

# Dimethyltryptamine and other hallucinogenic tryptamines exhibit substrate behavior at the serotonin uptake transporter and the vesicle monoamine transporter

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**Abstract** *N,N*-dimethyltryptamine (DMT) is a potent plant hallucinogen that has also been found in human tissues. When ingested, DMT and related *N,N*-dialkyltryptamines produce an intense hallucinogenic state. Behavioral effects are mediated through various neurochemical mechanisms including activity at sigma-1 and serotonin receptors, modification of monoamine uptake and release, and competition for metabolic enzymes. To further clarify the pharmacology of hallucinogenic tryptamines, we synthesized DMT, *N*-methyl-*N*-isopropyltryptamine (MIPT), *N,N*-dipropyltryptamine (DPT), and *N,N*-diisopropyltryptamine. We then tested the abilities of these *N,N*-dialkyltryptamines to inhibit [<sup>3</sup>H]5-HT uptake via the plasma membrane serotonin transporter (SERT) in human platelets and via the vesicle monoamine transporter (VMAT2) in Sf9 cells expressing the rat VMAT2. The tryptamines were also tested as inhibitors of [<sup>3</sup>H]paroxetine binding to the SERT and [<sup>3</sup>H]dihydrotrabenazine binding to VMAT2. Our results show that DMT, MIPT, DPT, and DIPT inhibit [<sup>3</sup>H]5-HT transport at the SERT with  $K_i$  values of  $4.00 \pm 0.70$ ,  $8.88 \pm 4.7$ ,  $0.594 \pm 0.12$ , and  $2.32 \pm$

$0.46 \mu\text{M}$ , respectively. At VMAT2, the tryptamines inhibited [<sup>3</sup>H]5-HT transport with  $K_i$  values of  $93 \pm 6.8$ ,  $20 \pm 4.3$ ,  $19 \pm 2.3$ , and  $19 \pm 3.1 \mu\text{M}$ , respectively. On the other hand, the tryptamines were very poor inhibitors of [<sup>3</sup>H]paroxetine binding to SERT and of [<sup>3</sup>H]dihydrotrabenazine binding to VMAT2, resulting in high binding-to-uptake ratios. High binding-to-uptake ratios support the hypothesis that the tryptamines are transporter substrates, not uptake blockers, at both SERT and VMAT2, and also indicate that there are separate substrate and inhibitor binding sites within these transporters. The transporters may allow the accumulation of tryptamines within neurons to reach relatively high levels for sigma-1 receptor activation and to function as releasable transmitters.

**Keywords** Biogenic amine · Dihydrotrabenazine · Dimethyltryptamine · DMT · Dipropyltryptamine · DPT · Diisopropyltryptamine · DIPT · Methylisopropyltryptamine · MIPT · Psychedelic · Paroxetine · Serotonin · Sigma-1 receptor · Trabenazine

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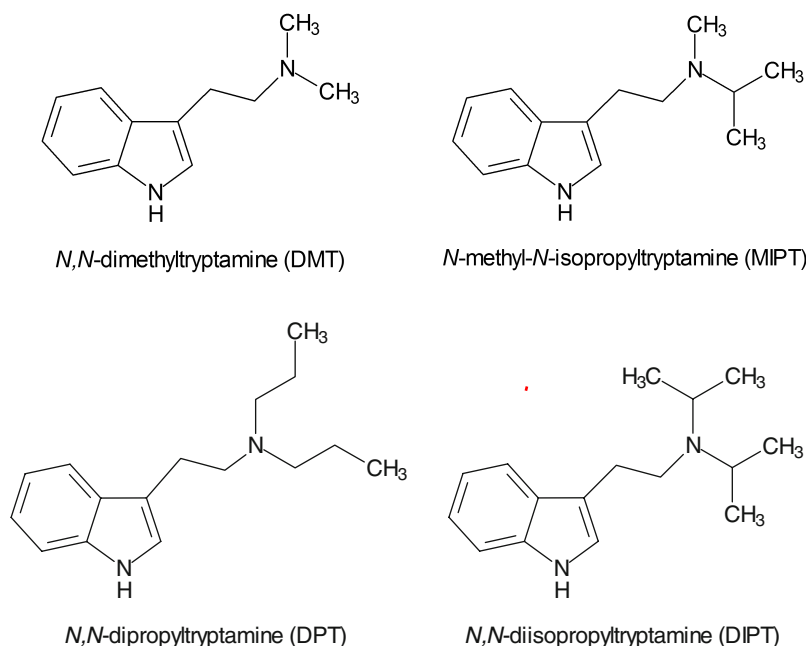
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## Introduction

The plant hallucinogen *N,N*-dimethyltryptamine (DMT; Fig. 1) has been used for religious and other purposes for many centuries (Pochettino et al. 1999). The psychological effects of ingested DMT are characterized as an intense dream-like state with colorful visual illusions, altered perceptions of time and space, changes in body image and sensations, and intense mood changes ranging from euphoria to sadness (Strassman 2001; Strassman et al. 1994). DMT occurs in hundreds of plants around the world, including *Psychotria viridis*, a plant used to make the sacramental hallucinogenic teas *ayahuasca* and *yagé*.

**Fig. 1** Chemical structures of *N,N*-dialkyltryptamines



In addition to occurring in plants, DMT is found in trace amounts in humans and other mammals (Angrist et al. 1976; Axelrod 1961; Corbett et al. 1978; Franzen and Gross 1965; Oon et al. 1977; Saavedra and Axelrod 1972; Smythies et al. 1979; Tanimukai et al. 1970; Wyatt et al. 1973). It is synthesized in mammals from tryptamine by two successive methylation reactions catalyzed by the enzyme indolethylamine-*N*-methyltransferase, using *S*-adenosyl methionine as a methyl donor (Mandell and Morgan 1971; Morgan and Mandell 1969; Thompson et al. 1999; Wyatt et al. 1973).

Numerous structural analogs of DMT have been synthesized in the laboratory and many of these substances are also hallucinogenic (Shulgin and Shulgin 1997). Interestingly, one of these tryptamines, *N,N*-diisopropyltryptamine (DIPT), selectively affects the sense of hearing (Shulgin and Carter 1980; Shulgin and Shulgin 1997). The psychoactive effects of tryptamines are mediated through various neurochemical mechanisms including binding to serotonin receptors (Deliganis et al. 1991; Fantegrossi et al. 2008; Glennon et al. 1978; McKenna et al. 1990), serotonin uptake transporters (Berge et al. 1983; Nagai et al. 2007), and monoamine oxidase enzymes (Reimann and Schneider 1993; Smith et al. 1962).

The sigma-1 receptor is the latest identified target for DMT. We recently reported that DMT binds to sigma-1 receptors at low micromolar concentrations, inhibits voltage-activated sodium ion channels via sigma-1 receptor interactions at higher concentrations, and induces a hypermobility reaction in wild-type mice that is abolished in sigma-1 receptor knockout mice (Fontanilla et al. 2009).

Sigma-1 receptors are ubiquitously expressed in mammalian tissues including human brain. Sigma-1 receptors have a well-defined distribution in the brain, with the highest densities found in cerebellum, orbitofrontal cortex, nucleus accumbens, occipital cortex, and frontal cortex (Weissman et al. 1988). At the cellular level, sigma-1 receptors have been localized to mitochondrial-associated endoplasmic reticulum membranes. When sigma-1 agonists are present at concentrations close to their  $K_1$  values, sigma-1 receptors function as ligand-activated calcium-sensitive chaperones for inositol 1,4,5-trisphosphate receptors (Hayashi and Su 2007). At higher agonist ligand concentrations (e.g., at tenfold  $K_1$ ), sigma-1 receptors move from the endoplasmic reticulum to the plasma membrane, where they interact with and inhibit several ion channels (Aydar et al. 2002; Fontanilla et al. 2009; Hayashi and Su 2003; Lupardus et al. 2000; Mavlyutov and Ruoho 2007; Zhang and Cuevas 2002).

Following our report on the interactions of DMT with sigma-1 receptors, a scheme for DMT signaling through sigma-1 receptors was proposed (Su et al. 2009). Still, because the sigma-1 receptor-mediated effects of DMT on ion channels required micromolar concentrations in vitro, and because DMT is only present in trace amounts in humans (Angrist et al. 1976; Oon et al. 1977) and is rapidly metabolized (Burchett and Hicks 2006), the question arises as to whether there exists a process whereby endogenous DMT could reach sufficiently high levels in vivo to account for its sigma-1 receptor-mediated effects, and, ultimately, some of its behavioral effects. In other words, is there a mechanism that would allow high concentrations of DMT

to be achieved locally within neurons, even though the global concentration in brain as a whole might be very low?

A two-step mechanism that would allow DMT to reach high local concentrations within neurons is via a process involving uptake across the plasma membrane followed by sequestration into synaptic vesicles. Because of earlier reports describing interactions of various hallucinogenic tryptamines with plasma membrane monoamine uptake transporters (Berge et al. 1983; Nagai et al. 2007; Whipple et al. 1983), we hypothesized that DMT and other hallucinogenic tryptamines are substrates for both the cell-surface serotonin uptake transporter (SERTs) and the neuronal vesicle monoamine transporter (VMAT2).

Drugs that affect SERT and VMAT2 are classified into two broad mechanistic categories: uptake blockers and substrate compounds. Uptake blockers bind to the transporters to interfere with their uptake function, but are not themselves transported (Olivier et al. 2000; Schloss and Williams 1998). Examples of drugs that act via this mechanism include the SERT uptake blockers imipramine (Talvenheimo et al. 1979) and paroxetine (Marcusson et al. 1988) and the VMAT2 uptake blocker tetrabenazine (Thiriou and Ruoho 2001). Substrates, on the other hand, are transported into the cytosol or vesicle by SERT or VMAT2, respectively. Examples of compounds that are transported include serotonin itself, methamphetamine, 3,4-methylenedioxymphetamine, and fenfluramine (Crespi et al. 1997; McKenna et al. 1991; Nelson and Rudnick 1979; Rothman et al. 2001). To classify compounds as either SERT uptake blockers or substrates, Rothman and colleagues developed a method that compares a compound's potency to compete for uptake blocker binding sites with its potency at inhibiting 5-HT transport (Rothman et al. 1993, 1999). For a series of known substrates and uptake inhibitors, these scientists observed that substrate compounds were more potent at inhibiting 5-HT transport than they were as competitive inhibitors of SERT uptake blocker binding. Conversely, the potency of known SERT uptake blockers to prevent 5-HT transport was similar to their potency as competitors of SERT uptake blocker binding. Thus, the ratio of a test drug's  $K_i$  for the inhibition of binding of the SERT uptake inhibitor [ $^{125}$ I]RTI-55 to its  $K_i$  value for the inhibition of [ $^3$ H]5-HT uptake (the binding-to-uptake ratio) was typically greater than 10 for known substrates such as 5-HT, fenfluramine, and amphetamine. The ratio was less than 2 for known uptake inhibitors such as clomipramine, fluoxetine, paroxetine, and cocaine (Rothman et al. 1999). If binding-to-uptake ratios for known transporter substrates are high compared to the ratios for known uptake inhibitors, evidence is provided that at least two nonoverlapping binding sites exist on the transporters, one for substrates and one for inhibitors.

If DMT and related tryptamines are substrates for SERT and VMAT2, they should also exhibit high binding-to-uptake ratios. To test this hypothesis, DMT, *N*-methyl-*N*-isopropyltryptamine (MIPT), *N,N*-dipropyltryptamine (DPT), and *N,N*-diisopropyltryptamine (DIPT) (Fig. 1) were examined for their abilities to inhibit [ $^3$ H]5-HT uptake via both SERT and VMAT2. We also tested the compounds as inhibitors of [ $^3$ H]paroxetine binding to SERT and [ $^3$ H]dihydrotetrabenazine binding to VMAT2. Based on the results of uptake inhibition, binding competition, and binding-to-uptake ratios, we then classified the tryptamines as substrates or uptake blockers.

## Materials and methods

### Drugs and reagents

[ $^3$ H]5-HT (specific activity 20.3 Ci/mmol), [ $^3$ H]paroxetine (specific activity 29.3 Ci/mmol), and [ $^3$ H]NaBH $_4$  (specific activity 75 Ci/mmol) were purchased from New England Nuclear, Boston, MA.

Tryptamines were synthesized by the method of Speeter and Anthony (1954) with minor modifications. Indole was condensed with oxalylchloride in diethyl ether to generate crystalline indol-3-yl glyoxylchloride. The glyoxylchloride was reacted with either *N,N*-dimethylamine, *N*-methyl-*N*-isopropylamine, *N,N*-dipropylamine, or *N,N*-diisopropylamine to yield the respective 3-indoleglyoxylamides. The glyoxylamides were then reduced to the *N,N*-disubstituted tryptamines with lithium aluminum hydride in refluxing dioxane as described (Brutcher and Vanderwerff 1958). Excess lithium aluminum hydride was decomposed by adding, in sequence (based on the weight  $X$  in grams of lithium aluminum hydride used),  $X$  mL H $_2$ O,  $X$  mL 15% NaOH, and  $4X$  mL H $_2$ O. The formed solids were removed by filtration, washed with dioxane, and discarded. The combined filtrate and dioxane washings were dried over anhydrous MgSO $_4$ . The free-base tryptamines were obtained after solvent removal by rotary evaporation. Analytical data (mass spectrometry, elemental analysis) confirmed the expected structures.

[ $^3$ H]Dihydrotetrabenazine was synthesized by reducing the 2-ketone of tetrabenazine as previously described (Sievert et al. 1998). Briefly, 5  $\mu$ mol of tetrabenazine in 300  $\mu$ l of dimethylformamide was reduced over 3 days at room temperature with [ $^3$ H]NaBH $_4$  (75 Ci/mmol). The reaction mixture was extracted with  $3 \times 1$  mL ethyl acetate and the combined organic extracts were concentrated under a stream of nitrogen to approximately 100  $\mu$ l. The concentrated extract was chromatographed using a silica gel-on-glass thin layer chromatography plate with ethyl acetate:methanol (5:1) as the mobile phase. The

silica gel containing the appropriate UV-quenching band ( $R_f \sim 0.8$ ) was removed from the plate and the silica was extracted with methanol. The isolated [ $^3\text{H}$ ]dihydro-tetrabenazine comigrated with authentic nonradioactive dihydro-tetrabenazine.

Fluoxetine, imipramine, ketanserin, norepinephrine, serotonin, reserpine, buffer components, and reagents for organic synthesis were purchased from various commercial sources.

#### SERT-mediated [ $^3\text{H}$ ]5-HT uptake and [ $^3\text{H}$ ]paroxetine binding

Outdated human platelets were obtained from the blood bank at the University of Wisconsin Clinical Sciences Center, Madison, WI. Platelets from 5–10 donors were pooled, 10% dimethylsulfoxide was added, and aliquots were stored frozen at  $-80^\circ\text{C}$  until use. Platelets were prepared for uptake or binding assays as described (Cozzi and Foley 2002).

For both uptake and binding assays, platelets were suspended in ice-cold Krebs-Ringer HEPES buffer containing (mM): NaCl (124.0), KCl (2.9),  $\text{MgSO}_4$  (1.3),  $\text{KH}_2\text{PO}_4$  (1.2),  $\text{CaCl}_2$  (2.4), D-glucose (5.2), HEPES (25.0), sodium ascorbate (0.1), and pargyline (0.1); pH = 7.4. For uptake assays, the ability of platelets to accumulate [ $^3\text{H}$ ]5-HT was measured in the absence and presence of various concentrations of test compounds as follows: a 490  $\mu\text{l}$  aliquot of the platelet suspension was added to glass tubes containing 5  $\mu\text{l}$  test drugs (dissolved in DMSO or water) or 5  $\mu\text{l}$  water (for total and nonspecific determinations). The assay tubes were preincubated in a  $37^\circ\text{C}$  water bath for 5 min. The tubes were then returned to the ice bath and chilled for 15 min. [ $^3\text{H}$ ]5-HT was added (5  $\mu\text{l}$  of stock solution; final concentration, 10 nM), giving a total incubation volume of 500  $\mu\text{l}$ . All tubes except nonspecific tubes were returned to the  $37^\circ\text{C}$  water bath to initiate [ $^3\text{H}$ ]5-HT uptake. After 5 min, uptake was stopped by returning the test tubes to the ice bath. After adding 3 ml ice-cold 300 mM NaCl, each assay tube was immediately vacuum filtered through glass fiber filters (Whatman GF/B) pretreated with 0.1% polyethyleneimine. Filters were washed twice with 3 ml ice-cold 300 mM NaCl, allowed to dry briefly under vacuum, then placed in liquid scintillation vials. Scintillation cocktail (3 ml Bio-Safe II, RPI Corporation) was added and the vials were sealed, vortexed, and allowed to stand overnight. Radioactivity was measured using liquid scintillation counting (Packard Tri-Carb 1600 CA). Specific uptake was defined as uptake at  $37^\circ\text{C}$  minus uptake at  $0^\circ\text{C}$  in the absence of drugs. Under these conditions, [ $^3\text{H}$ ]5-HT uptake was typically greater than 90% specific.

*N,N*-dialkyltryptamines, 5-HT (negative control), and imipramine (positive control) were screened for SERT uptake blocker competition by incubating the platelet preparations with Krebs-Ringer HEPES buffer containing 4 nM [ $^3\text{H}$ ]paroxetine in the presence or absence of 100  $\mu\text{M}$  concentrations of test compounds for 60 min at  $0^\circ\text{C}$ . Nonspecific [ $^3\text{H}$ ]paroxetine binding was defined with 10  $\mu\text{M}$  fluoxetine in the absence of test drugs. Labeled platelets were recovered by rapid vacuum filtration as described above. Radioactivity on the filters was counted and expressed as a percent of specific [ $^3\text{H}$ ]paroxetine binding.

#### VMAT2-mediated [ $^3\text{H}$ ]5-HT uptake and [ $^3\text{H}$ ] dihydro-tetrabenazine binding

Log-phase *Spodoptera frugiperda* (Sf9) cells were infected with recombinant baculovirus expressing rat VMAT2 and harvested 3 days later. The cells were suspended in sucrose-HEPES buffer, pH = 7.6, and passed through a custom-made cell cracker 30 times. The resulting suspension was subjected to centrifugation at low speed to remove cell debris and unbroken cells. The supernatant was used for uptake and binding assays.

Experiments testing for inhibition of substrate uptake were performed by first incubating vesicles in the presence of 10 mM ATP-Mg to form the proton gradient, then aliquots were added to wells containing 30 nM [ $^3\text{H}$ ]5-HT in the presence or absence of various concentrations of the test compounds. The suspension was incubated at  $32^\circ\text{C}$  for 8 min. The vesicles were rapidly collected on a 0.5% polyethyleneimine-soaked glass fiber filters (Whatman GF/B) and washed 3 times with sucrose-HEPES buffer in the absence of ATP-Mg. After adding scintillation cocktail, filters were counted for radioactivity. Specific uptake was defined as radioactivity accumulated in the absence of any test compound minus the amount of radioactivity accumulated in the presence of 10  $\mu\text{M}$  reserpine. The values obtained using the test compounds were normalized to specific [ $^3\text{H}$ ]5-HT uptake.

Studies to test for competition of VMAT2 uptake blocker binding were conducted in Sf9 vesicles expressing VMAT2 by incubating various concentrations of tryptamines, norepinephrine (negative control), or ketanserin (positive control) with 20 nM [ $^3\text{H}$ ]dihydro-tetrabenazine in sucrose-HEPES buffer for 60 min at  $32^\circ\text{C}$ . The vesicles were then rapidly filtered and washed using a cell harvester and collected on glass fiber filters (Whatman GF/B) filters soaked in 0.5% polyethyleneimine. The filters were counted for radioactivity using liquid scintillation counting. Nonspecific [ $^3\text{H}$ ]dihydro-tetrabenazine binding was defined with 10  $\mu\text{M}$  tetrabenazine in the absence of test drugs.

## Data analysis

The mean uptake  $IC_{50} \pm SEM$  at SERT and VMAT2 for each test drug was determined from displacement curves from 3–8 experiments using at least six drug concentrations, each run in triplicate. Data were transformed from decays per minute to percent specific uptake and fitted to a four-parameter logistic curve using commercial computer software. Uptake  $K_I$  values were calculated from the  $IC_{50}$  values using the Cheng–Prusoff equation (Cheng and Prusoff 1973).

Test drugs were screened for [ $^3H$ ]paroxetine (SERT) or [ $^3H$ ]dihydrotrabenazine (VMAT2) binding inhibition at 100 or 1,000  $\mu M$ , respectively. The percent inhibition of radioligand binding was determined, then the  $IC_{50}$  values were estimated from the percent inhibition of binding at the single 100 or 1,000  $\mu M$  concentrations according to the relationship:

$$\frac{D \times T}{DT} = \frac{D' \times T'}{DT'}$$

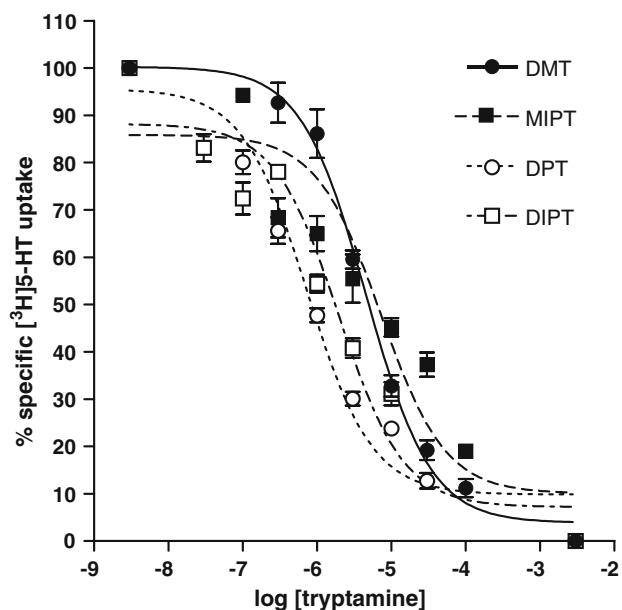
where  $D$  = drug concentration (i.e., 100  $\mu M$  [SERT] or 1,000  $\mu M$  [VMAT2]),  $T$  = % specific binding remaining,  $DT$  = % specific binding inhibited, with  $T + DT = 100\%$ .  $D'$ ,  $T'$ , and  $DT'$  represent the corresponding values at the derived  $IC_{50}$ , i.e.,  $D' = IC_{50}$ ,  $T' = DT' = 0.5$ . Binding  $K_I$  values were calculated from the estimated  $IC_{50}$  values using the Cheng–Prusoff equation. The binding-to-uptake ratios were then expressed as  $K_I$  binding/ $K_I$  uptake.

## Results

### Effect of *N,N*-dialkyltryptamines on uptake, binding, and release

Specific [ $^3H$ ]5-HT uptake into human platelets was typically greater than 90% of total uptake. All of the *N,N*-dialkyltryptamines in this study inhibited [ $^3H$ ]5-HT accumulation via the plasma membrane SERT (Fig. 2).  $K_I$  values for the SERT are in the low micromolar range. DPT was the most potent inhibitor of [ $^3H$ ]5-HT uptake with a  $K_I$  of  $0.594 \pm 0.12 \mu M$  (Table 1).

Specific [ $^3H$ ]paroxetine binding was greater than 60% of total binding as defined by 10  $\mu M$  fluoxetine. In contrast to the uptake experiments, only DIPT and imipramine showed greater than 50% inhibition of [ $^3H$ ]paroxetine binding at the screening concentration of 100  $\mu M$  (Fig. 3). DIPT inhibited [ $^3H$ ]paroxetine binding by 54% while the positive control imipramine inhibited [ $^3H$ ]paroxetine binding by greater than 99%. Serotonin, a negative control, decreased radioligand binding by less than 27% at 1,000  $\mu M$  (Fig. 3).



**Fig. 2** Drug inhibition of SERT-mediated [ $^3H$ ]5-HT uptake. The ability of *N,N*-dialkyltryptamines to inhibit accumulation of 10 nM [ $^3H$ ]5-HT was examined in human platelets. Specific uptake was defined as uptake at 37°C minus at 0°C. Data are the mean  $\pm$  SEM of 3–8 determinations, each run in triplicate. Data were fitted to a four-parameter logistic curve for  $IC_{50}$  determination and plotting

Specific uptake of [ $^3H$ ]5-HT into Sf9-derived vesicles containing VMAT2 was usually greater than 90% of total uptake. Curves for inhibition of [ $^3H$ ]5-HT uptake by tryptamines are shown in Fig. 4. While DPT, MIPT, and DIPT showed similar low micromolar  $K_I$  values ( $\sim 20 \mu M$ ) for inhibition of VMAT2-specific uptake, the  $K_I$  value of DMT is somewhat higher at  $93 \pm 6.8 \mu M$  (Table 1). When the test drugs were assayed for inhibition of [ $^3H$ ]dihydrotrabenazine binding, only the positive binding control ketanserin exhibited appreciable competition (Table 1). The known substrate (and negative binding control) norepinephrine did not inhibit [ $^3H$ ]dihydrotrabenazine binding at 1,000  $\mu M$ .

## Discussion

Hallucinogenic tryptamines such as DMT have a long and interesting history of use in various human cultures. Since the observation was made that DMT was present in human tissues, much speculation has ensued regarding its role in normal brain function and in mental disease (Jacob and Presti 2005; Lipinski et al. 1974; Strassman 2001; Wyatt et al. 1973). In the present work, we hypothesized that if DMT and related tryptamines could be taken up and stored within cells via SERT and VMAT2, they would exhibit high binding-to-uptake ratios. To test the hypothesis, several *N,N*-dialkyltryptamines were examined for their

**Table 1**  $K_i$  values ( $\mu\text{M}$ ) and binding-to-uptake ratios for various compounds at SERT and VMAT2

Compound	SERT			VMAT2		
	$[^3\text{H}]5\text{-HT}$ uptake	$[^3\text{H}]$ PXT binding	Ratio	$[^3\text{H}]5\text{-HT}$ uptake	$[^3\text{H}]$ TBZOH binding	Ratio
DMT	$4.00 \pm 0.70$	$>47$	$>11$	$93 \pm 6.8$	$>1,000$	$>10$
MIPT	$8.88 \pm 4.7$	$>97$	$>10$	$20 \pm 4.3$	$>1,000$	$>50$
DPT	$0.594 \pm 0.12$	$>32$	$>55$	$19 \pm 2.3$	$>1,000$	$>50$
DIPT	$2.32 \pm 0.46$	$>17$	$>7$	$19 \pm 3.1$	$>1,000$	$>50$
Imipramine	$0.017^a$	$0.009^b$	0.53	ND	ND	–
5-HT	$0.222^c$	$>540$	$>2400$	ND	ND	–
Norepinephrine	ND	ND	–	$1.3 \pm 0.3$	$>272$	$>200$
Ketanserin	ND	ND	–	$0.7 \pm 0.1$	0.086	0.1

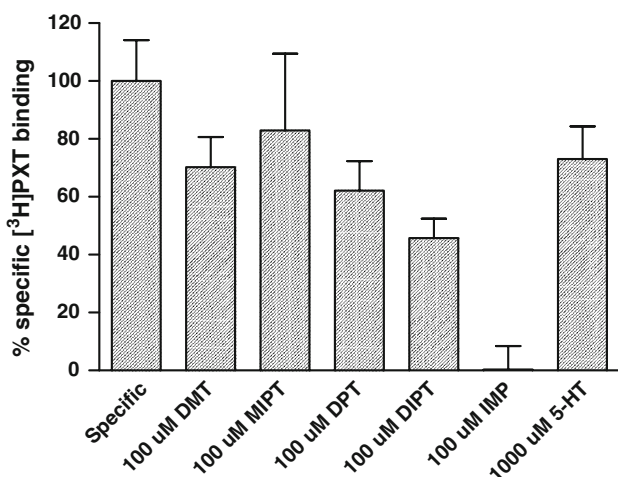
The ability of compounds to inhibit  $[^3\text{H}]5\text{-HT}$  uptake or radioligand binding was determined as described under “Materials and methods”

$[^3\text{H}]$ PXT =  $[^3\text{H}]$ paroxetine,  $[^3\text{H}]$ TBZOH =  $[^3\text{H}]$ dihydrotetrabenazine, ND not determined

<sup>a</sup> Data from Talvenheimo et al. (1979)

<sup>b</sup> Data from Marcusson et al. (1988)

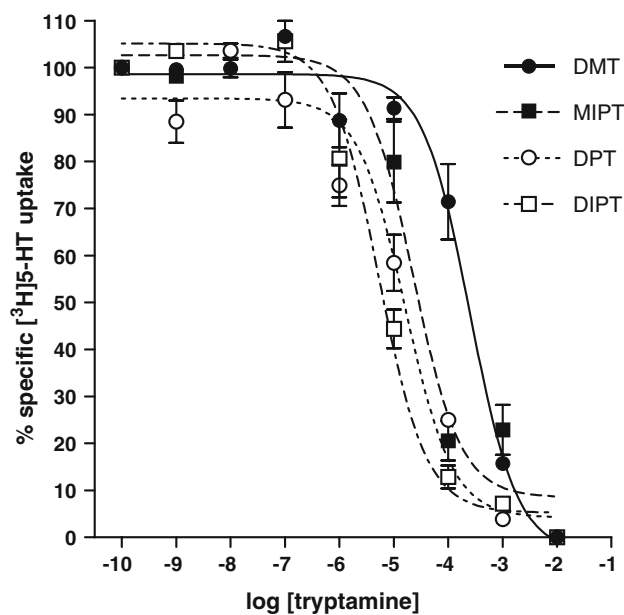
<sup>c</sup> Data from Nelson and Rudnick (1979)



**Fig. 3** Comparison of abilities of *N,N*-dialkyltryptamines, imipramine, and serotonin to inhibit  $[^3\text{H}]$ paroxetine binding to human platelets. The ability of test drugs, imipramine (IMP), and 5-HT to compete for 4 nM  $[^3\text{H}]$ paroxetine binding was examined in human platelets. Specific uptake was defined with 10  $\mu\text{M}$  fluoxetine. Data are the mean  $\pm$  SEM of 2–7 experiments run in triplicate

abilities to affect  $[^3\text{H}]5\text{-HT}$  uptake via SERT and VMAT2 and to competitively inhibit binding of known SERT and VMAT2 blockers.

All of the tryptamines tested inhibited  $[^3\text{H}]5\text{-HT}$  uptake via SERT and VMAT2 with  $K_i$  values in the micromolar range (Figs. 1, 2; Table 1). The tryptamines were between 2- and 32-fold more potent at SERT than they were at VMAT2 (Table 1). On the other hand, the tryptamines' abilities to interfere with uptake blocker binding at both transporters was considerably weaker (Table 1). Only the known SERT uptake blocker imipramine reduced  $[^3\text{H}]$ paroxetine binding to nonspecific levels (Fig. 3), and,



**Fig. 4** Drug inhibition of VMAT2-mediated  $[^3\text{H}]5\text{-HT}$  uptake. The ability of tryptamines to inhibit accumulation of 30 nM  $[^3\text{H}]5\text{-HT}$  was examined in Sf9 cells expressing the rat VMAT2. Specific uptake was defined with 10  $\mu\text{M}$  reserpine. Data are the mean  $\pm$  SEM of 3 determinations, each run in triplicate. Data were fitted to a four-parameter logistic curve for  $\text{IC}_{50}$  determination and plotting

at VMAT2, only the known uptake blocker ketanserin prevented  $[^3\text{H}]$ dihydrotetrabenazine binding (Table 1). As expected of substrates, serotonin did not inhibit  $[^3\text{H}]$ paroxetine binding to SERT and norepinephrine did not inhibit  $[^3\text{H}]$ dihydrotetrabenazine binding to VMAT2 (Fig. 3; Table 1). These results are consistent with the known pharmacology of these compounds and gave us confidence in the binding-to-uptake ratio method of analysis. When

the binding-to-uptake ratios for the *N,N*-dialkyltryptamines were calculated, the ratios ranged between 7 and 55 at the SERT and between 10 and 50 at VMAT2 (Table 1). The high binding-to-uptake ratios imply separate substrate and inhibitor sites at the SERT and VMAT2 and support the hypothesis that the tryptamine compounds are substrates for both transporters.

It is not immediately apparent whether the  $K_I$  values obtained in the present study have clinical relevance. In humans, effective hallucinogenic doses produce peak DMT plasma concentrations ranging between 12 and 90  $\mu\text{g/L}$  and with an apparent volume of distribution of 36–55 L/kg (Callaway et al. 1999; Riba et al. 2003; Yritia et al. 2002). The corresponding molar plasma concentrations of DMT are in the range of 0.060–0.500  $\mu\text{M}$  and this range is lower than the  $K_I$  values that were derived in the present study. However, the relatively high volume of distribution of DMT indicates significant movement of the drug from plasma into tissues and several reports have described the active accumulation of DMT and other tryptamines into rat brain following peripheral administration (Barker et al. 1982; Sangiah et al. 1979; Sitaram et al. 1987; Takahashi et al. 1985; Yanai et al. 1986). Similar active uptake processes in humans may plausibly concentrate DMT by severalfold or more, resulting in micromolar concentrations in the brain. The findings in the present study that the tryptamines exhibit substrate behavior at the SERT and VMAT2 are consistent with and strengthen this possibility.

In a report by Nagai et al., six substituted tryptamines inhibited 5-HT uptake in the low micromolar range and two of these compounds ( $\alpha$ -methyltryptamine and 5-methoxy- $\alpha$ -methyltryptamine) were classified as 5-HT releasers (Nagai et al. 2007). Based on the failure of other tryptamines to evoke 5-HT release, the authors speculated that, among psychoactive tryptamines, a primary amine group might be necessary for monoamine releasing activity (a property of substrates). Our results suggest that this is not necessarily the case, because the tryptamines in the present study are all tertiary amines and they display substrate behavior. Reasons for this seeming difference might be due to species and tissue differences in substrate recognition by SERT (Adkins et al. 2001; Barker et al. 1994, 1999). For example, Nagai et al. studied 5-HT uptake and release in rat brain synaptosomes, while we used human platelets and Sf9-derived vesicles containing rat VMAT2. Another important difference is in the chemical structures of the tryptamines tested. Those in the present study do not carry substitutions on the aromatic indole ring while most of the tryptamines studied by Nagai et al. had 5-methoxy substituents.

In summary, we report evidence for substrate-like properties for DMT, MIPT, DPT, and DIPT at the SERT

and VMAT2 uptake transporters. Together, the plasma membrane and vesicular uptake processes provide two mechanisms whereby high intracellular and vesicular concentrations of DMT and related compounds may be achieved within neurons. This is especially important regarding DMT interactions with sigma-1 receptors; DMT modulates sigma-1 chaperone activity and affects ion channels at micromolar concentrations. The transporters are two possible ways of attaining these concentrations. Once inside a neuron, these tryptamines can interact with intracellular sigma-1 receptors located in the mitochondrion-associated endoplasmic reticulum membrane (Hayashi and Su 2007) or they can be released into the synapse upon vesicular fusion to interact with cell-surface sigma-1 receptors, serotonin receptors, or other molecular targets. Interference with 5-HT uptake and the potential ability of the tryptamines to release 5-HT as substrate analogs at SERT and VMAT2 means that the psychoactive effects of these tryptamines cannot be explained solely through their already-known direct receptor actions. These results also suggest two mechanisms through which these drugs could reach intracellular binding sites and be stored within synaptic vesicles for subsequent release as transmitter substances. The hallucinogenic properties of these compounds likely arise from a complex interplay among all of these mechanisms.

## References

- Adkins EM, Barker EL, Blakely RD (2001) Interactions of tryptamine derivatives with serotonin transporter species variants implicate transmembrane domain I in substrate recognition. *Mol Pharmacol* 59:514–523
- Angrist B, Gershon S, Sathananthan G, Walker RW, Lopez-Ramos B, Mandel LR, Vandenheuevel WJ (1976) Dimethyltryptamine levels in blood of schizophrenic patients and control subjects. *Psychopharmacology (Berl)* 47:29–32
- Axelrod J (1961) Enzymatic formation of psychotomimetic metabolites from normally occurring compounds. *Science* 134:343
- Aydar E, Palmer CP, Klyachko VA, Jackson MB (2002) The sigma receptor as a ligand-regulated auxiliary potassium channel subunit. *Neuron* 34:399–410
- Barker SA, Beaton JM, Christian ST, Monti JA, Morris PE (1982) Comparison of the brain levels of *N,N*-dimethyltryptamine and alpha, alpha, beta, beta-tetra-deutero-*N,N*-dimethyltryptamine following intraperitoneal injection. The in vivo kinetic isotope effect. *Biochem Pharmacol* 31:2513–2516
- Barker EL, Kimmel HL, Blakely RD (1994) Chimeric human and rat serotonin transporters reveal domains involved in recognition of transporter ligands. *Mol Pharmacol* 46:799–807
- Barker EL, Moore KR, Rakhshan F, Blakely RD (1999) Transmembrane domain I contributes to the permeation pathway for serotonin and ions in the serotonin transporter. *J Neurosci* 19:4705–4717
- Berge OG, Chacho D, Hole K (1983) Inhibitory effect of 5-methoxy-*N,N*-dimethyltryptamine on the synaptosomal uptake of 5-hydroxytryptamine. *Eur J Pharmacol* 90:293–296

- Brutcher JF, Vanderwerff W (1958) Concerning a preparation of tryptamine. *J Org Chem* 23:146–147
- Burchett SA, Hicks TP (2006) The mysterious trace amines: protean neuromodulators of synaptic transmission in mammalian brain. *Prog Neurobiol* 79:223–246
- Callaway JC, McKenna DJ, Grob CS, Brito GS, Raymon LP, Poland RE, Andrade EN, Andrade EO, Mash DC (1999) Pharmacokinetics of *hoasca* alkaloids in healthy humans. *J Ethnopharmacol* 65:243–256
- Cheng Y, Prusoff WH (1973) Relationship between the inhibition constant ( $k_1$ ) and the concentration of inhibitor which causes 50 per cent inhibition (I50) of an enzymatic reaction. *Biochem Pharmacol* 22:3099–3108
- Corbett L, Christian ST, Morin RD, Benington F, Smythies JR (1978) Hallucinogenic *N*-methylated indolealkylamines in the cerebrospinal fluid of psychiatric and control populations. *Br J Psychiatry* 132:139–144
- Cozzi NV, Foley KF (2002) Rapid and efficient method for suspending cells for neurotransmitter uptake assays. *Biotechniques* 32:486–492
- Crespi D, Mennini T, Gobbi M (1997) Carrier-dependent and  $Ca^{2+}$ -dependent 5-HT and dopamine release induced by (+)-amphetamine, 3,4-methylenedioxymethamphetamine, *p*-chloroamphetamine and (+)-fenfluramine. *Br J Pharmacol* 121:1735–1743
- Deliganis AV, Pierce PA, Peroutka SJ (1991) Differential interactions of dimethyltryptamine (DMT) with 5-HT<sub>1A</sub> and 5-HT<sub>2</sub> receptors. *Biochem Pharmacol* 41:1739–1744
- Fantegrossi WE, Reissig CJ, Katz EB, Yarosh HL, Rice KC, Winter JC (2008) Hallucinogen-like effects of *N,N*-dipropyltryptamine (DPT): possible mediation by serotonin 5-HT<sub>1A</sub> and 5-HT<sub>2A</sub> receptors in rodents. *Pharmacol Biochem Behav* 88:358–365
- Fontanilla D, Johannessen M, Hajipour AR, Cozzi NV, Jackson MB, Ruoho AE (2009) The hallucinogen *N,N*-dimethyltryptamine (DMT) is an endogenous sigma-1 receptor regulator. *Science* 323:934–937
- Franzen F, Gross H (1965) Tryptamine, *N,N*-dimethyltryptamine, *N,N*-dimethyl-5-hydroxytryptamine and 5-methoxytryptamine in human blood and urine. *Nature* 206:1052
- Glennon RA, Liebowitz SM, Mack EC (1978) Serotonin receptor binding affinities of several hallucinogenic phenylalkylamine and *N,N*-dimethyltryptamine analogues. *J Med Chem* 21:822–825
- Hayashi T, Su TP (2003) Intracellular dynamics of  $\sigma$ -1 receptors ( $\sigma$ 1 binding sites) in NG108–15 cells. *J Pharmacol Exp Ther* 306:726–733
- Hayashi T, Su TP (2007) Sigma-1 receptor chaperones at the ER-mitochondrion interface regulate  $Ca^{2+}$  signaling and cell survival. *Cell* 131:596–610
- Jacob MS, Presti DE (2005) Endogenous psychoactive tryptamines reconsidered: an anxiolytic role for dimethyltryptamine. *Med Hypotheses* 64:930–937
- Lipinski JF, Mandel LR, Ahn HS, Vanden Heuvel WJ, Walker RW (1974) Blood dimethyltryptamine concentrations in psychotic disorders. *Biol Psychiatry* 9:89–91
- Lupardus PJ, Wilke RA, Aydar E, Palmer CP, Chen Y, Ruoho AE, Jackson MB (2000) Membrane-delimited coupling between sigma receptors and  $K^+$  channels in rat neurohypophysial terminals requires neither G-protein nor ATP. *J Physiol* 526(Pt 3):527–539
- Mandell AJ, Morgan M (1971) Indole(ethyl)amine *N*-methyltransferase in human brain. *Nat New Biol* 230:85–87
- Marcusson JO, Bergstrom M, Eriksson K, Ross SB (1988) Characterization of [<sup>3</sup>H]paroxetine binding in rat brain. *J Neurochem* 50:1783–1790
- Mavlyutov TA, Ruoho AE (2007) Ligand-dependent localization and intracellular stability of sigma-1 receptors in CHO-K1 cells. *J Mol Signal* 2:8
- McKenna DJ, Repke DB, Lo L, Peroutka SJ (1990) Differential interactions of indolealkylamines with 5-hydroxytryptamine receptor subtypes. *Neuropharmacology* 29:193–198
- McKenna DJ, Guan XM, Shulgin AT (1991) 3, 4-Methylenedioxymphetamine (MDA) analogues exhibit differential effects on synaptosomal release of [<sup>3</sup>H]dopamine and [<sup>3</sup>H]5-hydroxytryptamine. *Pharmacol Biochem Behav* 38:505–512
- Morgan M, Mandell AJ (1969) Indole(ethyl)amine *N*-methyltransferase in the brain. *Science* 165:492–493
- Nagai F, Nonaka R, Satoh Hisashi Kamimura K (2007) The effects of non-medically used psychoactive drugs on monoamine neurotransmission in rat brain. *Eur J Pharmacol* 559:132–137
- Nelson PJ, Rudnick G (1979) Coupling between platelet 5-hydroxytryptamine and potassium transport. *J Biol Chem* 254:10084–10089
- Olivier B, Soudijn W, van Wijngaarden I (2000) Serotonin, dopamine and norepinephrine transporters in the central nervous system and their inhibitors. *Prog Drug Res* 54:59–119
- Oon MC, Murray RM, Rodnight R, Murphy MP, Birley JL (1977) Factors affecting the urinary excretion of endogenously formed dimethyltryptamine in normal human subjects. *Psychopharmacology (Berl)* 54:171–175
- Pochettino ML, Cortella AR, Ruiz M (1999) Hallucinogenic snuff from northwestern Argentina: microscopical identification of *Anadenanthera colubrina* var *Cebil* (fabaceae) in powdered archaeological material. *Econ Bot* 53:127–132
- Reimann W, Schneider F (1993) The serotonin receptor agonist 5-methoxy-*N,N*-dimethyltryptamine facilitates noradrenaline release from rat spinal cord slices and inhibits monoamine oxidase activity. *Gen Pharmacol* 24:449–453
- Riba J, Valle M, Urbano G, Yritia M, Morte A, Barbanj MJ (2003) Human pharmacology of ayahuasca: subjective and cardiovascular effects, monoamine metabolite excretion, and pharmacokinetics. *J Pharmacol Exp Ther* 306:73–83
- Rothman RB, Becketts KM, Radesca LR, de Costa BR, Rice KC, Carroll FI, Dersch CM (1993) Studies of the biogenic amine transporters II A brief study on the use of [<sup>3</sup>H]DA-uptake-inhibition to transporter-binding-inhibition ratios for the in vitro evaluation of putative cocaine antagonists. *Life Sci* 53:PL267–PL272
- Rothman RB, Ayestas MA, Dersch CM, Baumann MH (1999) Aminorex, fenfluramine, and chlorphentermine are serotonin transporter substrates. Implications for primary pulmonary hypertension. *Circulation* 100:869–875
- Rothman RB, Baumann MH, Dersch CM, Romero DV, Rice KC, Carroll FI, Partilla JS (2001) Amphetamine-type central nervous system stimulants release norepinephrine more potently than they release dopamine and serotonin. *Synapse* 39:32–41
- Saavedra JM, Axelrod J (1972) Psychotomimetic *N*-methylated tryptamines: formation in brain in vivo and in vitro. *Science* 175:1365–1366
- Sangiah S, Gomez MV, Domino EF (1979) Accumulation of *N,N*-dimethyltryptamine in rat brain cortical slices. *Biol Psychiatry* 14:925–936
- Schloss P, Williams DC (1998) The serotonin transporter: a primary target for antidepressant drugs. *J Psychopharmacol* 12:115–121
- Shulgin AT and Shulgin A (1997) *TIHKAL: the continuation*. Transform Press, Berkeley, CA 94712
- Shulgin AT, Carter MF (1980) *N,N*-diisopropyltryptamine (DIPT) and 5-methoxy-*N,N*-diisopropyltryptamine (5-MeO-DIPT). Two orally active tryptamine analogs with CNS activity. *Commun Psychopharmacol* 4:363–369



- Sievert MK, Thiriot DS, Edwards RH, Ruoho AE (1998) High-efficiency expression and characterization of the synaptic-vesicle monoamine transporter from baculovirus-infected insect cells. *Biochem J* 330(Pt 2):959–966
- Sitaram BR, Lockett L, Talomsin R, Blackman GL, McLeod WR (1987) In vivo metabolism of 5-methoxy-*N,N*-dimethyltryptamine and *N,N*-dimethyltryptamine in the rat. *Biochem Pharmacol* 36:1509–1512
- Smith TE, Weissbach H, Udenfriend S (1962) Studies on the mechanism of action of monoamine oxidase: metabolism of *N,N*-dimethyltryptamine and *N,N*-dimethyltryptamine-*N*-oxide. *Biochemistry* 1:137–143
- Smythies JR, Morin RD, Brown GB (1979) Identification of dimethyltryptamine and *O*-methylbufotenin in human cerebrospinal fluid by combined gas chromatography/mass spectrometry. *Biol Psychiatry* 14:549–556
- Speeter ME, Anthony WC (1954) The action of oxalyl chloride on indoles: a new approach to tryptamines. *J Am Chem Soc* 76:6208–6210
- Strassman RJ (2001) DMT: the spirit molecule: a doctor's revolutionary research into the biology of near-death and mystical experiences. Park Street Press, Rochester, VT
- Strassman RJ, Qualls CR, Uhlenhuth EH, Kellner R (1994) Dose-response study of *N,N*-dimethyltryptamine in humans. II. Subjective effects and preliminary results of a new rating scale. *Arch Gen Psychiatry* 51:98–108
- Su T-P, Hayashi T, Vaupel DB (2009) When the endogenous hallucinogenic trace amine *N,N*-dimethyltryptamine meets the sigma-1 receptor. *Sci Signal* 2: pe12
- Takahashi T, Takahashi K, Ido T, Yanai K, Iwata R, Ishiwata K, Nozoe S (1985) <sup>11</sup>C-labeling of indolealkylamine alkaloids and the comparative study of their tissue distributions. *Int J Appl Radiat Isot* 36:965–969
- Talvenheimo J, Nelson PJ, Rudnick G (1979) Mechanism of imipramine inhibition of platelet 5-hydroxytryptamine transport. *J Biol Chem* 254:4631–4635
- Tanimukai H, Ginther R, Spaide J, Bueno JR, Himwich HE (1970) Detection of psychotomimetic *N,N*-dimethylated indoleamines in the urine of four schizophrenic patients. *Br J Psychiatry* 117:421–430
- Thiriot DS, Ruoho AE (2001) Mutagenesis and derivatization of human vesicle monoamine transporter 2 (VMAT2). Cysteines identifies transporter domains involved in tetrabenazine binding and substrate transport. *J Biol Chem* 276:27304–27315
- Thompson MA, Moon E, Kim UJ, Xu J, Siciliano MJ, Weinshilboum RM (1999) Human indolethylamine *N*-methyltransferase: cDNA cloning and expression, gene cloning, and chromosomal localization. *Genomics* 61:285–297
- Weissman AD, Su TP, Hedreen JC, London ED (1988) Sigma receptors in post-mortem human brains. *J Pharmacol Exp Ther* 247:29–33
- Whipple MR, Reinecke MG, Gage FH (1983) Inhibition of synaptic neurotransmitter uptake by hallucinogens. *J Neurochem* 40:1185–1188
- Wyatt RJ, Saavedra JM, Axelrod J (1973) A dimethyltryptamine-forming enzyme in human blood. *Am J Psychiatry* 130:754–760
- Yanai K, Ido T, Ishiwata K, Hatazawa J, Takahashi T, Iwata R, Matsuzawa T (1986) In vivo kinetics and displacement study of a carbon-11-labeled hallucinogen, *N,N*-[<sup>11</sup>C]dimethyltryptamine. *Eur J Nucl Med* 12:141–146
- Yritia M, Riba J, Ortuno J, Ramirez A, Castillo A, Alfaro Y, de la Torre R, Barbanoj MJ (2002) Determination of *N,N*-dimethyltryptamine and beta-carboline alkaloids in human plasma following oral administration of ayahuasca. *J Chromatogr B Analyt Technol Biomed Life Sci* 779:271–281
- Zhang H, Cuevas J (2002) Sigma receptors inhibit high-voltage-activated calcium channels in rat sympathetic and parasympathetic neurons. *J Neurophysiol* 87:2867–2879