drug was infused with methamphetamine after obtaining three samples of urine and plasma in rats infused with methamphetamine only. Thirty minutes after concomitant infusion of each drug with methamphetamine, urine and plasma samples were collected as described above. The infusion doses of cimetidine (48 mg/h/kg), tetraethylammonium (15.2–151.6 mg/h/kg) and *p*-aminohippuric acid (120 mg/h/kg) at a flow rate 2 ml/h were selected because they inhibited the function of cation transporters (Yano et al., 1997). The infusion dose of carnitine (264 mg/h/kg, flow rate 2 ml/h) was close to a saturating concentration of carnitine in this regimen because no pharmacokinetic data concerning carnitine in rats are available in the literature. In the experiments of urine alkalization, methamphetamine in 7.5% sodium bicarbonate was infused, as described in previous report (Batlle et al., 1986). After urine pH reached pH 8 (taking approximately 30 min), plasma and urine were collected three times as described above. Plasma samples were obtained by centrifugation of blood samples at 6000*g* for 5 min immediately after sampling. The volume of urine samples was measured using a specific gravity of 1.0. All plasma and urine samples were stored at −30 °C until analyzed.

2.5. The measurements of methamphetamine

2.5.1. Extraction of methamphetamine from plasma, urine and brain dialysate samples

Concentrations of methamphetamine in plasma, urine, tissue and brain dialysate samples were determined by high-performance liquid chromatography (HPLC) with the modification of Hayakawa et al. (1993). Tissue samples were homogenized in four equivalent volumes of water per wet weight, immediately before extraction. Then, 50 μl of plasma samples and homogenized tissue samples were vortexed with 350 μl of acetonitrile containing β-phenylethylamine (0.5 μg/ml) as an internal standard and 10 μl of 10% sodium hydroxide. After deproteinization by centrifugation at 12,000*g* for 5 min, the upper acetonitrile-rich layer was collected and evaporated to dryness under a stream of nitrogen gas at 45 °C. In the case of urine and brain dialysate samples, 50 μl of diluted urine samples or whole brain dialysate samples was vortexed with 350 μl of acetonitrile containing β-phenylethylamine, and evaporated to dryness under a stream of nitrogen gas at 45 °C. The dried residues were reconstituted with 100 μl of 10 mM sodium carbonate-sodium bicarbonate buffer (pH 9.0) and 100 μl of 2 mM dansyl chloride. Finally, samples were prepared for HPLC by heating them to 45 °C for 1 h in the dark to derivarize methamphetamine and β-phenylethylamine to dansyl-methamphetamine and dansyl-β-phenylethylamine, respectively. It should be noted that endogenous β-phenylethylamine in samples is unlikely to affect HPLC measurement of methamphetamine since the amount of β-phenylethylamine added as an internal standard is over 100-fold higher than that found in brain (Zametkin et al., 1984; Paterson et al., 1990).

2.5.2. Analysis of methamphetamine in plasma, urine and brain dialysate samples by HPLC

The HPLC apparatus was an LC-6A system (Shimadzu, Kyoto, Japan) consisting of an LC-6A liquid pump, an RF-

535 fluorescence detector and an SIL-6A autoinjector. A Cosmosil 5C18 column (4.6 × 150 mm; Nacalai Tesque, Kyoto, Japan) was used with a column oven (OTC-6A, Shimadzu) heated to 40 °C. The fluorescence detector was set at Em: 580 nm, Ex: 475 nm. The mobile phase was water/acetonitrile (1:2 vol/vol) containing 1 mM imidazole 30%, and the flow rate was 1.0 ml/min. Standard curves for measuring methamphetamine in the plasma, urine and brain dialysate samples were linear for concentrations ranging from 50 to 4000 ng/ml, with a correlation coefficient of 0.999. The intra- and inter-assay coefficients of variation for the HPLC assay were less than 6% at concentrations of 50–4000 ng/ml. The detection limit of methamphetamine was 5 ng/ml.

2.6. Pharmacokinetic data analysis

Plasma concentration–time data for methamphetamine in each rat were analyzed individually by noncompartmental methods. The area under the plasma concentration–time curve (AUC) and the area under the first moment curve (AUMC) were calculated by the trapezoidal rule method up to the last measured plasma concentration and were extrapolated to infinity by adding the following: the value of the last measured plasma concentration divided by the terminal elimination rate constant, which was calculated by determining the slope of the least-squares regression line from the terminal portion of the log concentration–time data. Systemic clearance (CL_{SYS}) was calculated by dividing the dose by the AUC. The steady-state volume of distribution (Vd_{SS}) was calculated as $Vd_{SS} = CL_{SYS} \times MRT$, where MRT represents the mean residence time, which was calculated as MRT = AUMC/AUC. The plasma half-life of methamphetamine ($t_{1/2}$) was also calculated as $0.693/k$, where k represents the elimination constant. All computer analyses were performed using WinNonlin (version 2.1, Pharsight Mountain View, CA) by weighting the data with the reciprocal of the concentration. Penetration of methamphetamine through the blood–brain barrier was estimated by AUC_{brain}/AUC_{plasma}, the ratio of AUC of unbound methamphetamine in plasma (AUC_{plasma}) to that in brain extracellular area (AUC_{brain}).

CL_R during the collection period (20 min) was calculated by dividing the amount of methamphetamine excreted into the urine by the plasma concentration of methamphetamine at the midpoint of urine collection period.

2.7. Estimation of protein binding

Protein binding of methamphetamine was determined by ultrafiltration. Briefly, methamphetamine dissolved in saline was diluted 50 times with rat plasma to give final concentrations of 0.1, 2.5 and 25 μg/ml. The mixture was incubated at 37 °C for 30 min to ensure binding equilibrium. Plasma was placed in an ultrafiltration apparatus (Ultrafree-MC; Milipore, Bedford, MA) with 10,000 molecular weight cutoff, and centrifuged at 6000 rpm for 60 min. After centrifugation, the concentration in the filtrate was determined as the unbound fraction. The plasma unbound fraction was calculated by dividing the unbound concentration by the total plasma concentration. Binding was normalized to the filter blank in the ultrafiltration apparatus assessed in the absence of plasma. The absorption of methamphetamine to the device was negligible.

2.8. Reverse transcriptase-polymerase chain reaction (RT-PCR)

Following the 21-day drug abstinence period, control and sensitized rats were anesthetized with sodium pentobarbital (25 mg/kg), and tissues of interest were removed, frozen on dry ice and stored at −80 °C until analysis.

Total RNA was isolated from frozen rat tissues using TRIzol Reagents (Gibco Laboratories, Grand Island, NY) according to the manufacturer's instructions. Single-strand cDNA was synthesized by reverse transcriptase (RT) using SuperScript Preamplification System for First Strand cDNA synthesis (Gibco Laboratories). Briefly, approximately 5 μg of total RNA was converted to cDNA using 125 ng random hexiamers, 10 U Superscript II RT in a buffer containing 20 mM Tris–HCl, 50 mM KCl, 2.5 mM $MgCl_2$, 10 mM dithiothreitol and 0.5 mM dNTP in a total volume of 50 μl. Reverse transcription was performed for 10 min at 25 °C and for 50 min at 42 °C, and samples were heated for 15 min at 70 °C to terminate the reaction. Residual RNA in the cDNA preparation was digested by RNase H (Gibco Laboratories) during a 40-min incubation at 37 °C.

Polymerase chain reaction (PCR) reaction was performed using 1 μl of cDNA preparation, 1 U *Taq* DNA polymerase, 1 U antibody against *Taq* DNA polymerase in a buffer containing 20 mM Tris–HCl, 50 mM KCl, 1.5 mM $MgCl_2$, 0.2 mM dNTP mix and 0.2 μM sense and antisense primers in a total volume of 20 μl. The tubes were incubated in a GeneAmp 9600 (Perkin-Elmer, Norwalk, CT) at 94 °C for 2 min to activate DNA polymerase. The cycling program was 94 °C for 30 s, 55/60 °C for 30 s, 72 °C for 1 min and for 7 min in the last cycle, and comprised of 28–36 cycles. For each primer set, an increasing number of cycles up to 36 cycles were applied to determine the optimal number of cycles. The number of cycles was 28 for β-actin, 36 for organic cation transporter 1 (OCT1), 36 for OCT2, 36 for OCT3 except heart (33 cycles), 36 for OCTN2, 33 for cytochrome (CYP) 2D1/5 and 34 for CYP2D2. Subsequent PCR products were loaded on 2% agarose gels and stained with ethidium bromide. The levels of PCR product of β-actin used as internal control were adjusted in control and sensitized animals before starting analysis of OCT mRNA.

Primer sequences and resulting PCR products were: β-actin: TTC TAC AAT GAG CTG CGT GTG GC (sense primer) and CTC ATA GCT CTT CTC CAG GGA GGA (antisense primer) (PCR products: 456 bp; Waki et al., 1999); OCT1: CCT GGG CTC CCT GGT TGT GGG TTA (sense

primer) and AAT GAG GGG CAG GGC TTG CCA AA (antisense primer) (989 bp; Grundemann et al., 1997); OCT2: CCG CTA TCC CTG GGC TGT GTC AAA (sense primer) and TGG CCC ACA GCT CCC TTG GGT ATT (antisense primer) (795 bp; Grundemann et al., 1997); OCT3: CCA CCA TCG TCA GCC AGT TT (sense primer) and ACA CGA CAC CCC TGC CAC TA (antisense primer) (851 bp; Wu et al., 1998); OCTN2: CCT ATG TGT TGG CCT GGC TG (sense primer) and AAC TTG CCC ACC ATC ACC AG (antisense primer) (153 bp; Spaniol et al., 2001).

2.9. Statistical analysis

All data are expressed as means ± S.E.M. One-way analysis of variance (one-way ANOVA) with repeated measures was used to test for a drug effect over time effect on methamphetamine-induced locomotor activity and on the concentration–time profile of methamphetamine in plasma and brain dialysate. One-way ANOVA was used to test for differences between groups in other experiments. When the ANOVA F ratios were significant ($p < 0.05$), post hoc analysis was done using the Scheffe's test. All statistics reported in these experiments were generated using StatView (version 4.5, Abacus Concepts, Berkeley, CA), and p-values less than 0.05 were considered statistically significant.

3. Results

3.1. Methamphetamine-induced locomotor activity

Repeated methamphetamine treatment, followed by a 21-day drug abstinence period (sensitized), did not affect habituation and/or basal locomotor activity [control rats: 1817 ± 135; sensitized rats: 1619 ± 302; $F(1,8) = 0.354$; NS]. One-way ANOVA with repeated measures showed a significant difference for methamphetamine-induced locomotor activity between control and sensitized rats [$F(1,88) = 13.07$, $p < 0.01$] (Fig. 1). Scheffe's post hoc test indicated significant differences between groups at 0–10, 30–40, 40–50 and 50–60 min after methamphetamine injection (Fig. 1). As shown in Fig. 1, there was a statistically significant difference in methamphetamine-induced locomotor activity between control and sensitized groups after methamphetamine injection for the 0- to 1-h period, but not for the 1- to 2-h period.

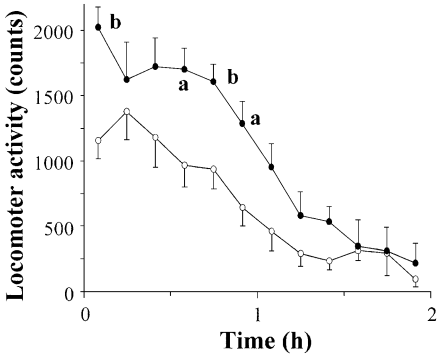

Fig. 1. Methamphetamine (0.8 mg/kg, i.v.)-induced locomotor activity in control (open circles) and sensitized (closed circles) rats. Sensitization was established as described in Materials and methods. Each point represents the mean ± S.E.M. for four to six animals. One-way ANOVA with repeated measure showed the significant difference between control and sensitized rats [$F(1,88) = 13.07$, $p < 0.01$]. $^{a}p < 0.05$, $^{b}p < 0.01$ vs. control (Scheffe's post hoc test).

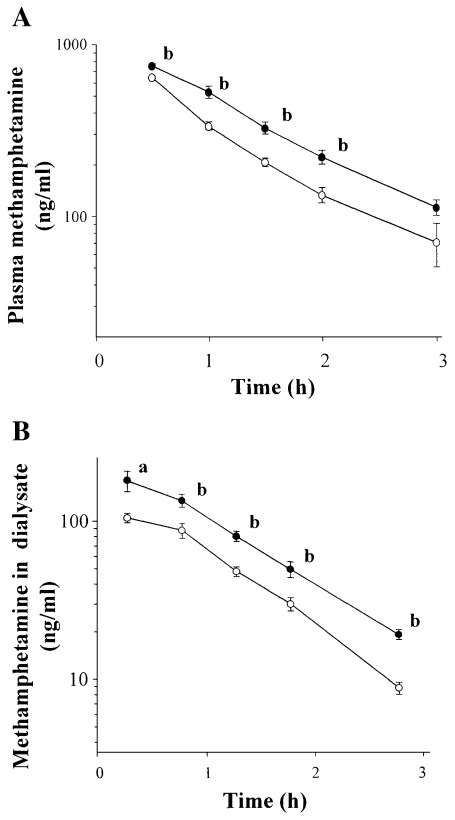

Fig. 2. (A) The plasma concentration and (B) brain microdialysate concentration–time profile of methamphetamine in control (open circles) and sensitized (closed circles) rats. Sensitization was established as described in Materials and methods. Each point represents the mean ± S.E.M. for five to seven animals. One-way analysis of variance (ANOVA) with repeated measures revealed a statistically significant difference between groups [plasma: $F(1,45) = 19.225$, $p < 0.01$; brain microdialysate: $F(1,55) = 15.257$, $p < 0.01$]. $^{a}p < 0.05$, $^{b}p < 0.01$ vs. control rats (Scheffe's post hoc test).

3.2. Plasma concentration–time profile of methamphetamine, pharmacokinetic parameters of methamphetamine and plasma protein binding of methamphetamine

Plasma concentration–time profiles of methamphetamine following bolus intravenous injection of methamphetamine (5 mg/kg) in control and sensitized rats are shown in Fig. 2A. Plasma concentrations of methamphetamine were significantly increased in sensitized rats

[$F(1,45) = 19.225$, $p < 0.01$], and Scheffe's post hoc test indicated significant differences between groups at all time points except 3 h after methamphetamine administration (Fig. 2A). In the pharmacokinetic analysis, a significant increase in AUC, a decrease in systemic clearance (CL_{SYS}) and Vd_{SS} and prolongation of $t_{1/2}$ of methamphetamine were observed in sensitized rats (Table 1). No significant difference in plasma protein binding to methamphetamine was observed (Table 1).

3.3. Brain dialysate concentration–time profile of methamphetamine

Brain dialysate concentration–time profiles of methamphetamine after bolus intravenous injection of methamphetamine (5 mg/kg) in control and sensitized rats are shown in Fig. 2B. Brain dialysate concentrations of methamphetamine in sensitized rats were significantly higher [$F(1,55) = 15.257$, $p < 0.01$] and Scheffe's post hoc test indicated significant differences between groups at all time points (Fig. 2B).

3.4. AUC_{plasma}, $AUC_{dialysis}$ and $AUC_{dialysis}/AUC_{plasma}$ in control and sensitized rats

At 0–1 h, $AUC_{dialysis}$ and AUC_{brain}/AUC_{plasma} were significantly increased in sensitized rats, whereas only AUC_{plasma} showed a significant difference at 0–2 h (Fig. 3).

3.5. Tissue/plasma ratio (Kp) of methamphetamine

The tissue distribution of methamphetamine is shown in Fig. 4, given as K*p* values. The tissues investigated showed a huge accumulation of methamphetamine, with 7–40 times higher concentration than plasma concentration of methamphetamine in control rats, which is consistent with the previous report (Riviere et al., 2000). The relative magnitude of accumulation, compared to plasma, was kidney (43 times)>lung>liver>brain>heart>muscle (7

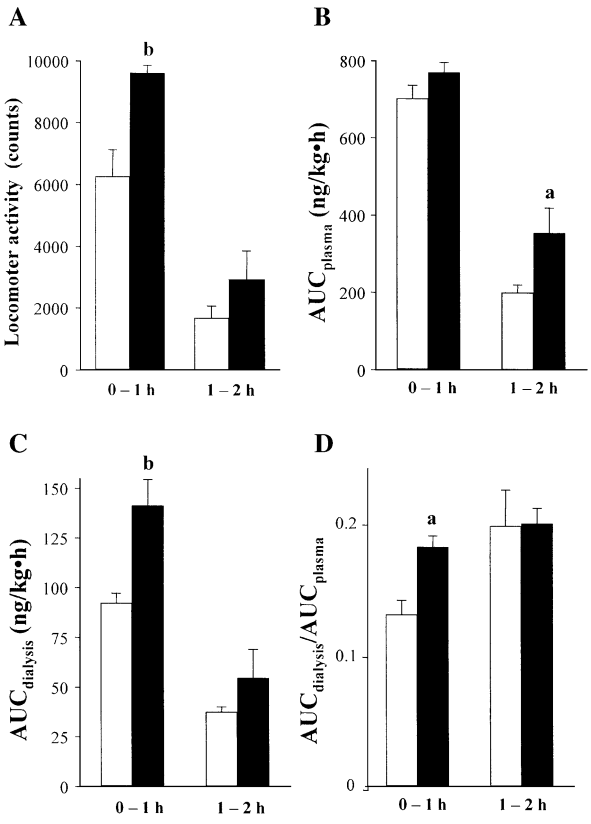

Fig. 3. (A) Methamphetamine (0.8 mg/kg, i.v.)-induced locomotor activity, (B) AUC of methamphetamine in plasma (AUC_{plasma}), (C) AUC of methamphetamine in brain dialysate ($AUC_{dialysis}$) and (D) the ratio of $AUC_{dialysis}$ to AUC_{plasma} ($AUC_{dialysis}/AUC_{plasma}$) in control (open columns) and sensitized (closed columns) rats. Data were calculated from the values presented in Figs. 1 and 2. [a] $p < 0.05$, [b] $p < 0.01$ vs. control rats (Scheffe's post hoc test).

Table 1
Pharmacokinetic parameters of methamphetamine (5 mg/kg) administered by bolus intravenous injection in control and sensitized rats

Parameters	Control (4)	Sensitized (6)
CL_{SYS} (l/h/kg)	5.56 ± 0.52	3.58 ± 0.22[a]
Vd_{SS} (l/kg)	7.29 ± 0.52	4.91 ± 0.22[a]
$t_{1/2}$ (min)	47.4 ± 4.4	57.1 ± 1.4[a]
AUC_{plasma} (ng h/ml)	1000 ± 148	1411 ± 93[b]
fu	0.46 ± 0.04	0.48 ± 0.05

Sensitization was established as described in Materials and methods. Pharmacokinetic parameters were obtained from data shown in Fig. 2A. Each value represents the mean ± S.E.M. The numbers in parentheses show the numbers of animals. AUC_{plasma}, area under the curve of methamphetamine; CL_{SYS}, systemic clearance of methamphetamine; Vd_{SS}, steady-state volume of distribution of methamphetamine; $t_{1/2}$, plasma half-life of methamphetamine; fu, unbound fraction.

[a] $p < 0.05$ vs. control rats (Scheffe's post hoc test, followed by one-way analysis of variance).

[b] $p < 0.01$ vs. control rats (Scheffe's post hoc test, followed by one-way analysis of variance).

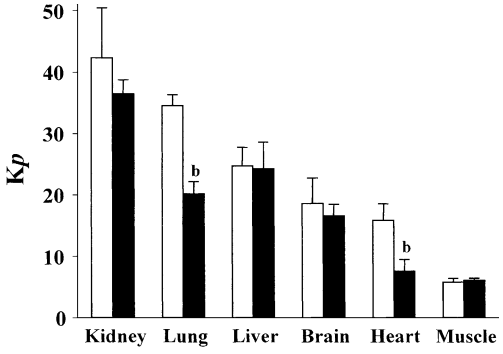

Fig. 4. The tissue/plasma ratio (K*p*) of methamphetamine in the various tissues of control (open columns) and sensitized (closed columns) rats. Two hours after methamphetamine (5 mg/kg, i.v.) administration, plasma and tissue samples were collected under sodium pentobarbital anesthesia (25 mg/kg body weight). Data represent the means ± S.E.M. for three animals. [b] $p < 0.01$ vs. corresponding control rats (Scheffe's post hoc test).

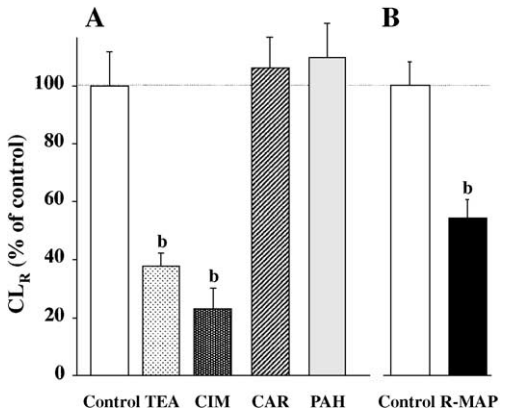

Fig. 5. (A) Effect of tetraethylammonium (TEA), cimetidine (CIM), carnitine (CAR), *p*-aminohippuric acid (PAH) on urinary clearance (CL_R) of methamphetamine after the constant-rate infusion of methamphetamine as described in Materials and methods. Each column represents the mean ± S.E.M. for four to six animals expressed as a percentage of CL_R before drug treatments. $^b p < 0.01$ vs. CL_R before drug treatments. (B) CL_R of methamphetamine in control and sensitized rats. Each column represents the mean ± S.E.M. for five animals expressed as a percentage of control. $^b p < 0.01$ vs. CL_R in control rats.

times) in control animals (Fig. 4). The K*p* value of methamphetamine in sensitized rats was significantly decreased in the lung and heart (Fig. 4).

3.6. The steady-state plasma concentration (C_{SS}) and renal clearance (CL_R) of methamphetamine in control and sensitized rats with or without urine alkalization and in rats treated with several drugs

The CL_R of methamphetamine in sensitized rats was lower than that in control rats (Fig. 5), although urine flow rate was unchanged [control rats: 12.2 ± 0.68 μl/min; sensitized rats: 10.3 ± 0.85 μl/min, $p > 0.05$]. The C_{SS} of methamphetamine in sensitized rats was significantly increased (control rats: 148.1 ± 3.9 ng/ml; sensitized rats: 200.1 ± 9.8 ng/ml, $p < 0.05$).

Urine alkalization decreased CL_R in both control and sensitized rats and there was no statistically significant difference when the data were expressed as a percentage of CL_R before urine alkalization (control rats: 16.3 ± 0.7%; sensitized rats: 16.8 ± 2.5 ng/ml, $p > 0.05$).

As in sensitized rats, in control rats, tetraethylammonium and cimetidine significantly decreased CL_R (Fig. 4). The increased C_{SS} of methamphetamine evoked by tetraethylammonium and cimetidine was also observed when the data were expressed as percentage of C_{SS} before drug treatment [tetraethylammonium: 137.8 ± 5.0% ($p < 0.01$); cimetidine: 216.1 ± 20.5% ($p < 0.01$)]. Nevertheless, carnitine and *p*-aminohippuric acid failed to affect CL_R (Fig. 5)

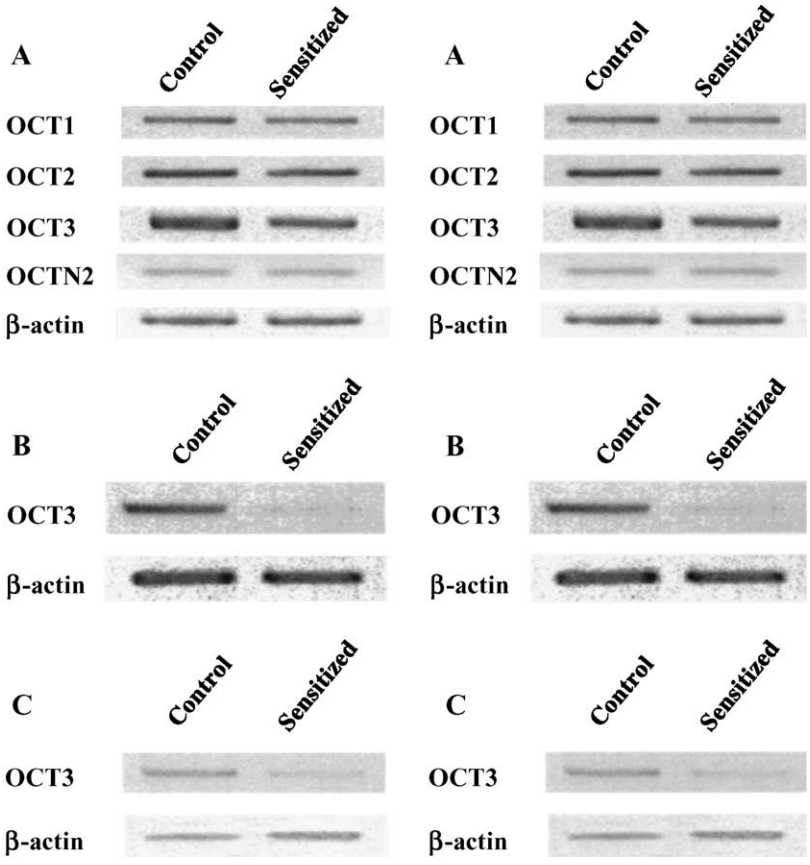

Fig. 6. The expression of OCTs and OCTN2 mRNA in kidney (A), lung (B) and brain (C) by RT-PCR in control and sensitized rats.

and C_{SS} [carnitine: 106.3 ± 10.7% (p>0.05); p-aminohippuric acid: 95.7 ± 4.8% (p>0.05)].

3.7. OCTs and OCTN2 mRNA expression in various tissues in control and sensitized rats

In each tissue, the levels of β-actin were adjusted to the same levels in control and sensitized animals (Fig. 6A–C). In control rats, using RT-PCR, OCT1 mRNA was detected in kidney, but not in the brain and lung, whereas OCT2 mRNA was detected in the kidney and brain, but not in the lung (Fig. 6A–C). OCT3 mRNA was found in all tissues investigated (Fig. 6A–C). Moreover, OCTN2 mRNA was also found in the kidney of control rats (Fig. 6A). OCT3 mRNA expression was decreased in the kidney, brain and lung of sensitized rats (Fig. 6A–C). The mRNA of the other OCTs and OCTN2 mRNA were not changed in sensitized rats. In the heart, only OCT3 mRNA was detected, and this was not altered in sensitized rats (data not shown).

4. Discussion

There are no reports in which methamphetamine levels were measured in plasma, brain and other tissues of methamphetamine-sensitized animals, except for those using positron emission tomography (PET) in experimental animals (Mizugaki et al., 1993; Numachi et al., 1992). These PET studies have demonstrated that ^{11}C-methamphetamine activity in the brain is increased in methamphetamine-sensitized animals, suggesting the importance of increased methamphetamine level in behavioral sensitization. However, it is difficult to exclude the possibility that the results of ^{11}C-methamphetamine activity from PET might be due to methamphetamine metabolites. Thus, it was deemed important to reevaluate more precisely the pharmacokinetic changes in methamphetamine in an experimental animal model.

Methamphetamine-sensitized rats, following subsequent bolus injection of methamphetamine, had increased levels of methamphetamine in plasma and brain microdialysate compared to control rats. Pharmacokinetic analysis of methamphetamine in plasma showed significant increases in AUC and $t_{1/2}$, accompanied by decreases in CL_{SYS} and Vd_{SS} in sensitized rats. Consistent with the decreased Vd_{SS}, the Kp values of methamphetamine in lung and heart were also decreased in sensitized rats. Considering these parameters, the observed increase in the plasma methamphetamine level might reflect a decreased tissue distribution and/or elimination of methamphetamine from plasma. It should be noted that the pharmacokinetic parameters and tissue distribution observed here in control rats were similar to those reported elsewhere (Burchfield et al., 1991; Riviere et al., 1999, 2000).

In the present study, we also demonstrated that the CL_R of methamphetamine was decreased in sensitized rats, suggesting that a key determinant of the altered pharmacokinetic parameters in sensitized rats is an impaired renal excretion of methamphetamine. Indeed, methamphetamine and its metabolites were mainly detected in the urine of rats (Hutchaleelaha and Mayersohn, 1996; Riviere et al., 1999) and humans (Cook et al., 1992, 1993). Taken together with the lack of change in methamphetamine-metabolizing enzyme in sensitized rats (Kitaichi and Hasegawa, personal communication), it would be of interest to investigate the nonhepatic elimination system, especially urinary excretion of methamphetamine, in sensitized rats. If CL_R is altered, changes in glomerular filtration rate, passive reabsorption and active tubular secretion/reabsorption by drug/nutrition transporters should be considered. Glomerular filtration rate was not altered in sensitized rats (data not shown). Moreover, urine alkalization decreased CL_R of methamphetamine to the same extent in control and sensitized rats, suggesting that there was no alteration of passive reabsorption of methamphetamine in sensitized rats. Taken together, these results suggest that the active transport system (i.e. active tubular secretion/reabsorption) might be of importance, as opposed to the other two factors.

Methamphetamine acts as an organic cation under physiological conditions. Thus, organic cation transporters might play a role in actively transporting methamphetamine into the kidney (from blood circulation into the renal tissue and/or from tubular lumen into the renal tissue). Five organic cation transporters (rOCT1–rOCT3, rOCTN1–rOCTN2) have been cloned in rats, and these mRNA signals were found in the kidney of rats (Burckhardt and Wolff, 2000). So far, it has been proposed that OCTs are responsible for the secretion of cationic drugs, and that OCTN2 is responsible for their reabsorption (Burckhardt and Wolff, 2000). Thus, we evaluated the effect of tetraethylammonium and cimetidine, substrates for all OCTs (Burckhardt and Wolff, 2000), and carnitine, a putative OCTN2 substrate (Burckhardt and Wolff, 2000), on the methamphetamine CL_R. Results revealed that tetraethylammonium and cimetidine decreased CL_R and increased C_{SS}, whereas carnitine failed to affect them. p-aminohippuric acid (a typical anion) also did not alter these parameters. Taken together, these results suggest that an altered OCT-type cation transport might play a role in the reduction of methamphetamine CL_R, subsequently increasing the methamphetamine level in plasma in sensitized rats. Our hypothesis is supported by the RT-PCR finding that the mRNA for OCT3 was decreased in the kidney of sensitized rats. The importance of OCT3 in the secretion of cationic drugs has already been proposed (Wu et al., 2000). In addition to the results reported here, future studies should more precisely investigate the nature of methamphetamine excretion into urine.

As in the kidney, the mRNA of OCT3 was decreased in the lung of sensitized rats. Considering the decreased Kp value in the lung of sensitized rats, OCT3 in the lung might affect the distribution/accumulation of methamphetamine. However, these changes did not occur in the heart of sensitized rats, which also showed the decreased Kp. Thus,

further studies are required to clarify how Vd_{SS} and K*p* in the lung are altered in sensitized rats.

Theoretically, the extracellular level of drug in the brain is important for determining its psychopharmacological action via receptor/transporters located on neuronal cells. Drug concentration is controlled by the influx/efflux transport of drug at brain vascular endothelial epithelial cells (the so-called blood–brain barrier), neurons and nonneuronal cells such as glial cells. In the brain, possible candidates that may alter the transport and/or elimination of methamphetamine include the neuronal monoamine transporters and neuronal/nonneuronal OCTs/OCTNs (Friedrich, 2001; Inazu et al., 1999; Page et al., 1998; Russ et al., 1996; Streich, 1996; Sweet, 2001; Wu et al., 1998). Thus, it is possible that decreased OCT3 expression in the brain (Fig. 5) might affect the brain penetration/elimination of methamphetamine in sensitized rats. Indeed, the ratio of $AUC_{dialysis}$ to AUC_{plasma} in sensitized rats at 0–1 h was higher than in control rats (Fig. 3D). Taken together, these data imply that methamphetamine might be differentially transported across the blood–brain barrier and/or be differentially eliminated from the extracellular area of the brain in sensitized rats. Further studies are necessary to understand the influx/efflux transport system of methamphetamine with regard to brain extracellular areas.

In summary, animals sensitized to methamphetamine showed an increase in the level of methamphetamine in plasma as well as in brain dialysate, and this was associated with a decreased excretion of methamphetamine into urine. Moreover, down-regulation of OCT3 in sensitized rats might be crucial for the changes in the renal excretion of methamphetamine in kidney, brain penetration and tissue distribution. Taken together, behavioral sensitization to methamphetamine might be partially affected by these changes, although there is no doubt that neuronal changes are still very important for the observed behavioral sensitization. Moreover, cation transporters might become novel molecular targets for elucidation of the mechanism(s) of the development and/or maintenance of behavioral sensitization to methamphetamine.

Acknowledgements

This work was supported by a grant-in-aid from the Ministry of Health and Welfare, Japan to T.K.

Preliminary data were presented at the Annual Meeting of the Society for Neuroscience, San Diego, 2001 and the 2001 CINP regional meeting, Hiroshima, Japan.

References

Änggård, E., Gunne, L.M., Niklasson, F., 1970. Pharmacokinetic and clinical studies on amphetamine dependent subjects. Eur. J. Clin. Pharmacol. 3, 3–11.

Batlle, D.C., Gutterman, C., Tarka, J., Prasad, R., 1986. Effect of short-term cyclosporine A administration on urinary acidification. Clin. Nephrol. 25 (Suppl. 1), S62–S69.

Beckett, A.H., Rowland, M., 1965a. Urinary excretion kinetics of amphetamine in man. J. Pharm. Pharmacol. 17, 628–639.

Beckett, A.H., Rowland, M., 1965b. Urinary excretion of methylamphetamine in man. Nature 206, 1260–1261.

Burchfield, D.J., Lucas, V.W., Abrams, R.M., Miller, R.L., DeVane, C.L., 1991. Disposition and pharmacodynamics of methamphetamine in pregnant sheep. JAMA 265, 1968–1973.

Burckhardt, G., Wolff, N.A., 2000. Structure of renal organic anion and cation transporters. Am. J. Physiol., Renal Physiol. 278, F853–F866.

Busto, U., Bendayan, R., Sellers, E.M., 1989. Clinical pharmacokinetics of non-opiate abused drugs. Clin. Pharmacokinet. 16, 1–26.

Cho, A.K., Melega, W.P., Kuczenski, R., Segal, D.S., 2001. Relevance of pharmacokinetic parameters in animal models of methamphetamine abuse. Synapse 39, 161–166.

Cook, C.E., Jeffcoat, A.R., Sadler, B.M., Hill, J.M., Voyksner, R.D., Pugh, D.E., White, W.R., Perez-Reyes, M., 1992. Pharmacokinetics of oral methamphetamine and effects of repeated daily dosing in humans. Drug Metab. Dispos., Biol. Fate Chem. 20, 856–862.

Cook, C.E., Jeffcoat, A.R., Hill, J.M., Pugh, D.E., Patetta, P.K., Sadler, B.M., White, W.R., Perez-Reyes, M., 1993. Pharmacokinetics of methamphetamine self-administered to human subjects by smoking *S*-(+)-methamphetamine hydrochloride. Drug Metab. Dispos., Biol. Fate Chem. 21, 717–723.

Crawford, C.A., Drago, J., Watson, J.B., Levine, M.S., 1997. Effects of repeated amphetamine treatment on the locomotor activity of the dopamine D1A-deficient mouse. NeuroReport 8, 2523–2527.

Day, J.C., Kornecook, T.J., Quirion, R., 2001. Application of in vivo microdialysis to the study of cholinergic systems. Methods 23, 21–39.

Ellinwood, E.H., King, G., Lee, T.H., 1995. Chronic amphetamine use and abuse. In: Bloom, F.E., Kupfer, D.J. (Eds.), Psychopharmacology—The Fourth Generation of Progress. Lippincott Williams & Wilkins, Philadelphia, PA.

Friedrich, A., 2001. Transport of choline and its relationship to the expression of the organic cation transporters in a rat brain microvessel endothelial cell line (RBE4). Biochim. Biophys. Acta 1512, 299–307.

Grundemann, D., Babin-Ebell, J., Martel, F., Ording, N., Schmidt, A., Schomig, E., 1997. Primary structure and functional expression of the apical organic cation transporter from kidney epithelial LLC-PK1 cells. J. Biol. Chem. 272, 10408–10413.

Hamamura, T., Akiyama, K., Akimoto, K., Kashihara, K., Okumura, K., Ujike, H., Otsuki, S., 1991. Co-administration of either a selective D1 or D2 dopamine antagonist with methamphetamine prevents methamphetamine-induced behavioral sensitization and neurochemical change, studied by in vivo intracerebral dialysis. Brain Res. 546, 40–46.

Hayakawa, K., Miyoshi, Y., Kurimoto, H., Matsushima, Y., Takayama, N., Tanaka, S., Miyazaki, M., 1993. Simultaneous determination of methamphetamine and its metabolites in the urine samples of abusers by high performance liquid chromatography with chemiluminescence detection. Biol. Pharm. Bull. 16, 817–821.

Hutchaleelaha, A., Mayersohn, M., 1996. Influence of activated charcoal on the disposition kinetics of methamphetamine enantiomers in the rat following intravenous dosing. J. Pharm. Sci. 85, 541–545.

Inazu, M., Kubota, N., Takeda, H., Zhang, J., Kiuchi, Y., Oguchi, K., Matsumiya, T., 1999. Pharmacological characterization of dopamine transport in cultured rat astrocytes. Life Sci. 64, 2239–2245.

Kiso, S., Cai, S.H., Kitaichi, K., Furui, N., Takagi, K., Takagi, K., Nabeshima, T., Hasegawa, T., 2000. Inhibitory effect of erythromycin on P-glycoprotein-mediated biliary excretion of doxorubicin in rats. Anticancer Res. 20, 2827–2834.

Kitaichi, K., Yamada, K., Yoneda, Y., Ogita, K., Hasegawa, T., Furukawa, H., Nabeshima, T., 1995. Risperidone prevents the development of supersensitivity, but not tolerance, to phencyclidine in rats treated with subacute phencyclidine. Life Sci. 56, 531–543.

Kitaichi, K., Hori, T., Srivastava, L.K., Quirion, R., 1999. Antisense oligodeoxynucleotides against the muscarinic m2, but not m4, receptor

supports its role as autoreceptors in the rat hippocampus. Brain Res., Mol. Brain Res. 67, 98–106.

Mizugaki, M., Hishinuma, T., Nakamura, H., Yamashita, M., Shimomura, K., Itoh, K., Hirose, A., Tomioka, Y., Aso, H., Edo, K., Takahashi, T., Ido, T., 1993. Distribution of carbon-11 labeled methamphetamine and the effect of its chronic administration in mice. Nucl. Med. Biol. 20, 487–492.

Numachi, Y., Yoshida, S., Inosaka, T., Sato, M., Mizugaki, M., Kimura, K., Hishinuma, T., 1992. Alterations in biodistribution of ^{11}C-methamphetamine (MAP), ^{14}C-MAP, and ^{123}I-*N*-isopropyl-iodoamphetamine (IMP) in MAP- and cocaine-sensitized animals. Ann. N.Y. Acad. Sci. 654, 153–159.

Page, G., Chalon, S., Barrier, L., Piriou, A., Huguet, F., 1998. Characterization of both dopamine uptake systems in rat striatal slices by specific pharmacological tools. Neurochem. Int. 33, 459–466.

Paterson, I.A., Juorio, A.V., Boulton, A.A., 1990. 2-Phenylethylamine: a modulator of catecholamine transmission in the mammalian central nervous system? J. Neurochem. 55, 1827–1837.

Paxinos, G., Watson, D., 1997. The Rat Brain in Stereotaxic Coordinates. Academic Press, New York.

Pierce, R.C., Kalivas, P.W., 1997. A circuitry model of the expression of behavioral sensitization to amphetamine-like psychostimulants. Brain Res., Brain Res. Rev. 25, 192–216.

Riviere, G.J., Byrnes, K.A., Gentry, W.B., Owens, S.M., 1999. Spontaneous locomotor activity and pharmacokinetics of intravenous methamphetamine and its metabolite amphetamine in the rat. J. Pharmacol. Exp. Ther. 291, 1220–1226.

Riviere, G.J., Gentry, W.B., Owens, S.M., 2000. Disposition of methamphetamine and its metabolite amphetamine in brain and other tissues in rats after intravenous administration. J. Pharmacol. Exp. Ther. 292, 1042–1047.

Robinson, T.E., Becker, J.B., 1986. Enduring changes in brain and behavior produced by chronic amphetamine administration: a review and evaluation of animal models of amphetamine psychosis. Brain Res. 396, 157–198.

Robinson, T.E., Berridge, K.C., 1993. The neural basis of drug craving: an incentive-sensitization theory of addiction. Brain Res., Brain Res. Rev. 18, 247–291.

Russ, H., Staust, K., Martel, F., Gliese, M., Schomig, E., 1996. The extraneuronal transporter for monoamine transmitters exists in cells derived from human central nervous system glia. Eur. J. Neurosci. 8, 1256–1264.

Sato, M., 1983. Long-lasting hypersensitivity to methamphetamine following amygdaloid kindling in cats: the relationship between limbic epilepsy and the psychotic state. Biol. Psychiatry 18, 525–536.

Sato, M., 1986. Acute exacerbation of methamphetamine psychosis and lasting dopaminergic supersensitivity—a clinical survey. Psychopharmacol. Bull. 22, 751–756.

Spaniol, M., Brooks, H., Auer, L., Zimmermann, A., Solioz, M., Stieger, B., Krahenbuhl, S., 2001. Development and characterization of an animal model of carnitine deficiency. Eur. J. Biochem. 268, 1876–1887.

Stewart, J., Badiani, A.A., 1993. Tolerance and sensitization to the behavioral effects of drugs. Behav. Pharmacol. 4, 289–312.

Streich, S., 1996. Expression of the extraneuronal monoamine transporter (uptake2) in human glioma cells. Naunyn-Schmiedeberg's Arch. Pharmacol. 353, 328–333.

Suzuki, H., Shishido, T., Watanabe, Y., Abe, H., Shiragata, M., Honda, K., Horikoshi, R., Niwa, S., 1997. Changes of behavior and monoamine metabolites in the rat brain after repeated methamphetamine administration: effects of duration of repeated administration. Prog. Neuropsychopharmacol. Biol. Psychiatry 21, 359–369.

Sweet, D.H., 2001. Ventricular choline transport: a role for organic cation transporter 2 expressed in choroid plexus. J. Biol. Chem. 276, 41611–41619.

Takayasu, T., Ohshima, T., Nishigami, J., Kondo, T., Lin, Z., Ohtsuji, M., Nagano, T., 1994. Toxicological analysis for drugs and poisons using the formalin-fixed organ tissues: 1. Methamphetamine. Nippon Hoigaku Zasshi 48, 33–37.

Ujike, H., 1989. Effects of selective D-1 and D-2 dopamine antagonists on development of methamphetamine-induced behavioral sensitization. Psychopharmacology 98, 89–92.

Ujike, H., Tsuchida, H., Kanzaki, A., Akiyama, K., Otsuki, S., 1992. Competitive and non-competitive *N*-methyl-D-aspartate antagonists fail to prevent the induction of methamphetamine-induced sensitization. Life Sci. 50, 1673–1681.

Vanderschuren, L.J., Schmidt, E.D., De Vries, T.J., Van Moorsel, C.A., Tilders, F.J., Schoffelmeer, A.N., 1999. A single exposure to amphetamine is sufficient to induce long-term behavioral, neuroendocrine, and neurochemical sensitization in rats. J. Neurosci. 19, 9579–9586.

Waki, Y., Nomura, M., Kasugai, S., Ohya, K., Miyamoto, K., 1999. Walker 256/S carcinosarcoma causes osteoporosis-like changes through ectopical secretion of luteinizing hormone-releasing hormone. Cancer Res. 59, 1219–1224.

Wu, X., Kekuda, R., Huang, W., Fei, Y.J., Leibach, F.H., Chen, J., Conway, S.J., Ganapathy, V., 1998. Identity of the organic cation transporter OCT3 as the extraneuronal monoamine transporter (uptake2) and evidence for the expression of the transporter in the brain. J. Biol. Chem. 273, 32776–32786.

Wu, X., Huang, W., Ganapathy, M.E., Wang, H., Kekuda, R., Conway, S.J., Leibach, F.H., Ganapathy, V., 2000. Structure, function, and regional distribution of the organic cation transporter OCT3 in the kidney. Am. J. Physiol., Renal Physiol. 279, F449–F458.

Xu, M., Guo, Y., Vorhees, C.V., Zhang, J., 2000. Behavioral responses to cocaine and amphetamine administration in mice lacking the dopamine D1 receptor. Brain Res. 852, 198–207.

Yano, I., Ito, T., Takano, M., Inui, K., 1997. Evaluation of renal tubular secretion and reabsorption of levofloxacin in rats. Pharm. Res. 14, 508–511.

Zametkin, A.J., Brown, G.L., Karoum, F., Rapoport, J.L., Langer, D.H., Chuang, L.W., Wyatt, R.J., 1984. Urinary phenethylamine response to D-amphetamine in 12 boys with attention deficit disorder. Am. J. Psychiatry 141, 1055–1058.