Structure-Activity Relationships and Mechanism of Action of Hallucinogenic Agents Based on Drug Discrimination and Radioligand Binding Studies

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The classical hallucinogens can be divided into several structural classes: phenalkylamine (PAA) derivatives and indolealkylamine (IAA) derivatives (Figure 1). Lysergic acid diethylamide (LSD) and related analogs have been classified as indolealkylamines, but might just as readily be classified as phenalkylamines based on their structural resemblance to these agents; as shown in Figure 1, they are accorded their own separate designation, i.e., lysergamides. Certain phenalkylamines [e.g., 1-(2,5-dimethoxy-4-methylphenyl)-2-aminopropane (DOM)] are hallucinogenic whereas others (e.g., amphetamine) are not. The same may be said of indolealkylamine derivatives. One of the early goals of our work was to to determine if a representative member of each of these three classes of hallucinogens was capable of producing a common behavioral (i.e., discriminative stimulus) effect in animals. After we had shown that this was indeed possible (Glennon et al., 1979; Young et al., 1981), our next goal was to determine which members within each of these classes of agents could produce similar behavioral effects. Once accomplished, attention could then be focused on (a) formulation of structure-activity relationships (SAR), (b) investigation of mechanism(s) of action, and (c) determination of a possible relationship between animal data and human hallucinogenic activity/potency.

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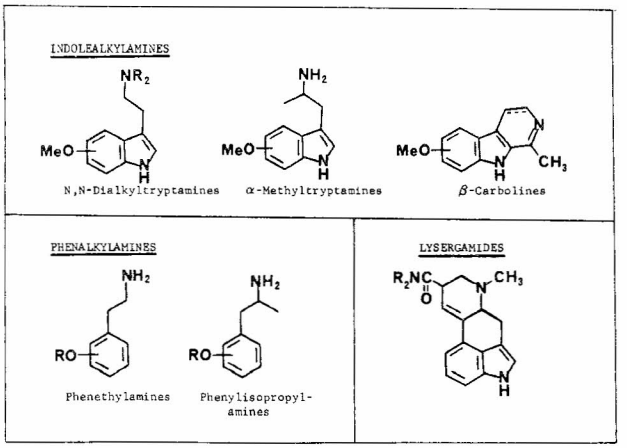
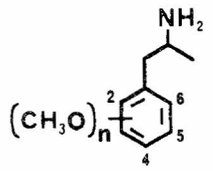


FIGURE 1. Structural classes of hallucinogenic agents.

Behavioral Studies

To accomplish the first goal, groups of rats were trained to discriminate either 1.0 mg/kg of racemic DOM hydrochloride or (+)−amphetamine sulfate from saline using a standard two-lever operant procedure. These animals, once trained, were then administered various doses of PAA and IAA derivatives in tests of stimulus generalization (i.e., transfer, substitution). In this way, it was possible to classify these agents as producing either DOM-like or amphetamine-like discriminative stimulus effects. Certain agents produced neither effect; that is, with some agents stimulus generalization did not occur with either the DOM- or the amphetamine-stimulus. Agents not resulting in stimulus generalization were of two types; some agents [e.g., 3,5-dimethoxyphenyl-2-aminopropane (3,5-DMA)] produced saline-appropriate responding (in both groups of animals) at the highest dose evaluated, whereas others (e.g., 2,6-DMA) resulted in partial generalization (in at least one of the two groups of animals) followed, at a slightly higher dose, by disruption of behavior. The present discussion will deal primarily with those agents that resulted in complete stimulus generalization (i.e., greater than 80 percent DOM-appropriate responding) in the DOM-trained animals. Table 1 presents qualitative results on selected

TABLE 1. Representative Results of Stimulus Generalization Studies Using Racemic DOM or (+)-Amphetamine as Training Drug.



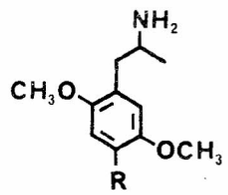
Monomethoxy Analogs:		
OMA = A	MMA = A	PMA = A
Dimethoxy Analogs:		
2,3-DMA = *	2,4-DMA = D	2,5-DMA = D
2,6-DMA = *	3,4-DMA = *	3,5-DMA = *
Trimethoxy Analogs:		
2,3,4-TMA = D	2,3,5-TMA = D	2,3,6-TMA = #
2,4,5-TMA = D	2,4,6-TMA = D	3,4,5-TMA = D
Other Analogs:		
AMPH = A	(+)AMPH = A	(−)AMPH = A
DOM = D	DOET = D	MDA = A/D
(−)DOM = D	DOPR = D	(−)MDA = D
(+)DOM = D	DOBU = D	(+)MDA = A
DOB = D	(−)DOB = D	(+)DOB = D
DOI = D	(−)DOI = D	(+)DOI = D

Racemic mixtures were used unless otherwise noted. OMA, MMA, and PMA are the 2-, 3- and 4-monomethoxy derivatives, respectively. AMPH = amphetamine. D = DOM-stimulus generalization. A = amphetamine-stimulus generalization. Agents not resulting in complete stimulus generalization (in either group of animals) are indicated with (*); a (#) denotes agent not tested. Data from Glennon et al., 1983; Young and Glennon, 1986.

PAA derivatives that were evaluated both in DOM-trained and amphetamine-trained animals. It can be seen that none of the monomethoxy analogs, two of the six dimethoxy analogs (DMAs), and five of the five trimethoxy analogs (TMAs) resulted in DOM-stimulus generalization. In like manner, various other derivatives also resulted in either DOM- or amphetamine-stimulus generalization. With one exception, there were no examples of agents that were recognized by both groups of animals; this one exception is 3,4-methylenedioxyphenylisopropylamine (3,4-MDA, MDA). [The DOM-stimulus generalized to racemic and R(−)-MDA but not to S(+)-MDA, whereas the amphetamine-stimulus generalized to racemic and S(+)-MDA but not to R(−)-MDA. It appears that each of the component optical isomers produces a different stimulus effect.] Quantitative data for a small number of agents that produced DOM-like effects are shown in Table 2. Substitution at the 4-position of 2,5-DMA results in some of the more potent DOM-like analogs. With respect to the alkyl substituted derivatives, potency increases as the length of the alkyl chain increases from methyl to ethyl (DOET) to *n*-propyl (DOPR). DOPR is the most potent of the alkyl series and a further increase in chain length to *n*-butyl (DOBU) results in a decrease in potency. 4-Halogenated analogs, such as the bromo analog (DOB) are also among the most potent agents (Table 2). Where optical isomers were evaluated (e.g., DOM, DOET, DOB), the R(−)-isomers were generally twice as potent as the racemates and 5- to 10-times more potent than their corresponding S(+)-enantiomers. Data such as these have allowed for the formulation of *in vivo* SAR for the phenalkylamines.

Various indolealkylamines were also evaluated in the DOM-trained animals (Glennon et al., 1983). IAA derivatives with a primary amine function (e.g., tryptamine, 5-methoxy tryptamine) did not produce DOM-like effects unless there was an alkyl group alpha to the amine. With respect to these alpha-alkyl tryptamines, alpha-methyl derivatives were more potent than their corresponding alpha-ethyl derivatives. Alpha-methyltryptamines,

TABLE 2. Potencies of Selected Phenalkylamine Derivatives in DOM-Trained Animals.



Agent		R	ED50 (μmoles/kg)
2,5-DMA		H	23.75
2,4,5-TMA		OCH_3	13.70
DOM	(±)	CH_3	1.79
	(−)		0.85
	(+)		6.91
DOET	(±)	C_2H_5	0.88
	(−)		0.36
	(+)		3.27
DOPR		nC_3H_7	0.62
DOBU		nC_4H_9	3.14
DOB	(±)	Br	0.64
	(−)		0.32
	(+)		2.60
DON[a]	(±)	NO_2	2.75
	(−)		2.02

Rats were trained to discriminate 1.0 mg/kg racemic DOM HCl from saline. Racemic mixtures were employed unless otherwise noted.
[a]1,(2,5-dimethoxy-4-nitrophenyl)-2-amino propane.

like the phenylisopropylamines mentioned above, also exist as optical isomers; S(+)-alpha-methyltryptamine was approximately twice as potent as racemic alpha-methyltryptamine whereas the R(−)-isomer did not produce DOM-like effects. Both isomers of 5-methoxy alpha-methyltryptamine produced DOM-like effects; the (+)-isomer was about twice as potent as its racemate and 5-times as potent as its (−)-enantiomer. *N,N*-Dimethyltryptamine (DMT) was half as potent as racemic alpha-methyltryptamine; both the 5- and 4-methoxy derivatives (i.e., 5-OMe DMT and 4-OMe DMT) were more potent than DMT whereas 6-OMe DMT was less potent than DMT. A variety of other tryptamine derivatives were examined. With respect to beta carbolines, several (e.g., harmaline, 6-methoxyharmalan) produced DOM-like effects, but none was more potent than DMT. As with the phenalkylamines, SAR were formulated for the IAA.

Mechanistic Studies

It has already been fairly well documented that serotonin (5-HT) plays an important role in the mechanism of action of hallucinogenic agents. With respect to our studies, several different approaches were taken. Using animals trained to discriminate DOM from saline, tests of stimulus generalization and stimulus antagonism were undertaken using known 5-HT agonists and antagonists. In the second approach, animals were trained to discriminate other 5-HT agonists from saline; DOM and several related analogs were then examined in tests of stimulus generalization. Finally, radioligand binding studies were conducted using rat brain homogenates.

(1) *DOM-Trained Animals.* Fenfluramine is known to release endogenous stores of 5-HT; administration of fenfluramine to DOM-trained animals resulted in stimulus generalization supporting the contention that 5-HT may play a mechanistic role in the stimulus effects produced by DOM. However, there is considerable evidence for the existence of multiple populations of central 5-HT binding sites. Peroutka and Snyder (1979) have demonstrated the existence of 5-HT_1 and 5-HT_2 sites, and Pedigo et al. (1981) have shown that the 5-HT_1 sites are heterogeneous and consist of several different subpopulations of sites. Currently, three sub-populations of 5-HT_1 sites have been identified: 5-HT_{1A}, 5-HT_{1B}, and 5-HT_{1C} (for a review, *see* Glennon, 1986). Although there are no 5-HT_{1C}-selective agonists, there is evidence that 8-hydroxy-*N,N*-(di-*n*-propylamino) tetralin (8-OH DPAT) is selective for 5-HT_{1A} sites whereas agents such as 1-(3-trifluoromethylphenyl)piperazine (TFMPP) and RU-24969 are selective for 5-HT_{1B} sites. Quipazine is a 5-HT agonist that appears to bind both to 5-HT_1 and 5-HT_2 sites. The DOM-stimulus generalized to quipazine, but not to 8-OH DPAT, TFMPP, or RU-24969. These results suggest that the DOM-stimulus might involve a 5-HT mechanism that is probably not 5-HT_{1A} or 5-HT_{1B} mediated.

Silverman and Ho (1980) demonstrated that the DOM-stimulus could be attenuated by pretreatment of the animals with the 5-HT antagonists cinanserin and methysergide; we later found that pizotyline (pizotifen, BC-105) was also an effective antagonist (Young et al., 1981). Shortly thereafter, Leysen et al. (1981) reported that agents such as cinanserin, methysergide, and pizotyline were 5-HT_2 antagonists; they also reported the development of two new agents, ketanserin and pirenperone, that were highly potent and selective 5-HT_2 antagonists. Subsequently, we demonstrated that ketanserin and pirenperone were very effective in blocking the stimulus effects of DOM, and DOM-stimulus generalization to mescaline, 5-OMe DMT, LSD, and quipazine. A 5-HT_2 mechanism had now been implicated, for the first time, as possibly being involved in the stimulus properties of DOM and agents to which the DOM-stimulus generalized.

One of the problems associated with ketanserin and pirenperone (and a number of other 5-HT_2 antagonists) is that although they are selective for 5-HT_2 vs 5-HT_1 sites, they are not necessarily selective for 5-HT; some of these agents display a high affinity for dopaminergic, histaminergic, and/or adrenergic binding sites. In order to determine if these other neurotransmitters might be playing a mechanistic role, antagonism studies were conducted with a variety of antagonists; none of these agents effectively antagonized or attenuated the DOM response. By this

time, several new 5-HT antagonists had become available; although these agents (e.g., tetrahydrotrazodone, LY-53857, dihydroflutroline, ritanserin) are not necessarily specific for 5-HT sites, they possess different binding profiles and have in common the ability to bind at 5-HT_2 sites. Each of these agents was an antagonist of the DOM-stimulus (Glennon & Hauck, 1985). Thus, evidence was mounting for 5-HT_2 involvement.

(2) *Site-Selective Serotonin Agonists.* In the next phase of our study, groups of animals were trained to discriminate the 5-HT_{1A} agonist 8-OH DPAT and the 5-HT_{1B} agonist TFMPP from saline. If DOM and related agents produce their stimulus effects via a 5-HT_2 agonist effect, stimulus generalization should not occur when these agents are administered to the 8-OH DPAT-trained or TFMPP-trained animals. Also, it should not be possible to block the stimulus effects of these two training drugs with 5-HT_2 antagonists. In rather limited tests of stimulus antagonism, it was found that ketanserin, pirenperone, pizotyline, and/or tetrahydrotrazodone were unable to antagonize the stimulus effects of 8-OH DPAT or TFMPP. In tests of stimulus generalization (Table 3), the 8-OH DPAT stimulus generalized to 8-methoxy DPAT, but not to the 5-HT_{1B} agonists TFMPP, 1-(3-chlorophenyl) piperazine (mCPP), or RU-24969, or to the (putative) 5-HT_2 agonists DOM or 1(2,5-dimethoxy-4-iodophenyl)-2-aminopropane (DOI). The TFMPP-stimulus generalized to the 5-HT_{1B} agonists mCPP and RU-24969 but not to the 5-HT_{1A} agonist 8-OH DPAT, or to DOM, DOB, or DOI. The DOM-stimulus generalized to LSD and quipazine; however, neither agent resulted in generalization in the 8-OH DPAT- or TFMPP-trained animals. Interestingly, both of these agents did result in partial generalization in each group of animals, supporting the finding that LSD and quipazine bind both at 5-HT_1 and 5-HT_2 sites.

TABLE 3. Results of Stimulus Generalization Studies Using Site-Selective Serotonin Agonists as Training Drugs.

	Training Drug		
Generalization to	8-OH DPAT (5-HT_{1A})	TFMPP (5-HT_{1B})	DOM (5-HT_2)
Fenfluramine	—	Yes	Yes
5-HT_{1A} Agonists			
8-OH DPAT	Yes	No	No
8-OMe DPAT	Yes	—	No
5-HT_{1B} Agonists			
TFMPP	No	Yes	No
RU-24969	No	Yes	No
mCPP	—	Yes	No
5-HT_2 Agonists			
DOM	No	No	Yes
DOB	—	No	Yes
DOI	No	No	Yes
Other Agents			
LSD	No (P)	No (P)	Yes
Quipazine	—	No (P)	Yes

Data from Glennon and McKenney, 1985.

TABLE 4. Representative 5-HT Binding Data for Phenalkylamine Analogs.

	K_i (nM)	
Agent	5-HT_1	5-HT_2
Amphetamine	7,660	43,000
OMA	3,500	8,130
MMA	2,660	7,850
PMA	79,400	33,600
3,4-DMA	64,600	43,300
2,5-DMA	1,020	5,200
3,4,5-TMA	12,800	16,500
2,4,5-TMA	46,800	1,650
DOF[a]	3,470	1,100
N-Me DOM	4,300	415
DON	14,100	300
DOM	2,890	100
DOM(−)	3,550	60
DOET	4,570	100
DOPR	3,170	69
DOBU	2,660	210
DOB	3,340	63
DOB(−)	4,200	60
DOB(+)	5,000	120
DOI	2,240	19
DOI(−)	2,290	10
DOI(+)	1,670	35

Binding data are for racemates unless otherwise stated. Data from Shannon et al., 1984; Glennon et al., 1984; Glennon et al., 1986.
[a]1(2,5-dimethoxy-4-fluorophenyl)-2-aminopropane.

(3) *Radioligand Binding Studies.* If DOM and related agents act as 5-HT_2 agonists, it should be possible to demonstrate binding of these agents at 5-HT_2 sites. Using rat cortical membranes, 5-HT_1 and 5-HT_2 binding data were obtained for a series of PAAs (Table 4) and a representative number of IAAs. Only data for the PAA analogs will be discussed at this time. In general, PAAs display micromolar affinities (i.e., K_i values) for 5-HT_1 sites; binding at 5-HT_2 sites seems to be very sensi-

tive to the presence and position of various substituents, and a number of agents possess nanomolar affinities. The 2,5-dimethoxy pattern appears to be an important feature for binding at 5-HT_2 sites; further substitution at the 4-position of 2,5-DMA can result in a further increase in affinity and selectivity for 5-HT_2 sites. With respect to 4-alkyl substituents, the *n*-propyl analog DOPR is more potent than either the methyl, ethyl, or butyl analogs. The 4-halogenated derivatives such as DOB and DOI are among the most potent agents in terms of potency/selectivity for 5-HT_2 sites. In the few instances where optical isomers were examined, the R(−)-isomers are several-fold more potent than their S(+)-enantiomers at 5-HT_2 sites, whereas stereoselective binding is less evident at 5-HT_1 sites.

Conclusions

It is difficult to separate mechanistic studies involving large numbers of compounds from studies of SAR in that both provide similar types of information. We have previously demonstrated that a significant correlation ($r = \geq 0.9$) exists between the human hallucinogenic potencies of a series of PAA and IAA derivatives and their ED_{50} values as determined in stimulus generalization studies using DOM as the training drug. We have also found significant correlations ($r = \geq 0.9$) between the affinities of a series of these agents for 5-HT_2 sites and both these ED_{50} values and hallucinogenic potencies (Glennon et al., 1984). Structure-activity relationships derived from all three types of studies afford similar results. The results of these studies also suggest that certain of these hallucinogenic agents may be producing their stimulus effects via a 5-HT_2-mediated mechanism, and, further, that they may be acting as 5-HT_2 agonists. It might be noted that certain agents to which the DOM-stimulus generalizes (e.g., fenfluramine, quipazine, lisuride) and/or that possess an affinity for 5-HT_2 sites (e.g., quipazine, lisuride) are not necessarily hallucinogenic in humans. A satisfactory explanation cannot yet be provided although several can be offered: these agents may not be sufficiently selective for 5-HT_2 sites, may be partial agonists, may possess a still higher affinity for some other binding sites, may produce other effects that mask their anticipated behavioral effects, and/or may be differently distributed or metabolized in humans vs rats.

A dividend of our work with hallucinogenic agents was the identification of the first examples of a series of 5-HT_2-selective agonists. This prompted us to conduct several additional studies. A detailed SAR study on DOB has recently been completed. Substituent groups were removed or transposed in a systematic manner and each of the resulting agents was evaluated with respect to its stimulus properties and binding characteristics. Most structural alterations served only to reduce potency in the discrimination studies, or reduce affinity/selectivity for 5-HT_2 sites (Glennon et al., 1986). Subsequently, [^{3}H]DOB was synthesized and evaluated as an agonist radioligand for 5-HT_2 sites (Titeler et al., 1985). Also, animals have now been trained to discriminate the more 5-HT_2-selective agent DOI from saline; with a training dose of 0.5 mg/kg, an ED50 value of 0.16 mg/kg (0.44 micromoles/kg) was obtained (Glennon and McKenney, 1985). The DOI-stimulus did not generalize with 8-OH DPAT or TFMPP, although generalization did occur with DOM (ED_{50} = 0.45 mg/kg; 1.83 micromoles/kg). Stimulus generalization also occurred with the optical isomers of DOI, with R(−)-DOI being about three times more potent than S(+)-DOI. The DOI-stimulus was blocked by pretreatment of the animals with the 5-HT_2 antagonist ketanserin (ID50 = 0.03 mg/kg).

The overall results of our work lead us to suggest that PAA and IAA hallucinogens may act primarily via a 5-HT_2 agonist mechanism.

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References

Glennon, R.A. Serotonin receptor agonists and antagonists. In: Williams, M., Glennon, R. A., and Timmermans, P. (eds.), *Receptor Function and Pharmacology*. New York: Marcel Dekker, 1986.

Glennon, R.A., and Hauck, A.E. Mechanistic Studies on DOM. *Pharmacol. Biochem. Behav.*, 23:937–941, 1985.

Glennon, R.A., and McKenney, J.D. Site-selective 5-HT agonists as discriminative stimuli. *Pharmacologist*, 27:194, 1985.

Glennon, R.A., Rosecrans, J.A., and Young, R. Drug-induced discrimination: A description of the paradigm and a review of its application in the study of hallucinogenic agents. *Med. Res. Rev.*, 3:289–340, 1983.

Glennon, R.A., Titeler, M., and McKenney, J.D. Evidence for 5-HT_2 involvement in the mechanism of action of hallucinogenic agents. *Life Sci.*, 35:2505–2511, 1984.

Glennon, R.A., McKenney, J.D., Titeler, M., and Lyon, R. 5-HT_1 and 5-HT_2 binding characteristics of DOB analogues. *J. Med. Chem.*, 29:194–199, 1986.

Glennon, R.A., Rosecrans, J.A., Young, R., and Gaines, J. Hallucinogens as discriminative stimuli. *Life Sci.*, 24:993–998, 1979.

Leysen, J.E., Niemegeers, C.J.E., Van Nueten, J.M., and Laduron, P.M. [^{3}H]Ketanserin (R41468), A selective ^{3}H-ligand for serotonin$_2$ receptor binding sites. *Mol. Pharmacol.*, 21:301–314, 1982.

Pedigo, N.W., Yamamura, H.I., and Nelson, D.L. Discrimination of multiple [^{3}H]5-hydroxytryptamine binding sites by the neuroleptic spiperone in rat brain. *J. Neurochem.*, 36:220–226, 1981.

Peroutka, S.J., and Snyder, S.H. Multiple serotonin receptors: Differential binding of [^{3}H]5-hydroxytryptamine, [^{3}H]lysergic acid diethylamide and [^{3}H]spiroperidol. *Mol. Pharmacol.*, 16:687–699, 1979.

Shannon, M., Battaglia, G., Glennon, R.A., Titeler, M. 5-HT_1 and 5-HT_2 binding properties of derivatives of the hallucinogen 1-(2,5-dimethoxyphenyl)-2-aminopropane (2,5-DMA). *Eur. J. Pharmacol.*, 102:23–29, 1984.

Silverman, P.B., and Ho, B.T. The discriminative stimulus properties of 2,5-dimethoxy-4-methylamphetamine (DOM): Differentiation from amphetamine. *Psychopharmacology*, 69:209–215, 1980.

Titeler, M., Herrick, K., Lyon, R.A., et al. [^{3}H]DOB: A specific radioligand for 5-HT_2 sites. *Eur. J. Pharmacol.*, 117:145–147, 1985.

Young, R., and Glennon, R.A. Discriminative stimulus properties of amphetamine and structurally related phenalkylamines. *Med. Res. Rev.*, 1986 (In press).

Young, R., Glennon, R.A., and Rosecrans, J.A. The hallucinogen DOM as a discriminative stimulus. *Commun. Psychopharm.*, 4:501–504, 1981.