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Psychoactive N,N-dialkyltryptamines modulate serotonin transport by at least two mechanisms

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Abstract

N,N-dimethyltryptamine (DMT) is a potent plant hallucinogen that has also been reported in human brain. In humans, DMT and related *N,N*-dialkyltryptamines produce an intense dream-like state with colorful visual imagery, altered perceptions of time and space, changes in body image and sensations, and intense mood changes ranging from euphoria to sadness. The hallucinogenic effects of these tryptamines are mediated through various neurochemical mechanisms. To further clarify the pharmacology of hallucinogenic tryptamines, we synthesized DMT, *N,N*-dipropyltryptamine (DPT), *N,N*-diisopropyltryptamine (DIPT), and *N*-methyl-*N*-isopropyltryptamine (MIPT); structures were confirmed by mass spectrometry. The drugs were tested for their abilities to inhibit [^{3}H]5-HT uptake via the plasma membrane serotonin transporter (SERT) and via the vesicle monoamine transporter (VMAT2). The tryptamines were also tested as inhibitors of [^{3}H]paroxetine ([^{3}H]PXT) binding to the SERT and [^{3}H]dihydrotetrabenazine ([^{3}H]TBZOH) binding to VMAT2. SERT-mediated [^{3}H]5-HT uptake and [^{3}H]PXT binding were assayed in human platelets, while VMAT2-mediated [^{3}H]5-HT uptake and [^{3}H]TBZOH binding were assayed in Sf9 cells infected with a recombinant baculovirus expressing the rat VMAT2. Our results show that DMT, DPT, DIPT, and MIPT inhibit [^{3}H]5-HT transport at SERT and VMAT2 at micromolar concentrations. The tryptamines inhibited [^{3}H]PXT binding to SERT at high micromolar or millimolar concentrations. At VMAT2, none of the tryptamines appreciably inhibited [^{3}H]TBZOH binding to VMAT2, even at millimolar concentrations. The resulting high binding-to-uptake ratios at the SERT and VMAT2 are consistent with substrate properties for the tryptamines at both of these transporters. Together, these studies reveal two mechanisms whereby hallucinogenic tryptamines modulate serotonin transport.

Introduction and Rationale

- *N,N*-Dimethyltryptamine (DMT) is an endogenous indole alkylamine found in trace amounts within human tissues. DMT also occurs in hundreds of plants around the world including the Illinois Bundleflower (*Desmanthus illinoensis*, common in the United States), chacruna (*Psychotria viridis*, native to Central and South America), and trees of the *Virola* genus, native to South American rainforests. Chacruna is used to make the hallucinogenic teas *ayahuasca* and *yagé* while *Virola* resin is used to make a hallucinogenic snuff known as *epená* in South America. Archeological evidence indicates that South American native cultures have used these plants for shamanistic rituals for at least 3000 years (ML Pochettino, AR Cortella, M Ruiz. *Econ. Bot., 53,* 127-132 [1999]).

- The psychological effects of absorbed DMT are characterized as an intense dream-like state with colorful visual imagery, altered perceptions of time and space, changes in body image and sensations, and intense mood changes ranging from euphoria to sadness (RJ Strassman, CR Qualls, EH Uhlenhuth, R Kellner. *Arch. Gen. Psychiat., 51*, 98-108 [1994]). Numerous structural analogs of DMT have been synthesized in the laboratory and many of these substances are also hallucinogenic. Interestingly, one of these tryptamines (*N,N*-diisopropyltryptamine; DIPT) selectively affects the sense of hearing in human subjects (AT Shulgin, MF Carter. *Commun. Psychopharmac., 4*, 363-369 [1980]).

- The psychoactive effects of tryptamines are mediated through various neurochemical mechanisms including activity at monoamine receptors, modification of monoamine uptake and release, and competition for monoamine oxidase enzymes. To further clarify the pharmacology of hallucinogenic tryptamines with respect to 5-HT transport, we tested DMT, *N,N*-dipropyltryptamine (DPT), DIPT, and *N*-methyl-*N*-isopropyltryptamine (MIPT) (Fig. 1) for their abilities to affect [^{3}H]5-HT uptake via the plasma membrane serotonin transporter, SERT, and via the vesicle monoamine transporter, VMAT2.

- To classify the tryptamines as either 5-HT uptake inhibitors or 5-HT substrate analogs, we also tested the compounds' abilities to compete for [^{3}H]paroxetine (PXT) binding to SERT and [^{3}H]dihydrotetrabenazine (TBZOH) binding to VMAT2. We then calculated the ratios of the K_i for PXT binding to the K_i for [^{3}H]5-HT uptake (SERT) or the K_i for TBZOH binding to the K_i for [^{3}H]5-HT uptake (VMAT2). These ratios were used to classify compounds as either substrates or uptake inhibitors at SERT and VMAT2. Known uptake inhibitors have similar potencies in competitive binding and in uptake inhibition assays, leading to low binding-to-uptake ratios (< 2). Recognized transporter substrates, on the other hand, are more potent at inhibiting uptake than they are as inhibitors in competitive binding assays and they typically display binding-to-uptake ratios greater than 10 (RB Rothman, MA Ayestas, CM Dersch, MH Baumann. *Circulation, 100*, 869-875 (1999).

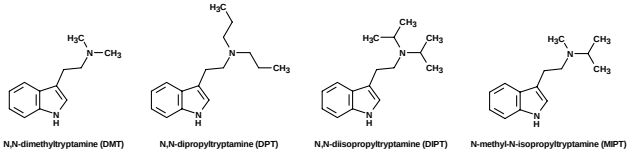

Fig. 1. Chemical structures of *N,N*-dialkyltryptamines used in this study.

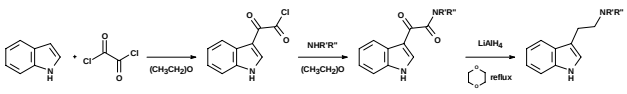

Fig. 2. Synthetic route to *N,N*-dialkyltryptamines. Tryptamines were synthesized by the method of Speeter and Anthony (ME Speeter, WC Anthony. *J. Am. Chem. Soc., 76*, 6208-6210 [1954]) with minor modifications. Indole was condensed with oxalyl chloride in diethyl ether to generate crystalline indol-3-ylglyoxyl chloride. The glyoxyl chloride was reacted with either *N,N*-dimethylamine, *N,N*-dipropylamine, *N,N*-diisopropylamine, or *N*-methyl-*N*-isopropylamine to yield the respective indol-3-glyoxylamides. The glyoxylamides were then reduced to the *N,N*-disubstituted tryptamines with lithium aluminum hydride in refluxing dioxane as described (FV Brutcher, WD Vanderwerff. *J. Org. Chem., 23*, 146-147 [1958]).

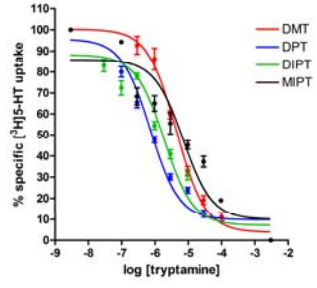

Fig. 3. *N,N*-Dialkyltryptamine inhibition of [^{3}H]5-HT uptake via SERT. Human platelets were suspended in modified Krebs-Ringer-HEPES (KRH) buffer with 10 nM [^{3}H]5-HT in the absence or presence of various concentrations of *N,N*-dialkyltryptamines. After a 10 min uptake incubation, the assays were vacuum filtered and filters were assessed for tritium content. Specific uptake was defined as uptake at 37 °C minus uptake at 0 °C in the absence of drugs. Data were transformed from DPM to percent specific uptake for curve fitting and analysis. Specific uptake was typically greater than 90% of total uptake.

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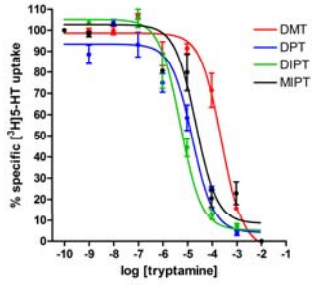

Fig. 4. *N,N*-Dialkyltryptamine inhibition of [^{3}H]5-HT uptake via VMAT2. Digitonin-permeabilized Sf9 cells containing vesicles expressing the rat VMAT2 were incubated in isotonic sucrose-HEPES uptake buffer containing Mg-ATP with 30 nM [^{3}H]5-HT in the absence or presence of various concentrations of *N,N*-dialkyltryptamines. After a 10 min uptake incubation, assays were vacuum filtered and filters were assessed for tritium content. Specific uptake was defined as reserpine-protectable (10μM) uptake. Data were transformed from DPM to percent specific uptake for curve fitting and analysis. Specific uptake was typically greater than 90% of total uptake.

Table 1. K_i values (μM) and binding-to-uptake ratios for various compounds at SERT and VMAT2.[a]

Compound	SERT			VMAT2		
	[^{3}H]5-HT uptake	[^{3}H]PXT binding	Binding-to-uptake ratio	[^{3}H]5-HT uptake	[^{3}H]TBZOH binding	Binding-to-uptake ratio
DMT	4.02 ± 0.70	> 300	> 75	93 ± 6.8	> 1000	> 10
DPT	0.596 ± 0.12	> 14	> 24	19 ± 2.3	> 1000	> 50
DIPT	2.33 ± 0.46	> 45	> 19	19 ± 3.1	> 1000	> 50
MIPT	8.90 ± 4.8	> 27	> 3	20 ± 4.3	> 1000	> 50
5-HT	0.222[b]	> 815	> 3600	ND	ND	--
Norepinephrine	ND	ND	--	1.3 ± 0.3	> 1000	> 600
Ketanserin	ND	ND	--	0.7 ± 0.1	0.086	0.1

[a]The ability of compounds to inhibit 4 nM [^{3}H]PXT binding or 20 nM [^{3}H]TBZOH binding was determined by testing compounds at various concentrations from 10^{-9} to 10^{-3} M. Binding assays for [^{3}H]PXT were performed by incubating platelets in ice-cold KRH buffer ± test compounds for 60 min followed by vacuum filtration and liquid scintillation counting of filters for retained tritium. Binding assays for [^{3}H]TBZOH were conducted by incubating Sf9-VMAT2 vesicles in isotonic sucrose-HEPES buffer ± test compounds for 60 min, followed by vacuum filtration and liquid scintillation counting. K_i values were calculated using the Cheng-Prusoff equation. When no competition for inhibitor binding was detected at drug concentrations of less than 1 mM, the K_i itself obviously could not be lower than 1 mM. When this occurred, binding-to-uptake ratios were estimated using a conservative value of 1 mM for the binding K_i. ND = not determined. [b]PJ Nelson, G Rudnick. *J. Biol. Chem., 254*, 10084-10089 (1979)

Conclusions

- All of the *N,N*-dialkyltryptamines tested inhibit [^{3}H]5-HT accumulation via both SERT and VMAT2. K_i values for SERT are in the nanomolar-to-low micromolar range with DPT being the most potent inhibitor of SERT-mediated [^{3}H]5-HT uptake. In contrast, the tryptamines were far weaker as inhibitors of [^{3}H]PXT binding at the SERT; this is consistent with substrate behavior. The binding-to-uptake ratio for MIPT was greater than 2, however, the ratio did not reach the predefined threshold ratio of 10 for substrates. As expected, 5-HT itself only inhibited [^{3}H]PXT binding at very high concentrations.

- At VMAT2, DPT, DIPT, and MIPT were equipotent at inhibiting [^{3}H]5-HT uptake. DMT, DPT, DIPT, MIPT, and norepinephrine did not show appreciable inhibition of [^{3}H]TBZOH binding to VMAT2 using concentrations up to 1 mM. Because the estimated binding-to-uptake ratios all exceed 10, DMT, DPT, DIPT, and MIPT appear to be substrates for VMAT2. Norepinephrine, a known VMAT2 substrate, had a binding-to-uptake ratio greater than 600 while the known inhibitor ketanserin had a ratio less than 1.

- Together, these studies reveal that hallucinogenic tryptamines modulate serotonin activity through at least two monoamine transporter mechanisms, acting as substrates at both SERT and VMAT2.