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Effect of 1-(3,4-methylenedioxyphenyl)-2-aminopropane and its optical isomers in PMMA-trained rats

Richard A. Glennon*, Richard Young

Department of Medicinal Chemistry, School of Pharmacy, Box 980540, Virginia Commonwealth University, Richmond, VA 23298-0540, USA

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Abstract

1-(3,4-Methylenedioxyphenyl)-2-aminopropane (MDA) is a drug of abuse that is known to produce stimulus effects similar to those of the stimulant phenylalkylamine (+)amphetamine and the hallucinogenic phenylalkylamine 1-(2,5-dimethoxy-4-methylphenyl)-2-aminopropane (DOM). Earlier, a working model was described to account for the stimulus effects produced by phenylalkylamines. Such agents can produce one or more of three distinct effects: an amphetamine effect, a DOM effect and a third effect that is typified by the agent *N*-methyl-1-(4-methoxyphenyl)-2-aminopropane (PMMA). Because MDA is known to produce two of the three effects, in the present investigation, we sought to determine if racemic MDA or either of its optical isomers could produce a PMMA-like effect in animals. Administration of *S*(+)MDA, *R*(−)MDA and (±)MDA to rats trained to discriminate 1.25 mg/kg of PMMA from saline vehicle under a VI 15-s schedule of reinforcement resulted in substitution in each case. (±)MDA and *S*(+)MDA were nearly equipotent and several fold more potent than *R*(−)MDA. The results are not only consistent with the proposed model but also identify (±)MDA as the first phenylalkylamine shown to produce all three types of stimulus effects (i.e., amphetamine-like, DOM-like and PMMA-like) in rats.

Keywords: Stimulants; Hallucinogens; Designer drugs

1. Introduction

1-(3,4-Methylenedioxyphenyl)-2-aminopropane (MDA) is a rather unique phenylalkylamine drug of abuse. It was popular during the late 1960s and early 1970s (*Love Drug*) and appears now to be making a comeback (e.g., see Christophersen, 2000; Furnari et al., 1998; Hegadoren et al., 1999; Kavanagh et al., 2001; Lora-Tamayo et al., 1997; Maurer et al., 2000; Ropero-Miller and Goldberger, 1998). MDA is both an amphetamine-like central stimulant (e.g., Braun et al., 1980) and a hallucinogenic agent (reviewed in Marquardt et al., 1978; Shulgin and Shulgin, 1991). Its effects in humans have been characterized as being similar to those of a combination of cocaine and LSD (Stafford, 1977). MDA exists as a pair of optical isomers and *S*(+)MDA seems primarily responsible for the stimulant actions of MDA in rodents. For example, it has been found that the gross behavioral effects of *S*(+)MDA are indistinguishable from those produced by (+)amphetamine (Marquardt et al., 1978). In contrast, the "hallucinogenic" effects of MDA appear to be associated with the *R*(−)isomer (Marquardt et al., 1978). Hence, one isomer seems responsible for one action and the opposite enantiomer seems responsible for a different action.

With respect to its discriminative stimulus effects, stimulus generalization occurs upon administration of racemic MDA to animals trained to discriminate the stimulant (+)amphetamine from vehicle and the hallucinogenic agent 1-(2,5-dimethoxy-4-methylphenyl)-2-aminopropane (DOM) from vehicle (reviewed in Glennon, 1989). Furthermore, it has been shown that the (+)amphetamine-like stimulus effects are associated with the *S*(+)enantiomer, whereas the DOM-like stimulus effects are associated with the *R*(−)isomer (Glennon, 1989). For example, using rats trained to discriminate either (+)amphetamine or cocaine from vehicle, stimulus generalization occurred with *S*(+)MDA but not with *R*(−)MDA (Glennon, 1989; Young and Glennon, 1997), whereas DOM stimulus generalization occurred to *R*(−)MDA but not to *S*(+)MDA (Glennon,

* Corresponding author. Tel.: +1-804-828-8487; fax: +1-804-828-7404.
E-mail address: glennon@hsc.vcu.edu (R.A. Glennon).

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1989). In addition, animals have been trained to discriminate racemic MDA from vehicle, and (±)MDA stimulus generalization was demonstrated to occur both to stimulants (e.g., amphetamine and cocaine) and hallucinogens (e.g., DOM and LSD; Glennon, 1989). Finally, using a three-lever drug discrimination paradigm with rats trained to discriminate *S*(+)MDA from *R*(−)MDA from saline vehicle, animals responded on the *S*(+)MDA-appropriate lever when administered stimulants and on the *R*(−)MDA-appropriate lever when administered hallucinogenic agents (Young and Glennon, 1996).

On the basis of their pharmacological actions, it was once suggested that phenylalkylamines exist on a behavioral continuum with stimulant phenylalkylamines such as (+)amphetamine existing at one end of the continuum, hallucinogenic phenylalkylamines such as DOM existing at the other extreme and MDA existing somewhere near the center (Glennon, 1989; Glennon et al., 1980). However, it was soon shown that MDA could produce yet another effect. *N*-Monomethylation of MDA results in *N*-methyl-MDA (MDMA; *Ecstasy* or *e*). MDMA possesses both central stimulant and empathogenic properties (i.e., increased sociability, enhanced sensory awareness and feelings of well being). In drug discrimination studies using MDMA-trained rats, MDA and both its optical isomers substituted for MDMA (Glennon, 1989; Nichols and Oberlender, 1989). Thus, the continuum model was revised to account for this (Glennon, 1989). More recently, this model has undergone further revision (Glennon et al., 1997) to account for the different and potentially overlapping stimulus actions of phenylalkylamines (Fig. 1). According to this new model, phenylalkylamines with abuse potential can produce one or more of at least three distinct effects: a (+)amphetamine-like effect, a DOM-like effect and a *N*-methyl-1-(4-methoxyphenyl)-2-aminopropane (PMMA)-like effect. Stimulus generalization occurs between MDMA and PMMA, independent of which is used as the training drug (Glennon et al., 1997; Rangisetty et al., 2001). However, because MDMA also produces (+)amphetamine stimulus effects, MDMA is best characterized as falling in the *A/P* intersect (see Fig. 1). *S*(+)- and *R*(−)-*N*-methyl-1(3,4-methylenedioxyphenyl)-2-aminobutane (MBDB; Rangisetty et al., 2001), *S*(+)- and *R*(−)-1-(3,4-dimethoxyphenyl)-2-aminopropane (3,4-DMA; Rangisetty et al., 2001) and *R*(−)-1-(4-methoxyphenyl)-2-aminopropane (PMA; Dukat et al., 2001) are examples of agents to which a PMMA stimulus, but not a (+)amphetamine stimulus or a DOM stimulus, generalizes.

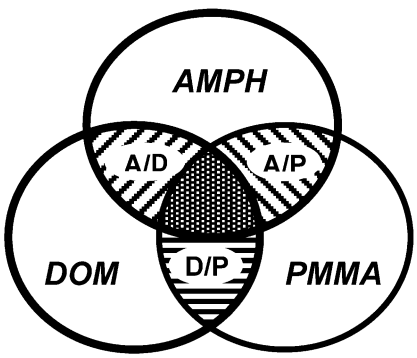

Fig. 1. Venn diagram showing the relationship between the stimulus effects produced by (+)amphetamine (AMPH; *A*), DOM (*D*) and PMMA (*P*). *A/P*, *A/D* and *D/P* reflect intersects. The common intersect is shaded. Adapted from Glennon et al. (1997).

MDA is an ideal agent with which to further challenge the model shown in Fig. 1. That is, it is already known that *S*(+)MDA produces (+)amphetamine-like and MDMA-like stimulus effects but not DOM-like stimulus effects, whereas *R*(−)MDA produces DOM-like and MDMA-like stimulus effects but not (+)amphetamine-like stimulus effects. On this basis, it might be expected that *S*(+)MDA will substitute for PMMA and result in classification as an agent that falls into the *A/P* intersect, whereas *R*(−)MDA should substitute for PMMA and hence be classified as an agent that falls into the *D/P* intersect. Because racemic MDA produces both (+)amphetamine-like and DOM-like stimulus effects, the possibility exists that (±)MDA might also substitute for PMMA. If generalization was to occur, (±)MDA would best be classified then as an agent falling into the common intersect (Fig. 1, shaded center area). These predictions were tested in the present study. Specifically, *S*(+)MDA, *R*(−)MDA and racemic or (±)MDA were examined in tests of stimulus generalization using animals trained to discriminate PMMA from saline vehicle.

2. Methods

2.1. Drug discrimination studies

The subjects were eight male Sprague–Dawley rats (Charles River Laboratories) weighing 250–300 g at the beginning of the study. The animals were trained to discriminate 1.25 mg/kg of PMMA from saline vehicle as previously described (Glennon et al., 1997). In brief, the animals were housed individually, and prior to the start of the study, their body weights were reduced to approximately 80% of their free-feeding weight. During the entire course of the study, the animals' body weights were maintained at this reduced level by restriction of food intake. The animals were allowed drinking water ad lib in their home cages. The animals were trained (15-min training session) to discriminate intraperitoneal injections (15-min presession injection interval) of PMMA from saline vehicle (sterile 0.9% saline) under a variable interval 15-s schedule of reward (i.e., sweetened milk) using standard two-lever Coulbourn Instruments operant equipment as previously described (Glennon et al., 1997). Daily training sessions were conducted with PMMA or saline. On every fifth day, learning was assessed during an initial 2.5-min nonrein-

forced (extinction) session followed by a 12.5-min training session. The left lever was designated the drug-appropriate lever for half the animals, whereas the situation was reversed for the remaining animals. Data collected during the extinction session included response rate (i.e., responses per minute) and number of responses on the drug-appropriate lever (expressed as a percent of total responses). Animals were not used in the subsequent stimulus generalization studies until they made $\geq 80\%$ of their responses on the drug-appropriate lever after administration of PMMA and $\leq 20\%$ of their responses on the same drug-appropriate lever after administration of saline.

Tests of stimulus generalization (i.e., substitution) were conducted in order to determine if the PMMA stimulus would generalize to *S*(+)MDA, *R*(−)MDA and (±)MDA. During this phase of the study, maintenance of the PMMA–saline discrimination was insured by continuation of the training sessions on a daily basis (except on a generalization test day; see below). On 1 of the 2 days before a generalization test, half of the animals would receive the training dose of PMMA and the remainder would receive saline. After a 2.5-min extinction session, training was continued for 12.5 min. Animals not meeting the original criteria (i.e., >80% of total responses on the drug-appropriate lever after administration of PMMA and <20% of total responses on the same lever after administration of saline) during the extinction session were excluded from the next generalization test session. During the investigations of stimulus generalization, test sessions were interposed among the training sessions. The animals were allowed 2.5 min to respond under nonreinforcement conditions. The animals were then removed from the operant chambers and returned to their home cages. An odd number of training sessions (usually 5) separated any two generalization test sessions. Doses of test drugs were administered in a random order using a 15-min presession injection interval to the rats with the proviso that if a particular dose of drug resulted in behavioral disruption, only lower doses would be investigated in subsequent sessions. Stimulus generalization was considered to have occurred when the animals, after a given dose of drug, made $\geq 80\%$ of their responses (group mean) on the training drug-appropriate lever. Animals making fewer than five total responses during the 2.5-min extinction session were considered as being disrupted. Where stimulus generalization occurred, ED_{50} values were calculated by the method of Finney (1952). The ED_{50} doses are doses at which the animals would be expected to make 50% of their responses on the drug-appropriate lever.

2.2. Drugs

Racemic MDA, *S*(+)MDA and *R*(−)MDA, as their hydrochloride salts, were obtained as gifts from NIDA. PMMA hydrochloride was synthesized as previously described (Glennon et al., 1997). Doses refer to the weight of the salt. All solutions were prepared fresh daily and intraperitoneal injections were made 15 min prior to testing.

3. Results and discussion

S(+)MDA, *R*(−)MDA and racemic MDA were administered to rats trained to discriminate 1.25 mg/kg of PMMA from saline vehicle. In each case, the PMMA stimulus generalized to the challenge agent in a dose-related manner (Fig. 2). Calculated ED_{50} values (followed by 95% CL) are as follows: *S*(+)MDA 0.7 mg/kg (0.2–2.1 mg/kg), *R*(−)MDA 1.5 mg/kg (0.4–4.8 mg/kg) and (±)MDA 0.5 mg/kg (0.2–0.4 mg/kg). At the doses of *S*(+)MDA and (±)MDA that produced $\geq 80\%$ PMMA-appropriate responding, the animals' response rates (Table 1) were reduced to nearly 50% of the response rate following administration of PMMA.

On the basis of previous results (see Introduction) and those presented here (Fig. 2), *S*(+)MDA has been shown to substitute for (+)amphetamine and PMMA but not for DOM and *R*(−)MDA has been shown to substitute for DOM and PMMA but not for (+)amphetamine. As expected, then, *S*(+)MDA is best categorized as an *A/P*-type agent, whereas *R*(−)MDA is best categorized as a *D/P*-type agent (see Fig. 1) with regard to the stimulus effects that they produce in rats under the assay conditions employed. The isomers of MDA are not alone in this classification scheme. For example, the scheme can be extended to indolealkylamines such as α-ethyltryptamine (α-ET). (−)α-ET has been classified as an *A/P*-type agent, whereas (+)α-ET has been classified as a *D/P*-type agent (Hong et al., 2001).

Administration of (±)MDA to animals trained to discriminate either (+)amphetamine, DOM (Glennon and Young, 1984) or PMMA (Fig. 2) from vehicle also results in stimulus generalization. It is possible that when an agent produces more than one type of stimulus effect, disruption of behavior might occur before stimulus generalization can occur. In other words, an agent might result in partial generalization in a given test of stimulus generalization and administration of slightly higher doses might result in disruption of the animals' behavior. Partial generalization is difficult to interpret but suggests that the agent might be capable of producing a stimulus effect other than or in addition to the one that is being studied (Glennon et al., 1983). Likewise, at a dose of drug that substitutes for another drug stimulus, the animals' response rates might be depressed and/or fewer than all animals might respond. These types of results are not uncommon with phenylalkylamines. Racemic MDA is a case in point. It now has been demonstrated that MDA can produce at least three distinct types of stimulus effects. As such, it is not surprising that at the dose of (±)MDA that produced $\geq 80\%$ PMMA-appropriate responding, the animals' response rates were depressed and only three of five animals responded. Indeed, what *is* surprising is that stimulus generalization occurred at

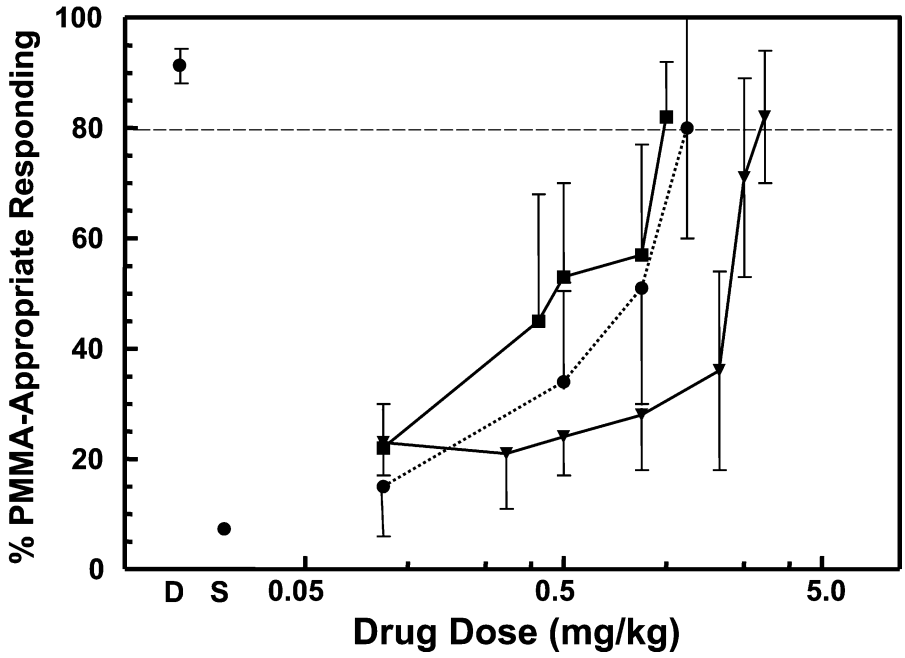

Fig. 2. Results (mean ± S.E.M. drug-appropriate responding) of stimulus generalization studies using rats trained to discriminate 1.25 mg/kg of PMMA from saline vehicle. $D = 1.25$ mg/kg of PMMA and S = saline. (±)MDA is represented by squares, S(+)MDA by circles, and R(−)MDA by triangles. Response rate data can be found in Table 1. Note: Four of the five animals treated with 1.5 mg/kg of S(+)MDA made 100% of their responses on the PMMA-appropriate lever.

all. This might be explained, however, by the fact that MDA seems to produce each of its stimulus effects at roughly comparable doses (Glennon and Young, 1984). Had one of the three effects been manifested at substantially lower doses, it is likely that this effect would have obscured one or more of the other effects by either disrupting the animals' behavior or resulting in partial generalization in other stimulus generalization studies.

Generally, drug stimuli are thought to be quite selective. However, they can be no more selective than the drug that is producing the training stimulus (Glennon and Young, 1984). Had, for example, racemic MDA been examined only in (+)amphetamine-trained animals, the conclusion reached would have been that MDA is an amphetamine-like agent. Although this conclusion would be correct, it is not an accurate description of the MDA stimulus because it is incomplete. A similar argument can be made had racemic MDA been administered only to DOM- or PMMA-trained animals. The model shown in Fig. 1 suggests that arylalkylamines (i.e., phenylalkylamines and indolealkylamines) can produce one or more of several different stimulus effects in animals. It further suggests that the most accurate assessment of a novel arylalkylamine will only follow studies that use several different training drugs (as shown, for example, in Fig. 1).

In summary, the PMMA stimulus was shown to generalize to both optical isomers of MDA and to racemic MDA. As such, racemic MDA is the first phenylalkylamine demonstrated to produce three distinct types of stimulus effects in animals. It is also unique in being the first agent identified that corresponds to the common intersect of a model proposed to define the different stimulus effects that might be produced by phenylalkylamines with abuse potential.

Table 1
Drug doses and number of animals used in and response rate from the present study

Agent	Dose (mg/kg)	n[a]	Responses per minute ± S.E.M.[b]
S(+)MDA	0.1	5/5	13.6 (3.8)
	0.5	5/6	10.2 (3.4)
	1.0	5/5	6.3 (2.1)
	1.5	5/5	5.5 (1.3)
R(−)MDA	0.1	7/8	14.2 (3.1)
	0.3	5/8	10.9 (4.3)
	0.5	5/8	14.7 (3.7)
	1.0	6/8	10.7 (4.3)
	2.0	5/5	11.5 (3.7)
	2.5	4/5	7.5 (3.7)
	3.0	4/5	8.5 (4.5)
(±)MDA	0.1	5/5	11.4 (1.6)
	0.4	4/4	9.0 (1.4)
	0.5	5/5	9.0 (2.3)
	1.0	4/5	10.1 (2.3)
	1.25	3/5	5.4 (2.6)
PMMA	1.25	8/8	10.7 (1.2)
Saline (1 ml/kg)		8/8	13.6 (2.1)

[a] Number of animals responding/number administered drug.
[b] Data obtained during a 2.5-min extinction session reflect results only from those animals that made ≥ 5 responses.

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References

Braun U, Shulgin AT, Braun G. Centrally active *N*-substituted analogs of 3,4-methylenedioxyphenylisopropylamine (3,4-methylenedioxyamphetamine). J Pharm Sci 1980;69:192–5.

Christophersen A. Amphetamine designer drugs — an overview and epidemiology. Toxicol Lett 2000;112–3:127–31.

Finney D. Probit analysis. London: Cambridge Univ. Press, 1952.

Furnari C, Ottaviano V, Rosati F, Tondi V. Identification of 3,4-methylenedioxyamphetamine analogs encountered in clandestine tablets. Forensic Sci Int 1998;92:49–58.

Dukat M, Young R, Glennon RA. Discriminative stimulus properties of 4-MTA and the optical isomers of PMA. MDMA/ecstasy research conference abstracts, July 19–20. Bethesda, MD: NIH, 2001. p. 47.

Glennon RA. Stimulus properties of hallucinogenic phenylalkylamines and related designer drugs: formulation of structure–activity relationships. NIDA Res Monogr 1989;94:43–67.

Glennon RA, Young R. MDA: a psychoactive agent with dual stimulus effects. Life Sci 1984;34:379–83.

Glennon RA, Liebowitz SM, Anderson GM. Serotonin receptor affinities of psychoactive phenalkylamine analogs. J Med Chem 1980;23:294–9.

Glennon RA, Rosecrans JA, Young R. Drug-induced discrimination: a description of the paradigm and a review of its specific application to the study of hallucinogenic agents. Med Res Rev 1983;3:289–340.

Glennon RA, Young R, Dukat M, Cheng Y. Initial characterization of PMMA as a discriminative stimulus. Pharmacol, Biochem Behav 1997; 57:151–8.

Hegadoren KM, Baker GB, Bourin M. 3,4-Methylenedioxy analogues of amphetamine: defining the risks to humans. Neurosci Biobehav Rev 1999;23:539–53.

Hong S-S, Young R, Glennon RA. Discriminative stimulus properties of α-ethyltryptamine (α-ET) optical isomers. Pharmacol, Biochem Behav, 2001;70:311–6.

Kavanagh P, Dunne J, Feely J, Maguire R, Corrigan D, Keating JJ, Meegan JM, Clancy JM, Burdett J. Phenylalkylamine abuse among opiate addicts attending a methadone treatment programme in the Republic of Ireland. Addict Biol 2001;6:177–81.

Lora-Tamayo C, Tena T, Rodriguez A. Amphetamine derivative related deaths. Forensic Sci Int 1997;85:149–57.

Marquardt GM, DiStefano V, Ling LL. Pharmacological effects of (±)-, (*S*)-, and (*R*)-MDA. In: Stillman RC, Willette RE, editors. The psychopharmacology of hallucinogens. New York: Pergamon, 1978. pp. 84–104.

Maurer HH, Bickeboeller-Friedrich J, Kraemer T, Peters FT. Toxicokinetics and analytical toxicology of amphetamine-derived designer drugs (“Ecstasy”). Toxicol Lett 2000;112–3:133–42.

Nichols DE, Oberlender R. Structure–activity relationships of MDMA-like substances. NIDA Res Monogr 1989;94:1–29.

Rangisetty JB, Bondarev ML, Chang-Fong J, Young R, Glennon RA. PMMA-stimulus generalization to the optical isomers of MBDB and 3,4-DMA. Pharmacol, Biochem Behav 2001;69:261–7.

Ropero-Miller JD, Goldberger B. A recreational drugs. Current trends in the 90s. Clin Lab Med 1998;18:727–46.

Shulgin A, Shulgin A. Pihkal. Berkeley, CA: Transform Press, 1991.

Stafford P. Psychedelics encyclopedia. Berkley, CA: And/Or Press, 1977. p. 307.

Young R, Glennon RA. A three-lever operant procedure differentiates the stimulus effects of *R*(−)-MDA from *S*(+)-MDA. J Pharmacol Exp Ther 1996;276:594–601.

Young R, Glennon RA. Cocaine-stimulus generalization to MDA optical isomers: a reevaluation. Pharmacol, Biochem Behav 1997;57: 115–8.