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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 [^{3}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 [^{3}H]paroxetine binding to the SERT and [^{3}H]dihydrotetrabenazine binding to VMAT2. Our results show that DMT, MIPT, DPT, and DIPT inhibit [^{3}H]5-HT transport at the SERT with K_I values of 4.00 ± 0.70, 8.88 ± 4.7, 0.594 ± 0.12, and 2.32 ± 0.46 μM, respectively. At VMAT2, the tryptamines inhibited [^{3}H]5-HT transport with K_I values of 93 ± 6.8, 20 ± 4.3, 19 ± 2.3, and 19 ± 3.1 μM, respectively. On the other hand, the tryptamines were very poor inhibitors of [^{3}H]paroxetine binding to SERT and of [^{3}H]dihydrotetrabenazine 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 · Dihydrotetrabenazine · Dimethyltryptamine · DMT · Dipropyltryptamine · DPT · Diisopropyltryptamine · DIPT · Methylisopropyltryptamine · MIPT · Psychedelic · Paroxetine · Serotonin · Sigma-1 receptor · Tetrabenazine

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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*.

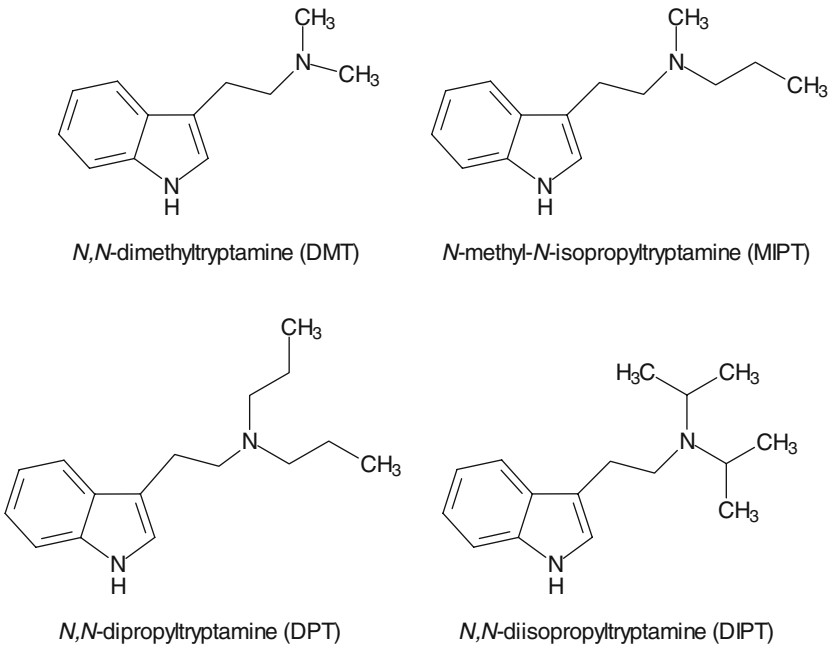

Fig. 1 Chemical structures of *N,N*-dialkyltryptamines

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_I 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_I), 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 (Thiriot 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-methylenedioxyamphetamine, 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_2O, *X* mL 15% NaOH, and 4*X* mL H_2O. 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 µmol of tetrabenazine in 300 µl 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 × 1 mL ethyl acetate and the combined organic extracts were concentrated under a stream of nitrogen to approximately 100 µl. 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 ~0.8) was removed from the plate and the silica was extracted with methanol. The isolated [^{3}H]dihydrotetrabenazine comigrated with authentic nonradioactive dihydrotetrabenazine.

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

SERT-mediated [^{3}H]5-HT uptake and [^{3}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°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), $MgSO_4$ (1.3), KH_2PO_4 (1.2), $CaCl_2$ (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}H]5-HT was measured in the absence and presence of various concentrations of test compounds as follows: a 490 µl aliquot of the platelet suspension was added to glass tubes containing 5 µl test drugs (dissolved in DMSO or water) or 5 µl water (for total and nonspecific determinations). The assay tubes were preincubated in a 37°C water bath for 5 min. The tubes were then returned to the ice bath and chilled for 15 min. [^{3}H]5-HT was added (5 µl of stock solution; final concentration, 10 nM), giving a total incubation volume of 500 µl. All tubes except nonspecific tubes were returned to the 37°C water bath to initiate [^{3}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°C minus uptake at 0°C in the absence of drugs. Under these conditions, [^{3}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}H]paroxetine in the presence or absence of 100 µM concentrations of test compounds for 60 min at 0°C. Nonspecific [^{3}H]paroxetine binding was defined with 10 µM 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}H]paroxetine binding.

VMAT2-mediated [^{3}H]5-HT uptake and [^{3}H] dihydrotetrabenazine 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}H]5-HT in the presence or absence of various concentrations of the test compounds. The suspension was incubated at 32°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 µM reserpine. The values obtained using the test compounds were normalized to specific [^{3}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}H]dihydrotetrabenazine in sucrose–HEPES buffer for 60 min at 32°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}H]dihydrotetrabenazine binding was defined with 10 µM tetrabenazine in the absence of test drugs.

Data analysis

The mean uptake IC_{50} ± 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 [^{3}H]paroxetine (SERT) or [^{3}H]dihydrotetrabenazine (VMAT2) binding inhibition at 100 or 1,000 μM, 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 μM concentrations according to the relationship:

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

where D = drug concentration (i.e., 100 μM [SERT] or 1,000 μM [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 [^{3}H]5-HT uptake into human platelets was typically greater than 90% of total uptake. All of the *N,N*-dialkyltryptamines in this study inhibited [^{3}H]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 [^{3}H]5-HT uptake with a K_I of 0.594 ± 0.12 μM (Table 1).

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

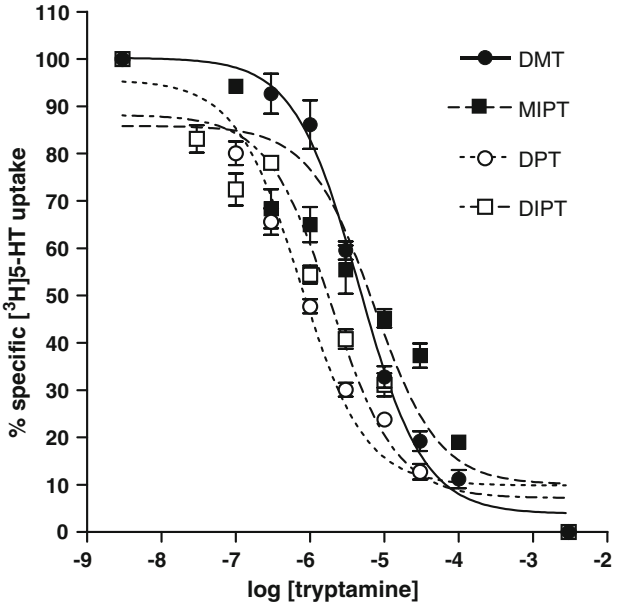

Fig. 2 Drug inhibition of SERT-mediated [^{3}H]5-HT uptake. The ability of *N,N*-dialkyltryptamines to inhibit accumulation of 10 nM [^{3}H]5-HT was examined in human platelets. Specific uptake was defined as uptake at 37°C minus at 0°C. Data are the mean ± SEM of 3–8 determinations, each run in triplicate.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

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

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 (μM) and binding-to-uptake ratios for various compounds at SERT and VMAT2

Compound	SERT			VMAT2		
	[^{3}H]5-HT uptake	[^{3}H]PXT binding	Ratio	[^{3}H]5-HT uptake	[^{3}H]TBZOH binding	Ratio
DMT	4.00 ± 0.70	>47	>11	93 ± 6.8	>1,000	>10
MIPT	8.88 ± 4.7	>97	>10	20 ± 4.3	>1,000	>50
DPT	0.594 ± 0.12	>32	>55	19 ± 2.3	>1,000	>50
DIPT	2.32 ± 0.46	>17	>7	19 ± 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 ± 0.3	>272	>200
Ketanserin	ND	ND	–	0.7 ± 0.1	0.086	0.1

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

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

[a] Data from Talvenheimo et al. (1979)

[b] Data from Marcusson et al. (1988)

[c] Data from Nelson and Rudnick (1979)

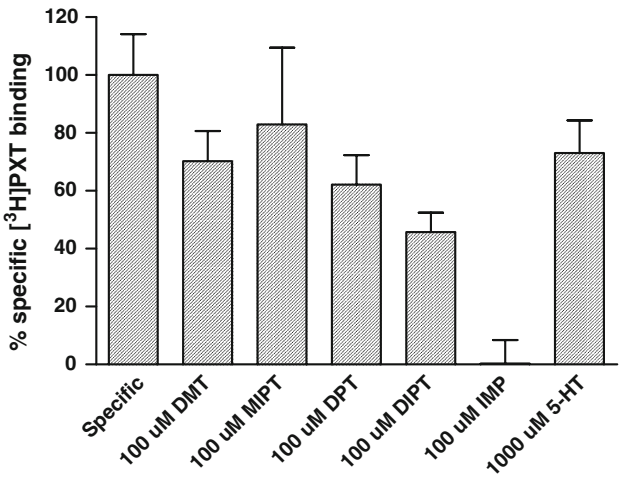

Fig. 3 Comparison of abilities of *N,N*-dialkyltryptamines, imipramine, and serotonin to inhibit [^{3}H]paroxetine binding to human platelets. The ability of test drugs, imipramine (IMP), and 5-HT to compete for 4 nM [^{3}H]paroxetine binding was examined in human platelets. Specific uptake was defined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

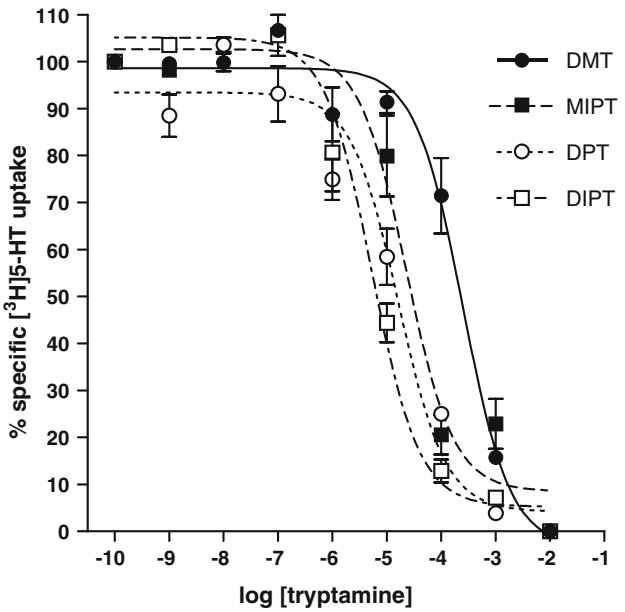

Fig. 4 Drug inhibition of VMAT2-mediated [^{3}H]5-HT uptake. The ability of tryptamines to inhibit accumulation of 30 nM [^{3}H]5-HT was examined in Sf9 cells expressing the rat VMAT2. Specific uptake was defined with 10 μM reserpine. Data are the mean ± SEM of 3 determinations, each run in triplicate.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

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

All of the tryptamines tested inhibited [^{3}H]5-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}H]paroxetine binding to nonspecific levels (Fig. 3), and, at VMAT2, only the known uptake blocker ketanserin prevented [^{3}H]dihydrotetrabenazine binding (Table 1). As expected of substrates, serotonin did not inhibit [^{3}H]paroxetine binding to SERT and norepinephrine did not inhibit [^{3}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 μg/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 μM 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 (α-methyltryptamine and 5-methoxy-α-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.

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