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Further characterization of the stimulus properties of 5,6,7,8-tetrahydro-1,3-dioxolo[4,5-*g*]isoquinoline

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Abstract

This investigation is based on the premise that conformational restriction of abused phenylalkylamines in a tetrahydroisoquinoline conformation alters their pharmacology in such a manner that their original action is lost and that a new action emerges. TDIQ or 5,6,7,8-tetrahydro-1,3-dioxolo[4,5-*g*]isoquinoline, is a conformationally constrained phenylalkylamine that serves as a discriminative stimulus in animals. Although TDIQ bears structural resemblance to phenylalkylamine stimulants (e.g., amphetamine), hallucinogens (e.g., 1-(2,5-dimethoxy-4-methylphenyl)-2-aminopropane [DOM]), and designer drugs (e.g., *N*-methyl-1-(3,4-methylenedioxyphenyl)-2-aminopropane [MDMA], *N*-methyl-1-(4-methoxyphenyl)-2-aminopropane [PMMA]), the TDIQ stimulus failed to generalize to (+)amphetamine or MDMA. In the present investigation, further evaluations were made of the stimulus nature of TDIQ. Specifically, the stimulus similarities of TDIQ, PMMA, and DOM were examined. In no case was stimulus generalization (substitution) observed. The results confirm that TDIQ produces stimulus effects distinct from those of the abovementioned phenylalkylamines. We also examined the structure–activity relationships of a series of TDIQ analogs, including several that might be viewed as conformationally restricted (CR) analogs of phenylalkylamine hallucinogens, stimulants, and designer drugs. These agents were examined in rats trained to discriminate either DOM (1.0 mg/kg), (+)amphetamine (1.0 mg/kg), MDMA (1.5 mg/kg), or TDIQ (5.0 mg/kg) from saline vehicle. Whereas we have demonstrated that none of these agents retains their respective phenylalkylamine stimulus actions, several of these agents were found to substitute for TDIQ. *N*-Methylation abolished TDIQ-stimulus action. These results, coupled with previous findings, imply that TDIQ derivatives represent a novel class of phenylalkylamines analogs with unique stimulus properties. Preliminary radioligand binding studies suggest that an α_2-adrenergic mechanism might underlie the stimulus effects produced by TDIQ.

Keywords: Stimulants; Hallucinogens; Designer drugs; MDMA; PMMA; Amphetamine

1. Introduction

Psychoactive phenylalkylamines can produce one or more of several distinct stimulus effects in animals (Glennon, 1989; Glennon et al., 1997). For example, rats can be trained to discriminate both the phenylalkylamine stimulant (+)amphetamine and the structurally related phenylalkylamine hallucinogen 1-(2,5-dimethoxy-4-methylphenyl)-2-aminopropane (DOM) from vehicle. Stimulus generalization (i.e., substitution) does not occur when DOM is administered to (+)amphetamine-trained animals, nor does generalization occur when (+)amphetamine is administered to DOM-trained animals (Glennon, 1989). This is consistent with the proposed stimulus mechanisms of action of (+)amphetamine (primarily dopaminergic) (e.g., Goudie, 1991) and DOM (primarily serotonergic) (e.g., Glennon, 1991). *N*-Methyl-1-(4-methoxyphenyl)-2-aminopropane (PMMA), a third type of phenylalkylamine, produces a stimulus effect that is not yet well defined; however, stimulus generalization does not occur between PMMA, (+)amphetamine, and DOM regardless of which of the three is used as training drug (Glennon et al., 1997).

Certain phenylalkylamines are capable of producing more than one type of stimulus effect. *N*-Methyl-1-(3,4-methylenedioxyphenyl)-2-aminopropane (MDMA; "XTC," "e," "Ecstasy") is a widely abused phenylalkylamine (Schedules of Controlled Substances, 1986) that produces an empathogenic effect in humans (see, for example, Christophersen, 2000; Collin and Godfrey, 1997; Hegadoren et al., 1999; Peroutka, 1990 for a description and history of

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MDMA use). The effects of MDMA can be related to those of PMMA and (+)amphetamine; that is, stimulus generalization occurs upon administration of MDMA to either PMMA-trained or (+)amphetamine-trained rats (Glennon, 1989; Glennon et al., 1988a, 1997). Although PMMA and MDMA share a similarity in their stimulus effects, PMMA has been shown to lack the amphetaminergic stimulant component of action associated with MDMA (Glennon and Young, 2000). Furthermore, slight structural modification of MDMA to its α-ethyl homolog, *N*-methyl-1-(3,4-methylenedioxyphenyl)-2-aminobutane (MBDB), abolishes amphetaminergic character such that MBDB no longer substitutes for (+)amphetamine (Nichols and Oberlender, 1989; Nichols et al., 1986) but does substitute for PMMA (Rangisetty et al., 2001).

Recently, we described a series of conformationally restricted (CR) tetrahydroisoquinoline analogs of the above phenylalkylamines (Malmusi et al., 1996a,b; Young and Glennon, in press). In particular, 5,6,7,8-tetrahydro-1,3-dioxolo[4,5-*g*]isoquinoline (TDIQ; see Fig. 1 for structure) can be viewed as a conformationally constrained analog of MDMA. Curiously, administration of TDIQ to MDMA-trained animals resulted in a high degree (75%) of partial generalization but failed to completely substitute (i.e., failed to produce $\geq$ 80% drug-appropriate responding) for MDMA (Malmusi et al., 1996b). Conversely, using animals trained to discriminate TDIQ from vehicle, administration of MDMA resulted in 76% TDIQ-appropriate responding; but again, complete substitution failed to occur (Young and Glennon, 2002). Thus, although there seem to be some similarities between the stimulus effects produced by TDIQ and MDMA, the two agents can be distinguished. In addition, it should be noted that stimulus generalization did not occur between TDIQ and (+)amphetamine regardless of which was used as the training stimulus (Malmusi et al., 1996b). One interpretation of the above results is that stimulus generalization is incomplete between MDMA and TDIQ because TDIQ lacks amphetamine-like action (Malmusi et al., 1996b; Young and Glennon, 2002). Since the stimulus effect of PMMA is also like that of MDMA — except that PMMA is devoid of amphetamine-like actions (Glennon et al., 1988b, 1997) — perhaps PMMA and TDIQ have more stimulus properties in common than they do with MDMA. That is, TDIQ might behave in a manner similar to MBDB, a PMMA-like agent that also lacks amphetaminergic activity (Rangisetty et al., 2001). If so, it might be expected that stimulus generalization would occur between PMMA and TDIQ regardless of which is used as the training drug. This hypothesis was evaluated in the first study of the present investigation. Specifically, TDIQ and PMMA were examined in animals trained to discriminate either PMMA or TDIQ from saline vehicle.

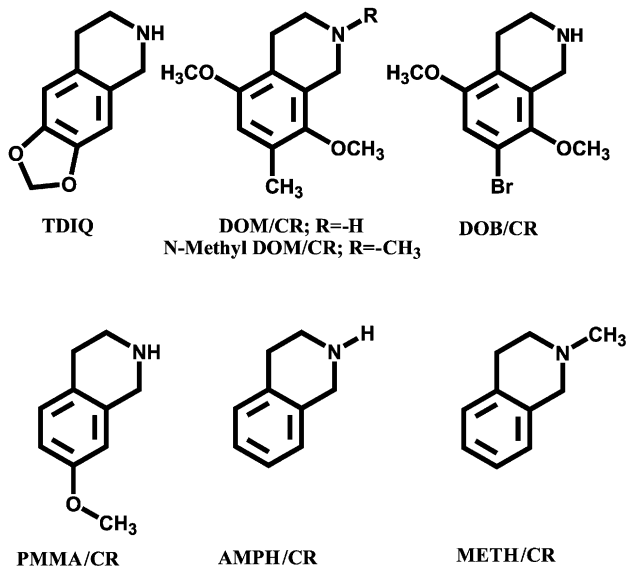

Fig. 1. Chemical structures of agents evaluated in the present study: TDIQ, 5,8-dimethoxy-7-methyl-1,2,3,4-tetrahydroisoquinoline (DOM/CR) and its *N*-methyl analog (*N*-methyl DOM/CR), 5,8-dimethoxy-7-bromo-1,2,3,4-tetrahydroisoquinoline (DOB/CR), 7-methoxy-1,2,3,4-tetrahydroisoquinoline (PMMA/CR), 1,2,3,4-tetrahydroisoquinoline (also known as TIQ or AMPH/CR) and its *N*-methyl counterpart METH/CR.

In the second study, further evaluations were made of the stimulus nature of TDIQ. It has been demonstrated that unlike its conformationally flexible counterpart (i.e., MDMA) (Glennon et al., 1988a), TDIQ does not behave as a locomotor stimulant in rodents (Malmusi et al., 1996b). Furthermore, stimulus generalization did not occur upon administration of TDIQ to (+)amphetamine- or (−)ephedrine-trained animals, nor did it occur upon administration of the stimulant phenylalkylamines (+)amphetamine or (−)ephedrine to TDIQ-trained animals (Young and Glennon, 2002). In contrast, the TDIQ stimulus completely generalized to cocaine (Young and Glennon, 2002). Thus, TDIQ appears to differentiate among the stimuli produced by (+)amphetamine, (−)ephedrine, and cocaine. Apparently, conformational restriction in the form of a tetrahydroisoquinoline structure alters the pharmacological properties of the phenylalkylamines in an unexpected manner. In Study 2, the stimulus properties of several TDIQ analogs were examined in animals trained to discriminate either DOM, (+)amphetamine, MDMA, or TDIQ from saline vehicle. The test compounds (see Fig. 1 for structures) included, for example, CR tetrahydroisoquinoline analogs of the hallucinogenic phenylalkylamines DOM (DOM/CR) and DOB (DOB/CR), the stimulant phenylalkylamines amphetamine (AMPH/CR) and methamphetamine (METH/CR), and the phenylalkylamine designer drug PMMA (PMMA/CR). DOB/CR is a CR analog of the bromo counterpart of DOM (i.e., DOB or 1-(4-bromo-2,5-dimethoxyphenyl)-2-aminopropane). Restraining the conformation of the alkylamino portion of these phenylalkylamines by incorporation into a tetrahydroisoquinoline ring reduces or abolishes their respective activities. That is, DOM/CR and DOB/CR failed to result in stimulus generalization when

administered to DOM-trained animals (Malmusi et al., 1996a), AMPH/CR and METH/CR failed to result in stimulus generalization when administered to (+)amphetamine-trained animals (Malmusi et al., 1996a), and PMMA/CR failed to substitute for training drug when administered to PMMA-trained animals (Young et al., 1999b). In the present study, the CR derivatives were examined to determine their stimulus similarity to other phenylalkylamine training drugs and to a TDIQ training stimulus. In particular, it was of interest to determine if TDIQ-like actions would be found for other tetrahydroisoquinolines and, if so, whether such actions are associated simply with the presence of a tetrahydroisoquinoline structure or whether pendant substituents influence their TDIQ-like action. These results could eventually be used in formulating structure–activity relationships for TDIQ-like activity. Finally, a radioligand binding profile was obtained for TDIQ in an attempt to determine which neurotransmitter system(s) might underlie its stimulus actions.

2. Methods

2.1. Drug discrimination studies

The subjects were 32 male Sprague–Dawley rats (Charles River Laboratories, Wilmington, MA) weighing 250–300 g at the beginning of the study. The animals were divided into five groups and trained to discriminate either 1.25 mg/kg of PMMA ($n=6$), 1.5 mg/kg of MDMA ($n=7$), 1.0 mg/kg of (+)amphetamine ($n=7$), 1.0 mg/kg of DOM ($n=6$), or 5.0 mg/kg of TDIQ ($n=6$) from saline vehicle as previously described (Glennon et al., 1997; Malmusi et al., 1996a; Young and Glennon, 2000, 2002; Young et al., 1999a). In brief, the animals were housed individually in standard animal care facilities with a 12-h light (06:00–18:00 h)/dark cycle, and testing was typically conducted between 10:00 and 13:00 h. 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 partial food deprivation; the animals were allowed drinking water ad lib in their home cages. The rats were trained (15-min training session) to discriminate intraperitoneal injections (15-min presession injection interval) of training drug 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., 1988b, 1997). Daily training sessions were conducted with training drug or saline. On every fifth day, learning was assessed during an initial 2.5-min nonreinforced (extinction) session followed by a 12.5-min training session. The left lever was designated the drug-appropriate lever for approximately half the animals, whereas the situation was reversed for the remaining animals. Data collected during the extinction session included responses per minute (i.e., response rate) 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 training drug, 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 training drug stimulus would generalize to the challenge drugs. During this phase of the study, maintenance of the training drug–saline discrimination was insured by continuation of the training sessions on a daily basis (except on a generalization test day; see below). On one of the two days before a generalization test, approximately half of the animals would receive the training dose of the training drug 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., $\geq$80% of total responses on the drug-appropriate lever after administration of training drug, and $\leq 20\%$ 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 groups of rats. 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. Radioligand binding studies

Radioligand binding studies were conducted by the NIMH Psychoactive Drug Screening Program in the laboratory of Dr. B. Roth (Case Western Reserve University). Each agent was initially evaluated for its ability to inhibit binding of radioligand at a concentration of 10,000 nM. Where >50% inhibition was observed, a K_i value was obtained in triplicate; K_i values are reported along with their SEM. Details of the binding assays, and specific radioligands and procedures employed, can be found at: http://pdsp.cwru.edu/pdsp.htm.

2.3. Drugs

PMMA, MDMA (Glennon et al., 1988b) and all the tetrahydroisoquinolines, as their hydrochloride salts (Malmusi et al., 1996a,b), were previously synthesized in our laboratories, and (+)amphetamine sulfate was available from earlier investigations. DOM was a gift from NIDA. 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

3.1. Study 1

Doses of TDIQ and PMMA were administered to animals trained to discriminate either TDIQ (5.0 mg/kg) or PMMA (1.25 mg/kg) from saline (Table 1). Administered to animals trained to discriminate PMMA from vehicle, TDIQ produced saline-appropriate responding at doses of up to 5.0 mg/kg. The animals' mean response rate was substantially reduced following 5.0 mg/kg of TDIQ, and administration of 6.0 mg/kg disrupted the animals' responding behavior. Similarly, administration of 0.1 to 0.5 mg/kg of PMMA to TDIQ-trained animals produced a maximum of 13% TDIQ-appropriate responding with a reduced response rate at 0.5 mg/kg of PMMA. PMMA doses of 0.75 and 1.0 mg/kg produced disruption of behavior (i.e., no responding).

Table 1
Stimulus effects of TDIQ and PMMA in rats trained to discriminate either TDIQ (5.0 mg/kg) or PMMA (1.25 mg/kg) from saline

Training drug/treatment	Dose (mg/kg)	N[a]	% Drug-appropriate responding (±S.E.M.)[b]	Response rate (resp/min±S.E.M.)[b]
PMMA-trained animals				
TDIQ	2.0	5/5	3 (1)	10.9 (2.1)
	4.0	4/4	11 (6)	6.5 (1.9)
	5.0	2/4	20 (6)	3.8 (1.0)
	6.0	1/5	–[c]	
PMMA	1.25	6/6	92 (4)	16.5 (5.9)
Saline (1 ml/kg)		6/6	4 (1)	21.1 (5.7)
TDIQ-trained animals				
PMMA	0.1	5/5	0	9.3 (2.1)
	0.3	4/5	10 (4)	8.1 (2.8)
	0.5	3/5	13 (7)	4.5 (3.0)
	0.75	0/5	–[c]	
	1.0	0/5	–[c]	
TDIQ	5.0	6/6	96 (2)	9.9 (2.3)
Saline (1 ml/kg)		6/6	5 (3)	10.6 (1.9)

[a] N=number of animals responding/number of animals administered drug.
[b] Data obtained during a 2.5-min extinction session. Results reflect data only from animals that made ≥5 responses during the extinction session.
[c] Disruption of behavior; animals made fewer than five responses during the extinction period.

Table 2
Results of stimulus generalization studies using DOM (1.0 mg/kg) as training drug

Treatment	Dose (mg/kg)	N[a]	% Drug-appropriate responding (±S.E.M.)[b]	Response rate (resp/min±S.E.M.)[b]
DOM	1.0	6/6	98 (1)	5.3 (0.8)
Saline (1 ml/kg)		6/6	4 (2)	7.1 (2.2)
TDIQ	1.0	5/5	1 (1)	8.1 (2.6)
	1.5	4/5	5 (5)	8.9 (3.4)
	2.0	2/5	–[c]	
AMPH/CR	0.3	6/6	0	10.3 (2.7)
	1.0	4/6	0	4.5 (1.5)
	2.0	4/6	0	5.1 (1.8)
	3.0	2/6	–[c]	
METH/CR	1.0	6/6	0	6.7 (1.3)
	3.0	6/6	0	3.3 (0.3)
	4.5	3/6	0	2.4 (0.4)
PMMA/CR	2.0	5/6	12 (9)	3.5 (0.5)
	2.5	2/6	–[c]	
	3.0	1/6	–[c]	
	4.0	0/6	–[c]	

[a] N=number of animals responding/number of animals administered drug.
[b] Data obtained during a 2.5-min extinction session. Results reflect data only from animals that made ≥5 responses during the extinction session.
[c] Disruption of behavior; animals made fewer than five responses during the extinction period.

3.2. Study 2

3.2.1. DOM stimulus (Table 2)

In DOM-trained animals, doses of 1.0 and 1.5 mg/kg of TDIQ produced a maximum of 5% DOM-appropriate responding; administration of 2.0 mg/kg resulted in disruption of behavior. Similarly, doses of 0.3 to 2.0 mg/kg of AMPH/CR, 1.0 to 4.5 mg/kg of METH/CR, and 2.0 mg/kg of PMMA/CR produced <20% drug-appropriate responding. Higher doses of these agents either severely depressed the animals' response rate (i.e., METH/CR) or disrupted the animals' behavior (i.e., AMPH/CR and PMMA/CR).

3.2.2. (+)Amphetamine stimulus (Table 3)

Administered to (+)amphetamine-trained animals, DOM/CR, *N*-methyl DOM/CR, DOB/CR, and PMMA/CR produced <20% (+)amphetamine-appropriate responding at doses evaluated. At the highest doses evaluated, the agents either severely depressed the animals' response rate (i.e., *N*-methyl DOM/CR) or disrupted the animals' behavior (i.e., DOM/CR, DOB/CR, PMMA/CR).

3.2.3. MDMA stimulus (Table 4)

Administered to MDMA-trained animals, AMPH/CR (1.0–4.5 mg/kg), METH/CR (3.0–5.0 mg/kg), DOM/CR (1.0–3.0 mg/kg), *N*-methyl DOM/CR (2.0–5.0 mg/kg), and DOB/CR (1.0 and 2.0 mg/kg) produced no more than 25% MDMA-appropriate responding. Higher doses of these agents either markedly reduced mean response rate (i.e.,

Table 3
Results of stimulus generalization studies using (+)amphetamine (1.0 mg/kg) as training drug

Treatment	Dose (mg/kg)	N[a]	% Drug-appropriate responding (±S.E.M.)[b]	Response rate (resp/min±S.E.M.)[b]
(+)Amphetamine	1.0	7/7	95 (3)	8.2 (2.1)
Saline (1 ml/kg)		7/7	6 (3)	9.9 (1.7)
DOM/CR	1.0	5/5	3 (2)	12.4 (2.1)
	2.0	4/7	15 (9)	10.9 (5.4)
	2.5	3/5	13 (3)	5.3 (3.4)
	3.0	2/7	–[c]	
N-Methyl DOM/CR	1.0	6/6	3 (2)	12.3 (2.1)
	3.0	5/6	5 (3)	11.8 (6.5)
	4.0	4/6	3 (3)	6.1 (2.3)
	5.0	3/6	2 (2)	4.0 (0.8)
DOB/CR	1.0	4/4	4 (2)	13.8 (2.1)
	2.0	5/7	12 (12)	14.4 (5.4)
	3.0	3/6	15 (8)	6.7 (3.2)
	3.5	3/7	–[c]	
PMMA/CR	0.1	3/3	0	4.4 (1.8)
	0.5	2/3	0	4.6 (2.2)
	1.0	1/4	–[c]	
	2.0	0/4	–[c]	

[a] N = number of animals responding / number of animals administered drug.
[b] Data obtained during a 2.5-min extinction session. Results reflect data only from animals that made ≥5 responses during the extinction session.
[c] Disruption of behavior; animals made fewer than 5 responses during the extinction period.

METH/CR, *N*-methyl DOM/CR) or disrupted the animals' behavior (i.e., AMPH/CR, DOM/CR, DOB/CR). MDMA-stimulus generalization occurred to PMMA/CR in a dose-related manner (ED_{50} = 2.4 mg/kg). A dose of 3.0 mg/kg of PMMA/CR generated 83% MDMA-appropriate responding; at this dose, three of four animals responded and the animals' response rate was severely reduced.

3.2.4. TDIQ stimulus (Table 5)

The CR DOM analog DOM/CR (ED_{50} = 4.2 mg/kg) and its bromo counterpart DOB/CR (ED_{50} = 3.4 mg/kg) generalized in the TDIQ-trained animals, as did the CR analogs of PMMA (PMMA/CR, ED_{50} = 1.6 mg/kg) and amphetamine (AMPH/CR, ED_{50} = 1.5 mg/kg). The *N*-methyl analog of DOM/CR and AMPH/CR (i.e., *N*-methyl DOM/CR and METH/CR, respectively) failed to result in stimulus generalization. *N*-Methyl DOM/CR produced 0% TDIQ-appropriate responding at 2.5 mg/kg and disruption of behavior at 3.0 mg/kg, whereas METH/CR produced a maximum of 6% TDIQ-appropriate responding at 0.5 mg/kg and disruption of behavior at 1.0 mg/kg.

3.3. Radioligand binding assays

TDIQ was examined at more than 30 different receptor populations. Specifically, TDIQ failed to bind (i.e., K_i >10,000 nM) at most populations of serotonin (5-HT) receptors (i.e., 5-HT_{1A}, r5-HT_{1B}, h5-HT_{1B}, r5-HT_{2A}, r5-HT_{2C}, 5-HT_3, 5-HT_{5A}, 5-HT_6) and displayed low affinity for h5-HT_{1D} (K_i = 7130 ± 1800 nM) and 5-HT_7 (K_i = 1750 ± 40 nM) receptors. TDIQ also failed to bind at most populations of dopamine receptors (D1, rD2, rD4, D5) but possessed modest affinity for rD3 receptors (K_i = 1440 ± 70 nM). In addition, TDIQ showed no affinity for the norepinephrine transporters (NET), dopamine transporters (DAT), or serotonin transporters (SERT), for rPCP receptors, rBZ receptors, or muscarinic (m_1–m_5) receptors. TDIQ also lacked affinity for β_1- and β_2-adrenergic receptors, α_{1A}- and α_{1B}-adrenergic receptors, but displayed high affinity for α_2-adrenergic receptors (α_{2A} K_i = 75 ± 5 nM; α_{2B} K_i = 95 ± 5 nM; α_{2C} K_i = 65 nM). For comparison, oxymetazoline binds at α_{2A}-adrenergic receptors with K_i = 0.3 ± 0.2 nM, and prazosin binds at

Table 4
Results of stimulus generalization studies using MDMA (1.5 mg/kg) as training drug

Treatment	Dose (mg/kg)	N[a]	% Drug-appropriate responding (±S.E.M.)[b]	Response rate (resp/min±S.E.M.)[b]
MDMA	1.5	7/7	99 (1)	7.6 (1.8)
Saline (1 ml/kg)		7/7	5 (3)	8.9 (2.1)
AMPH/CR	1.0	5/5	0	6.2 (2.1)
	3.0	4/5	1 (1)	5.7 (1.7)
	4.0	3/5	18 (9)	3.5 (0.3)
	4.5	3/5	25 (13)	4.4 (0.6)
	5.0	2/5	–[c]	
METH/CR	3.0	4/4	24 (5)	3.1 (0.3)
	4.0	3/4	6 (6)	2.8 (0.2)
	5.0	2/4	5 (5)	3.2 (0.8)
DOM/CR	1.0	4/4	0	5.4 (1.7)
	3.0	3/4	10 (10)	4.8 (1.0)
	4.0	1/4	–[c]	
	5.0	1/4	–[c]	
N-Methyl DOM/CR	2.0	4/4	4 (3)	6.5 (2.2)
	4.0	3/4	4 (4)	4.3 (1.3)
	5.0	2/4	9 (9)	2.4 (0.0)
DOB/CR	1.0	3/3	0	3.1 (0.4)
	2.0	2/4	17 (17)	4.8 (1.2)
	2.5	1/3	–[c]	
	3.0	0/4	–[c]	
PMMA/CR	2.0	3/4	19 (19)	2.9 (0.5)
	2.5	3/4	73 (14)	3.1 (0.1)
	3.0	3/4	83 (17)	2.9 (0.5)
			ED_{50} = 2.4 (1.8–3.0) mg/kg[d]	

[a] *N* = number of animals responding/number of animals administered drug.
[b] Data obtained during a 2.5-min extinction session. Results reflect data only from animals that made ≥5 responses during the extinction session.
[c] Disruption of behavior; animals made fewer than five responses during the extinction period.
[d] ED_{50} value followed by 95% confidence limits.

Table 5
Results of stimulus generalization studies using TDIQ (5.0 mg/kg) as training drug

Treatment	Dose (mg/kg)	N[a]	% Drug-appropriate responding (±S.E.M.)[b]	Response rate (resp/min±S.E.M.)[b]
TDIQ	5.0	6/6	97 (2)	11.3 (2.6)
Saline (1 ml/kg)		6/6	6 (3)	10.9 (1.9)
DOM/CR	2.0	4/5	3 (2)	20.0 (8.2)
	3.0	5/5	22 (13)	4.6 (1.4)
	4.0	5/5	66 (17)	9.4 (4.2)
	5.0	4/5	57 (10)	3.9 (1.1)
	5.5	4/5	70 (14)	3.8 (1.5)
	6.0	3/5	63 (13)	4.8 (2.0)
	6.5	4/5	95 (3)	3.4 (0.5)
			ED_{50}=4.2 (3.3–5.4) mg/kg[c]	
N-Methyl DOM/CR	1.5	6/6	0	9.9 (4.6)
	2.0	3/6	6 (6)	2.6 (0.3)
	2.5	3/6	0	13.6 (6.1)
	3.0	1/6	–[d]	
DOB/CR	3.0	4/6	20 (20)	4.0 (0.7)
	3.5	4/6	65 (24)	4.8 (1.2)
	4.0	4/6	90 (6)	4.3 (1.3)
			ED_{50}=3.4 (2.9–3.8) mg/kg	
PMMA/CR	1.0	4/5	29 (13)	5.3 (1.6)
	3.0	5/5	63 (21)	6.2 (2.1)
	4.0	4/5	96 (4)	4.4 (1.8)
			ED_{50}=1.6 (0.8–3.3) mg/kg	
AMPH/CR	1.0	6/6	30 (12)	13.0 (5.9)
	2.0	6/6	44 (15)	5.7 2.2)
	3.0	6/6	96 (3)	13.6 (7.3)
			ED_{50}=1.5 (1.0–2.4) mg/kg	
METH/CR	0.1	5/6	8 (6)	11.0 (4.4)
	0.5	3/6	6 (4)	12.7 (0.9)
	1.0	1/6	–[d]	

[a] N=number of animals responding/number of animals administered drug.
[b] Data obtained during a 2.5-min extinction session. Results reflect data only from animals that made $\geq$5 responses during the extinction session.
[c] ED_{50} value followed in parenthesis by 95% confidence limits.
[d] Disruption of behavior; animals made fewer than five responses during the extinction period.

α_{2B}- and α_{2C}-adrenergic receptors with K_i=3.9±0.7 and 16.2±3.2 nM, respectively.

4. Discussion

PMMA can be considered the structural parent of the MDMA family of phenylalkylamines and may be a somewhat more "selective" agent than MDMA in that, unlike MDMA, it lacks amphetamine-like effects (Glennon et al., 1988b, 1997). TDIQ bears structural resemblance to PMMA and MDMA, and it has been shown that stimulus generalization occurs between PMMA and MDMA regardless of which is used as the training drug (Malmusi et al., 1996b; Young and Glennon, 2002). The fact that TDIQ does not have an amphetamine-like stimulus element of action known to exist with MDMA (Glennon, 1989; Young and Glennon, 2002) may account for the failure to achieve complete cross-generalization between TDIQ and MDMA. Might the stimulus effects of TDIQ and PMMA be more mutual than they are with MDMA? Study 1 of the present investigation tested this idea by evaluating the stimulus properties (i.e., substitution) of TDIQ in PMMA-trained animals. Stimulus generalization did not occur (Table 1). Similar results were obtained upon administration of PMMA to TDIQ-trained animals (Table 1). When administered to the TDIQ-trained animals, PMMA doses comparable to, or higher than, its ED_{50} dose (0.44 mg/kg) (Glennon et al., 1997) in PMMA-trained animals depressed the animals' response rate or disrupted lever-pressing behavior. These results were unexpected because it was thought that TDIQ might have substituted in the PMMA-trained animals, and vice versa, and therefore its profile of stimulus activities would have been similar to that of the PMMA/MDMA-like agent MBDB. For example, Nichols and Oberlender (1989), Nichols et al. (1986), and Rangisetty et al. (2001) have found that in animals trained to discriminate either MDMA, PMMA, (+)amphetamine, or a hallucinogen from vehicle, MBDB generalizes only to MDMA (Nichols and Oberlender, 1989; Nichols et al., 1986) and PMMA (Rangisetty et al., 2001). In addition, when *S*(+)MBDB is used as training drug, stimulus generalization occurred to MDMA but not to DOM or (+)amphetamine (Oberlender and Nichols, 1990). Similar results were expected here. In the case of TDIQ, however, it is clear from the results of the present study and from an earlier investigation (Young and Glennon, 2002) that its stimulus effects are distinct from those of PMMA (Table 3) (and also from those of (+)amphetamine and DOM). What, then, might explain the partial generalization that occurs between TDIQ and MDMA?

MDMA is thought to produce its behavioral effects through a complex mechanism of action that includes serotonergic, dopaminergic, and adrenergic components. As such, numerous studies have employed a variety of agents (neurotransmitter agonists and antagonists) in attempts to define the specificity of the MDMA stimulus and to evaluate more fully the relative contribution of each neurotransmitter system to the actions of this agent. Perhaps not unexpectedly, this approach has shown that partial generalization occurs quite often to agents from different drug and chemical classes. For example, in animals trained to discriminate MDMA from vehicle, marked partial (i.e., >50% to <80% drug-appropriate responding) generalization has been observed with the nonselective serotonin (5-HT) receptor agonist quipazine and the nonselective dopamine receptor agonist apomorphine, and partial or complete (i.e., $\geq$80% drug-appropriate responding) generalization has been observed with the 5-HT_{1A} receptor agonists 8-OH DPAT (8-hydroxy-2-di-*N*-propylaminotetralin), *S*(−)8-OH DPAT, and *R*(+)8-OH DPAT (e.g., Glennon and Young, 2000; Schechter, 1989). Similar results were obtained when other drugs have been used as training stimuli and MDMA

used as the challenge agent. Thus, animals trained to discriminate apomorphine, ethanol, TFMPP (a nonselective serotonin agonist), Δ^9-THC, or the 5-HT releasing agent fenfluramine from vehicle, exhibit a high degree of partial generalization in tests of substitution (e.g., Barrett et al., 1995; Meehan et al., 1995; Schechter, 1986, 1988, 1989). Taken together, these results are difficult to reconcile in a cohesive manner and reemphasize the view that partial generalization is difficult to interpret (Glennon et al., 1983). The partial generalization that occurs between TDIQ and MDMA is another such example. However, the lack of cross-generalization between TDIQ and PMMA or (+)amphetamine, coupled with the absence of a locomotor stimulant effect by TDIQ, suggests that while some overlap might exist in the mechanism(s) of action of TDIQ and MDMA, it is unlikely that TDIQ is an agent with the potential to produce ''Ecstasy-like'' effects.

Study 2 examined the specificity of the TDIQ stimulus and explored some initial structure–activity considerations using CR tetrahydroisoquinoline analogs of phenylalkylamine hallucinogens (e.g., DOM, DOB), phenylalkylamine stimulants (e.g., amphetamine and methamphetamine), and the phenylalkylamine designer drug PMMA. This was accomplished by evaluating these derivatives to determine their stimulus similarity to certain phenylalkylamine training drugs (i.e., DOM, amphetamine, and MDMA) and a TDIQ training stimulus. The results are shown in Tables 2–5 and are summarized in Table 6.

It is rather interesting that with one exception (i.e., PMMA/CR in MDMA-trained animals) the CR analogs produced $\leq$25% phenylalkylamine-appropriate lever responding in rats trained to discriminate DOM, (+)amphetamine, or MDMA from vehicle (Tables 1–4), but that nearly all the tetrahydroisoquinolines generalized to a TDIQ stimulus (Table 5). Only the *N*-methyl derivatives *N*-methyl DOM/CR and METH/CR failed to substitute in the TDIQ-trained animals. The latter two agents also failed to result in stimulus generalization in the DOM-trained, (+)amphetamine-trained, and MDMA-trained animals (Tables 2–4, respectively). Also shown in the tables is that whereas DOM/CR and DOB/CR failed to produce DOM-like, (+)amphetamine-like, or MDMA-like stimulus effects, they substituted in TDIQ-trained animals. Likewise, although AMPH/CR failed to substitute in (+)amphetamine-trained animals and did not generalize in DOM-trained or MDMA-trained animals (Tables 2 and 4), it completely substituted for TDIQ (Table 5). Finally, whereas PMMA/CR failed to substitute for DOM and (+)amphetamine, it resulted in stimulus generalization in MDMA-trained and TDIQ-trained animals (Tables 2–5). The generalization of PMMA/CR in the MDMA-trained animals is unusual given that PMMA/CR has been shown to result only in partial generalization in PMMA-trained animals (Young et al., 1999a,b).

Nonetheless, it appears that conformational restriction of phenylalkylamine hallucinogens, stimulants, and designer drugs into a tetrahydroisoquinoline structure usually abolishes their respective actions (Malmusi et al., 1996b; Young et al., 1999a,b). However, from the results of the present investigation, it can be concluded that such restriction does not necessarily abolish their TDIQ-like stimulus effects (Table 5). Indeed, when these CR analogs are evaluated for their stimulus similarity to TDIQ and the phenylalkylamine training drugs, they appear to be most like TDIQ (Table 6).

Although DOM/CR (ED_{50} = 4.2 mg/kg) and DOB/CR (ED_{50} = 3.4 mg/kg) substituted for TDIQ, they are about four to five times less potent than TDIQ (ED_{50} = 0.9 mg/kg). The structurally simpler PMMA/CR and AMPH/CR (ED_{50} = 1.6 and 1.5 mg/kg, respectively) also substituted for, and were nearly as potent as, TDIQ. Evidently, the methylenedioxy functionality of TDIQ is not required for TDIQ-like stimulus effects, and its presence contributes only minimally when compared to PMMA/CR and AMPH/CR. On the other hand, unlike TDIQ, PMMA/CR completely substituted for MDMA (Table 4), and administration of AMPH/CR resulted in partial generalization in (+)amphetamine-trained animals (Malmusi et al., 1996a). Hence, the methylenedioxy group of TDIQ might be contributing to selectivity of action. It is also evident that a tertiary amine (as found in *N*-methyl DOM/CR and METH/CR) detracts from TDIQ-like stimulus action.

The results of the present studies, coupled with our earlier reports (Malmusi et al., 1996a,b; Young et al., 1999b), indicate that under the conditions employed the CR phenylalkylamine analog TDIQ produces stimulus effects in animals that are different than those produced by the conformationally flexible psychoactive phenylalkylamines (i.e., as typified by DOM, amphetamine, and PMMA; see Introduction). Costall et al. (1976) have reported that METH/CR produces hyperactivity in rats when injected directly into the nucleus accumbens. However, we have found that neither AMPH/CR nor its *N*-methyl analog METH/CR produces locomotor stimulation when administered to mice via the

Table 6
Summary of stimulus generalization results of agents used in the present investigation

	Training drug			
Agent	(+)AMPH	MDMA	DOM	TDIQ
TDIQ	NG[a]	PG[b]	NG	G[a]
AMPH/CR	PG[c]	NG	NG	G
METH/CR	NG[c]	NG	NG	NG
DOM/CR	NG	NG	NG[c]	G
N-Methyl DOM/CR	NG	NG	NG[c]	NG
DOB/CR	NG	NG	NG[c]	G
PMMA/CR	NG	G	NG	G

NG = no stimulus generalization (i.e., saline-appropriate responding), PG = partial generalization, G = generalization: see Results, Discussion, and Glennon and Young, 2000 for further details).

Data are from the present study (Tables 2–5) unless otherwise noted.

[a] Data from Young and Glennon (in press).

[b] Data from Malmusi et al. (1996b).

[c] Data from Malmusi et al. (1996a).

intraperitoneal route (Malmusi et al., 1996a). Results with AMPH/CR are consistent with those recently reported by Vetulani et al. (2001). Furthermore, neither AMPH/CR nor METH/CR completely substituted for (+)amphetamine in (+)amphetamine-trained rats (Malmusi et al., 1996a) and only AMPH/CR, but not METH/CR (present study), substituted for TDIQ.

Prior stimulus generalization and antagonism studies suggested a role for adrenergic receptors in the actions of TDIQ; possible involvement of dopaminergic and/or serotonergic mechanisms, although somewhat less likely, was also implicated (Young and Glennon, 2002). In an attempt to gain further clues as to possible mechanisms underlying the actions of TDIQ, TDIQ was examined at more than 30 different receptor populations. TDIQ displayed little to no affinity for the various subpopulations of dopamine or serotonin receptors, and did not bind at either the NET, DAT, or SERT. TDIQ also lacked affinity for α_1- and β-adrenergic receptors but displayed high affinity ($K_i < 100$ nM) for α_{2A}-, α_{2B}-, and α_{2C}-adrenergic receptors. Interestingly, AMPH/CR, an agent to which the TDIQ stimulus generalized, has also been shown to lack affinity for dopamine receptors but to bind ($K_i = 206$ nM) at α_2-adrenergic receptors (Vetulani et al., 2001). These preliminary results, then, suggest that TDIQ might be producing its stimulus effects primarily via an adrenergic mechanism. Obviously, additional studies will be necessary to further explore this issue.

We can conclude, at this time, that TDIQ produces stimulus effects, and presumably subjective effects, in rats that differ from those produced by the phenylalkylamines amphetamine, DOM, and PMMA. Furthermore, even though partial cross-generalization occurs between TDIQ and MDMA, it is unlikely that TDIQ is an agent that produces what might be considered a typical MDMA-like or ''Ecstasy-like'' effect. Finally, the TDIQ stimulus generalized to several TDIQ analogs — ''inactive'' CR analogs of DOM, DOB, PMMA, and amphetamine (i.e., DOM/CR, DOB/CR, PMMA/CR, and AMPH/CR) — with potencies roughly comparable to that of TDIQ. And yet, the TDIQ stimulus generalized to cocaine, but is not blocked by dopaminergic antagonists that block the stimulus effects of cocaine (Young and Glennon, in press). Thus, these TDIQ analogs represent a novel class of interesting centrally acting agents whose actions and mechanism of action are still not fully understood, but that deserve further attention.

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