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The stimulus effect of 5,6,7,8-tetrahydro-1,3-dioxolo[4,5-*g*]isoquinoline is similar to that of cocaine but different from that of amphetamine

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Abstract

5,6,7,8-Tetrahydro-1,3-dioxolo[4,5-*g*]isoquinoline (TDIQ) is a conformationally restricted phenylalkylamine related in structure to amphetamine and *N*-methyl-1-(3,4-methylenedioxyphenyl)-2-aminopropane (MDMA) that does not act as a locomotor stimulant. To further evaluate this agent, a group of six rats was trained to discriminate 5.0 mg/kg of TDIQ from vehicle and tests of stimulus generalization were conducted to define the stimulus. The TDIQ stimulus ($ED_{50} = 0.9$ mg/kg) failed to generalize to the central stimulants (+)amphetamine, methylphenidate or (−)ephedrine but, curiously, generalized to cocaine ($ED_{50} = 1.5$ mg/kg). When administered to rats ($n = 5$) trained to discriminate 1.0 mg/kg of (+)amphetamine from vehicle, TDIQ produced a maximum of 7% (+)amphetamine-appropriate responding, whereas when administered to rats ($n = 7$) trained to discriminate 4.0 mg/kg of (−)ephedrine from vehicle, TDIQ produced a maximum of 57% drug-appropriate responding. Administration of MDMA to TDIQ-trained animals resulted in 76% TDIQ-appropriate responding. Tests of stimulus generalization were also conducted with fenfluramine, nisoxetine, clenbuterol, imipramine and buspirone, and tests of antagonism were conducted with haloperidol and *R*(+)SCH-23390 using the TDIQ-trained animals. Results were inconclusive in that these agents either failed to completely substitute for or failed to completely antagonize the TDIQ stimulus. Nevertheless, the generalization seen with cocaine, the partial generalization seen with (−)ephedrine, MDMA, nisoxetine, clenbuterol and buspirone and the partial antagonism seen with haloperidol suggest that TDIQ might be acting through a mixed mechanism that involves adrenergic, dopaminergic and/or serotonergic systems. Given that TDIQ is an agent that seems to differentiate among the stimuli produced by amphetamine, methylphenidate, ephedrine and cocaine, it is proposed that further tests be undertaken, using animal models of cocaine abuse, to evaluate the potential usefulness of TDIQ as pharmacotherapy in cocaine dependence.

Keywords: Amphetamine cocaine; MDMA; Ephedrine; Stimulants

1. Introduction

N-Methyl-1-(3,4-methylenedioxyphenyl)-2-aminopropane (MDMA) is a so-called designer drug that produces an empathogenic effect in humans (Hegadoren et al., 1999; Nichols and Oberlender, 1989) and serves as a discriminative stimulus in animals (e.g., Glennon, 1989, 1991). At this time, the stimulus mechanism of action of MDMA is unknown and is believed to be rather complex. For example, there is evidence both for serotonergic and dopaminergic involvement (Glennon and Higgs, 1992; Schechter, 1989). Furthermore, it already has been demonstrated that MDMA substitutes for (+)amphetamine in rats trained to discriminate 1.0 mg/kg of (+)amphetamine from vehicle (e.g., Glennon et al., 1988), and because there is evidence that amphetamine possesses adrenergic as well as dopaminergic properties (reviewed in Goudie, 1991; Young and Glennon, 1986 but see also Darracq et al., 1998; Fleckenstein et al., 2000; Rothman et al., 2001), the possibility exists that in addition to dopaminergic involvement, MDMA and amphetamine could share some adrenergic character.

Recently, we prepared and evaluated several different types of conformationally constrained analogs of MDMA in a drug discrimination paradigm with rats trained to discriminate either MDMA or several other phenylalkylamines from vehicle (Malmusi et al., 1996a,b; Young et al., 1999). It was determined that an aminotetralin conformation, rather than a tetrahydroisoquinoline conformation, better accounts for the MDMA-like stimulus effects of these analogs (Malmusi et al., 1996b; Young et al., 1999). How-

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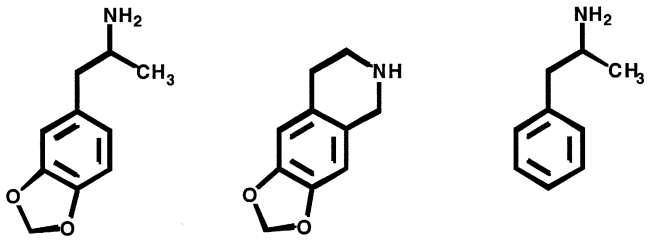

Fig. 1. Chemical structures of MDMA (left), TDIQ (center) and amphetamine (right).

ever, one of the tetrahydroisoquinoline analogs produced some unusual results. 5,6,7,8-Tetrahydro-1,3-dioxolo[4,5-*g*]isoquinoline (TDIQ; see Fig. 1 for chemical structures) administered to rats trained to discriminate MDMA from saline vehicle resulted in 75% MDMA-appropriate responding at 5.0 mg/kg (Malmusi et al., 1996b). Administration of slightly higher doses (5.2 and 5.25 mg/kg) of TDIQ elicited 63% and 59% MDMA-appropriate responding, whereas 5.5 mg/kg of TDIQ disrupted the animals' responding. Hence, although there was an indication that TDIQ might share some stimulus character with MDMA, the specified generalization criteria (i.e., $\geq$80% MDMA-appropriate responding) were not met (Malmusi et al., 1996b).

The curious results (i.e., the high degree of partial generalization yet failure to meet generalization criteria) obtained with TDIQ in MDMA-trained animals prompted this further examination of TDIQ's behavioral effects. In the present study, animals were trained to discriminate TDIQ from vehicle in a two-lever drug discrimination paradigm. Once the animals were trained, stimulus generalization (i.e., substitution) studies were conducted to determine if TDIQ-trained animals would recognize MDMA. (+)Amphetamine and several other stimulants (i.e., methylphenidate, ephedrine and cocaine) were also examined. The possible involvement of serotonergic, dopaminergic and adrenergic mechanisms in mediating the stimulus effects of TDIQ were explored by examining agents known to influence such mechanisms. In a series of companion studies, TDIQ was also examined in groups of animals trained to discriminate either (+)amphetamine or (−)ephedrine from vehicle.

2. Methods

2.1. Drug discrimination studies

Eighteen male Sprague–Dawley rats (Charles River Laboratories), weighing 250–300 g at the beginning of the study, were trained to discriminate either 5.0 mg/kg of TDIQ ($n=6$), 1.0 mg/kg of (+)amphetamine ($n=5$) or 4.0 mg/kg of (−)ephedrine ($n=7$) from saline vehicle. 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 partial food deprivation. The animals were allowed drinking water ad libitum in their home cages. The training procedure and equipment are exactly the same as previously reported for (−)ephedrine (Young and Glennon, 1998) and will be illustrated here, in detail, only for TDIQ. The rats were trained (15-min training session) to discriminate intraperitoneal injections (15-min presession injection interval) of TDIQ from saline vehicle (sterile 0.9% saline) under a variable interval 15-s schedule of reward (i.e., sweetened milk) using standard (Coulbourn Instruments) two-lever operant equipment. Daily training sessions were conducted with training drug or saline. On every second and fifth days, learning was assessed during an initial 2.5-min nonreinforced (extinction) session followed by a 12.5-min training session. For half the animals, the left lever was designated the drug-appropriate lever, whereas the situation was reversed for the remaining animals. Data collected during the extinction session included responses per minute (i.e., response rate as expressed as resp/min) 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 or antagonism studies until they consistently 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 various agents. 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 2 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 five) separated any two generalization test sessions. Doses of the test drugs were administered in a random order, using a 15-min presession injection interval, to groups of rats. Stimulus generalization was said 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.

The time-course test investigated the effects of changing the pretreatment interval of the training dose of TDIQ (i.e., 5.0 mg/kg) and initiation of the 2.5-min extinction session. In addition to the standard 15-min delay, the effects of 5-, 60-, 180- and 300-min pretreatment intervals were examined in a random order.

The antagonism studies using the TDIQ-trained animals were conducted in the same manner as the stimulus generalization studies, except that on "test days," the training dose of TDIQ was administered 45 min after a dose of either haloperidol or *R*(+)SCH-23390 was administered. A subsequent 15-min interval elapsed before the animals were tested under extinction conditions. Antagonism was said to have occurred when animals administered the drug combination made $\leq 20\%$ of their responses on the drug-appropriate lever.

2.2. Drugs

We have previously reported the synthesis of TDIQ hydrochloride (Malmusi et al., 1996b). MDMA was previously synthesized in our laboratories, and (+)amphetamine sulfate was available from earlier investigations. Methylphenidate HCl, (−)ephedrine HCl, clenbuterol HCl, imipramine HCl, desipramine HCl and trazodone HCl were purchased from Sigma–Aldrich (St. Louis, MO) and buspirone HCl, haloperidol and *R*(+)SCH-23390 HCl were purchased from Research Biochemicals (Natick, MA). Fenfluramine HCl was a gift from A.H. Robins (Richmond, VA), diazepam was a gift from Hoffman La Roche (Nutley, NJ) and nisoxetine HCl was a gift from Eli Lilly and Co. (Indianapolis, IN). Except for diazepam and haloperidol, which were

Table 1
Results of stimulus generalization studies with MDMA and central stimulants using animals trained to discriminate 5.0 mg/kg of TDIQ from saline vehicle

Treatment	Dose	*n*[a]	% Drug-appropriate responding (±S.E.M.)[b]	Response rate (resp/min ± S.E.M.)[b]
TDIQ	0.25	4/5	19 (9)	8.4 (0.6)
	0.5	5/5	31 (10)	8.5 (2.0)
	1.0	5/5	46 (11)	6.6 (1.0)
	2.5	5/5	78 (11)	9.9 (4.3)
	5.0	6/6	98 (1)	8.6 (2.1)
			ED_{50} = 0.9 (0.4–3.1) mg/kg[c]	
Saline (1 ml/kg)		6/6	8 (2)	10.2 (1.7)
MDMA	0.5	5/5	26 (2)	4.4 (0.2)
	1.0	5/5	76 (10)	6.6 (1.2)
	1.15	3/5	74 (14)	4.0 (0.6)
	1.25	0/5	–[d]	
	1.5	1/5	–[d]	
(+)Amphetamine	0.01	4/5	0	7.0 (2.8)
	0.05	5/5	27 (20)	5.6 (2.4)
	0.1	6/6	25 (17)	4.3 (1.6)
	0.3	3/5	2 (2)	4.8 (0.4)
	0.5	1/6	–[d]	
Methylphenidate	0.5	5/5	0	8.5 (2.6)
	1.5	4/5	14 (14)	9.8 (4.6)
	2.0	0/5	–[d]	
	2.5	1/5	–[d]	
	3.0	0/5	–[d]	
(−)Ephedrine	2.0	5/5	12 (5)	8.6 (1.2)
	3.0	5/5	33 (10)	7.4 (3.1)
	5.0	3/5	47 (14)	3.9 (2.1)
	5.5	2/5	–[d]	
	6.0	0/5	–[d]	
Cocaine	1.0	5/5	2 (2)	9.4 (5.4)
	1.5	4/5	38 (24)	5.6 (2.0)
	1.75	4/5	67 (24)	3.3 (0.1)
	2.0	4/5	99 (1)	7.3 (3.0)
	3.0	0/5	–[d]	
			ED_{50} = 1.5 (1.3–1.8) mg/kg[c]	

[a] *n* = number of animals responding/number administered drug.

[b] Data obtained during a 2.5-min extinction session. Percent drug-appropriate responding and response rates are group means and reflect results from those animals that made at least five responses during the entire extinction session.

[c] ED_{50} value followed by 95% confidence limits.

[d] Disruption of behavior. Fewer than half the animals made five responses during the extinction session following administration of this drug dose.

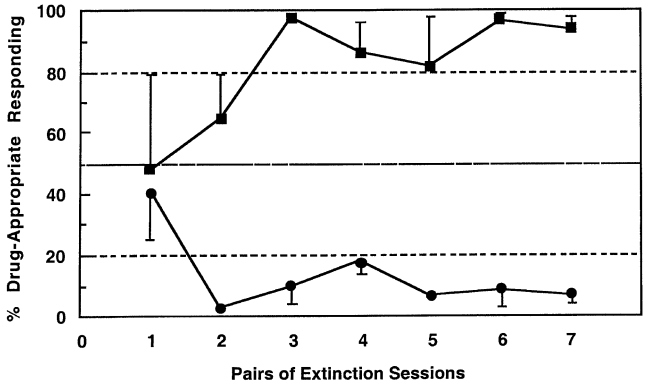

Fig. 2. Learning curve for acquisition of the TDIQ versus saline discrimination. *Ordinate:* Mean ($n=6$) percent (±S.E.M.) of responses made on the TDIQ-designated lever after the intraperitoneal administration of 5.0 mg/kg of TDIQ (solid squares) and 1.0 ml/kg of 0.9% saline (solid circles). Data were collected during 2.5-min extinction periods. *Abscissa:* Each number represents a pair of extinction sessions conducted during that week (total of 7 weeks).

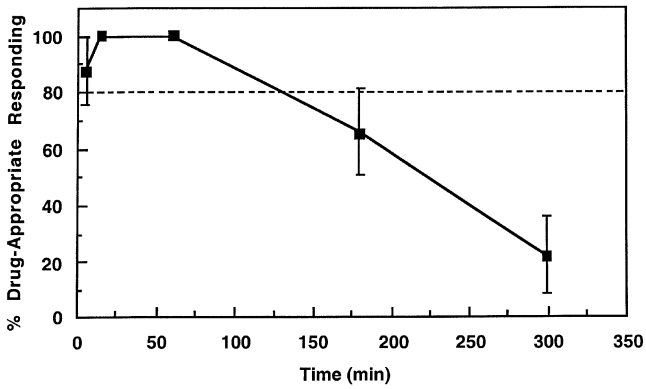

Fig. 3. Results of a time-course study using 5.0 mg/kg ip of TDIQ. *Ordinate:* Mean ($n=6$) percent (±S.E.M.) TDIQ-appropriate lever responding. *Abscissa:* pretreatment interval (min).

used as their free bases, doses refer to the weight of the salt. Solutions were made in saline, except that diazepam and haloperidol were suspended in saline to which one drop of Tween 80 was added. All solutions were prepared fresh daily and intraperitoneal injections (1 ml/kg) were made 15 min prior to testing unless otherwise specified.

3. Results

Six rats were trained to discriminate 5.0 mg/kg of TDIQ from vehicle. Fig. 2 shows that the animals learned the discrimination very quickly. Specifically, the rats demonstrated substantial separation in their drug-appropriate responding at the end of their second set of TDIQ/saline extinction sessions (i.e., within 2 weeks of training that included extinction sessions) and met the criteria for drug discrimination (i.e., $\geq 80\%$ drug-appropriate responding after TDIQ administration and $\leq 20\%$ drug-appropriate responding after administration of saline injection) by the third week of training. Once achieved, the animals maintained the TDIQ versus saline discrimination for the remainder of the study.

Administration of doses of TDIQ lower than the training dose resulted in the animals making a reduced number of responses on the drug-correct lever (Table 1). The calculated ED_{50} dose for TDIQ is 0.9 mg/kg. A time-course study revealed that even at 60 min postinjection, the animals made $>80\%$ of their responses on the drug-appropriate

Table 2
Results of stimulus generalization studies with TDIQ using rats trained to discriminate either 1.0 mg/kg of (+)amphetamine or 4.0 mg/kg of (−)ephedrine from vehicle

Treatment	Dose	n[a]	% Drug-appropriate responding (±S.E.M.)[b]	Response rate (resp/min±S.E.M.)[b]
(+)Amphetamine-trained animals				
TDIQ	2.0	5/5	5 (5)	6.1 (0.8)
	4.0	3/5	0	6.9 (4.7)
	5.0	3/4	7 (4)	5.5 (1.5)
	6.0	2/5	–[c]	
(+)Amphetamine	1.0	5/5	95 (2)	11.7 (2.3)
Saline (1 ml/kg)		5/5	7 (3)	13.8 (1.7)
(−)Ephedrine-trained animals				
TDIQ	1.0	7/7	9 (5)	16.7 (4.2)
	3.0	7/7	32 (16)	6.9 (1.5)
	4.0	5/7	57 (22)	7.0 (2.2)
	4.25	4/6	55 (20)	2.9 (0.4)
	4.5	2/7	–[c]	
	5.0	2/7	–[c]	
(−)Ephedrine	4.0	7/7	98 (1)	9.5 (1.9)
Saline (1 ml/kg)		7/7	5 (2)	10.2 (2.3)

[a] n=number of animals responding/number administered drug.

[b] Data obtained during a 2.5-min extinction session. Percent drug-appropriate responding and response rates are group means and reflect results from those animals that made at least five responses during the entire extinction session.

[c] Disruption of behavior. Fewer than half the animals made five responses during the extinction session following administration of this drug dose.

Table 3
Results of stimulus generalization and antagonism studies using animals trained to discriminate 5.0 mg/kg of TDIQ from saline vehicle

Treatment	Dose	n[a]	% Drug-appropriate responding (±S.E.M.)[b]	Response rate (resp/min±S.E.M.)[b]
Fenfluramine	0.1	5/6	19 (15)	7.3 (3.4)
	0.3	3/5	33 (33)	4.0 (1.8)
	0.5	1/6	–[c]	
Nisoxetine	1.0	5/5	6 (3)	6.8 (2.9)
	2.0	4/5	48 (25)	6.8 (1.8)
	2.5	2/5	–[c]	
	3.0	0/5	–[c]	
Clenbuterol	0.001	5/6	9 (8)	7.5 (2.1)
	0.005	5/6	42 (24)	16.4 (5.8)
	0.01	3/5	59 (30)	8.4 (5.2)
	0.02	2/5	–[c]	
	0.05	1/6	–[c]	
	0.1	0/6	–[c]	
Imipramine	0.1	6/6	0	7.4 (3.2)
	0.2	3/6	59 (15)	6.8 (2.3)
	0.3	1/6	–[c]	
	0.5	1/6	–[c]	
	1.0	0/6	–[c]	
Desipramine	0.1	5/5	3 (3)	4.6 (1.5)
	0.3	2/5	–[c]	
	0.5	1/5	–[c]	
Trazodone	0.1	5/5	0	8.9 (4.3)
	0.5	4/6	10 (4)	11.4 (4.5)
	1.0	1/5	–[c]	
Buspirone	0.001	4/5	0	11.0 (8.5)
	0.005	5/6	53 (14)	5.2 (2.3)
	0.01	1/5	–[c]	
	0.1	2/5	–[c]	
	1.0	0/5	–[c]	
Diazepam	0.1	6/6	0	8.7 (1.4)
	0.2	4/6	15 (5)	4.6 (1.7)
	0.3	0/6	–[c]	
	0.5	0/6	–[c]	
Haloperidol + TDIQ (5.0 mg/kg)	0.0005	4/6	88 (12)	3.3 (0.6)
	0.001	4/6	50 (14)	7.8 (1.9)
	0.01	3/6	46 (27)	2.8 (0.4)
	0.02	2/6	–[c]	
	0.03	1/6	–[c]	
	0.1	1/6	–[c]	
	0.3	2/6	–[c]	
R(+)SCH-23390 + TDIQ (5.0 mg/kg)	0.01	5/6	93% (6.6)	6.8 (2.8)
	0.05	2/6	–[c]	
	0.1	1/6	–[c]	

[a] n = number of animals responding/number administered drug.
[b] Data obtained during a 2.5-min extinction session. Percent drug-appropriate responding and response rates are group means and reflect results from those animals that made five responses during the entire extinction session.
[c] Disruption of behavior. Fewer than half the animals made five responses during the extinction session following administration of this drug dose.

lever following administration of the training dose of TDIQ (Fig. 3). Drug-appropriate responding was substantially reduced at 180 and 300 min postadministration. Response rates were not appreciably different after TDIQ (doses or postinjection intervals) and saline treatments.

Tests of stimulus generalization were conducted with MDMA, (+)amphetamine, methylphenidate, (−)ephedrine and cocaine. Doses of MDMA were administered to five animals (Table 1). MDMA (1.0 mg/kg) elicited 76% drug-appropriate responding, whereas administration of 1.15 mg/kg of MDMA resulted in 74% drug-appropriate responding. Higher MDMA doses (i.e., 1.25 and 1.5 mg/kg) disrupted the animals' behavior. At a dose of 0.1 mg/kg, (+)amphetamine elicited 25% drug-appropriate responding (response rate = 4.3 resp/min; Table 1). Higher doses of (+)amphetamine did not increase percent responding on the TDIQ-designated lever. Administration of 0.3 mg/kg of (+)amphetamine resulted in 2% TDIQ-appropriate responding (three of five animals) and 0.5 mg/kg resulted in disruption of behavior (the single animal that responded at the latter dose made 4% of its responses on the TDIQ-appropriate lever). Likewise, administration of 0.5 and

1.5 mg/kg of methylphenidate produced a maximum of 14% drug-appropriate responding, whereas at doses of 2.0, 2.5 and 3.0 mg/kg, the animals' behavior was disrupted (Table 1). At doses of 3.0 and 5.0 mg/kg, (−)ephedrine engendered 33% and 47% TDIQ-appropriate responding, respectively. The animals' response rates were reduced following the latter dose, and administration of 5.5 and 6.0 mg/kg of (−)ephedrine disrupted the animals' responding behavior. The TDIQ stimulus generalized to cocaine in a dose-related manner (Table 1). A dose of 2.0 mg/kg of cocaine elicited 99% drug-appropriate responding. At this dose, four of five animals responded and the response rate was 7.3 resp/min. Administration of 3 mg/kg of cocaine resulted in disruption of the animals' behavior with none of five animals making ≥ 5 responses during the extinction session.

Administered to (+)amphetamine-trained animals, TDIQ produced a maximum of 7% drug-appropriate responding at 5.0 mg/kg, with three of four animals responding. At 6.0 mg/kg, TDIQ disrupted the animals' behavior (Table 2). Administered to (−)ephedrine-trained animals, TDIQ produced a maximum of 57% drug-appropriate responding at 4.0 mg/kg. At this dose, only five of seven animals responded (Table 2). A higher dose of TDIQ (4.25 mg/kg) produced 55% drug-appropriate responding with a decrease in the animals' response rate, and doses of 4.5 and 5.0 mg/kg disrupted the animals' behavior.

The serotonin-releasing agent fenfluramine produced a maximum of 33% TDIQ-appropriate responding, and the norepinephrine reuptake inhibitor nisoxetine produced a maximum of 48% drug-appropriate responding (Table 3). The adrenergic agonist clenbuterol partially substituted for the TDIQ stimulus (maximal TDIQ-appropriate responding followed by dose: 59%, 0.01 mg/kg), as did imipramine (59%, 0.2 mg/kg) and buspirone (53%, 0.005 mg/kg). Desipramine, trazodone and diazepam (Table 3) failed to produce >20% TDIQ-appropriate responding. The dopaminergic antagonist haloperidol partially antagonized the TDIQ stimulus, whereas *R*(+)SCH-23390 was without effect on percent TDIQ responding (Table 3).

4. Discussion

The results of the study presented here demonstrate that TDIQ serves as an effective discriminative stimulus in rats. The animals very quickly learned to distinguish between the stimulus effects produced by the administration of 5.0 mg/kg of TDIQ and the administration of saline vehicle, and learning remained stable over time (Fig. 2). In fact, when acquisition data for TDIQ versus saline were compared to 20 or so different training stimuli (from different drug or chemical classes versus saline that the present laboratory has employed during the past 20 years), none approached the minimal amount of time required to establish TDIQ as a training stimulus. For comparison, animals can be fairly readily trained to discriminate (+)amphetamine from vehicle (approximately 3 months), whereas at the other extreme, just over 1 year was required to train rats to discriminate racemic 1-(3,4-methylenedioxyphenyl)-2-aminopropane (MDA) from vehicle (Glennon and Young, 1984). At this time, we have no explanation why TDIQ is such an effective training drug and why the animals acquired the discrimination so rapidly. It was noted during initial training sessions, however, that TDIQ did not seem to produce disruptive effects on behavior typical of other training drugs. Perhaps, tolerance develops very rapidly to the mild disruptive effects of the drug or the impact of TDIQ on behavior might involve a mechanism of action that exerts a positive effect on learning or memory processes. Obviously, further studies will be needed to address these possibilities.

The administration of TDIQ to MDMA-trained animals was previously shown to result in a maximum of 75% drug-appropriate responding (Malmusi et al., 1996b). Administration of MDMA to TDIQ-trained animals (Table 1) resulted in a maximum of 76% drug-appropriate responding. In both instances, administration of slightly higher drug doses resulted in no further increase in drug-appropriate responding. Nonetheless, the results are consistent regardless of which agent is used as the training drug. One explanation that can be offered for the lack of complete stimulus generalization is that TDIQ does not possess an amphetamine-like stimulus component of action that is known to exist with MDMA. A possible lack of amphetaminergic character by TDIQ might account for subtle differences between the stimulus properties of the two agents. Previous studies have shown that MDMA substitutes for a (+)amphetamine stimulus (e.g., Glennon and Higgs, 1992). However, (+)amphetamine failed to substitute for TDIQ in TDIQ-trained rats (Table 1) and disrupted the animals' behavior at relatively low doses. Also, TDIQ, unlike (+)amphetamine and MDMA, does not stimulate locomotor activity in mice (Glennon et al., 1988; Malmusi et al., 1996b). Consistent with these findings, administration of TDIQ to (+)amphetamine-trained rats failed to result in >7% drug-appropriate responding (Table 2). Thus, although some similarity might exist between the stimulus properties of MDMA and TDIQ, the two agents produce effects that are sufficiently different (i.e., the presence versus the absence of amphetamine-like effects?) to prevent occurrence of complete stimulus generalization. The lack of TDIQ stimulus generalization to methylphenidate, an agent that cross-generalizes to (+)amphetamine (reviewed in Young and Glennon, 1986), adds further support to this argument. In contrast, similarities between TDIQ and MDMA might be related to their effect on adrenergic or serotonergic systems. For example, MDMA is at least as potent (if not more potent) at releasing norepinephrine and 5-HT than it is in releasing dopamine (Rothman et al., 2001).

(−)Ephedrine is another example of a phenylalkylamine central stimulant. Although stimulus generalization occurs between (−)ephedrine and (+)amphetamine regardless of

which is used as training drug (Young and Glennon, 1998, 2000), there is evidence that the two stimuli are not identical. For example, (a) (−)ephedrine stimulus generalization occurred to six of eight isomeric phenylpropanolamines, whereas (+)amphetamine stimulus generalization occurred only to two of the eight isomers (Young and Glennon, 2000) and (b) the (−)ephedrine stimulus did not generalize to (+)methamphetamine, whereas the (+)amphetamine stimulus did (Glennon et al., 1988; Young and Glennon, 1998). It was suggested that the (−)ephedrine stimulus might involve more of an adrenergic component of action than does the (+)amphetamine stimulus (Young and Glennon, 1998). Consistent with this hypothesis, it has been recently shown that (−)ephedrine is much more effective at releasing norepinephrine (and blocking norepinephrine reuptake) than it is at producing the corresponding dopaminergic effects, whereas (+)amphetamine causes release of norepinephrine and dopamine (and blocks reuptake of norepinephrine and dopamine) at roughly comparable concentrations (Rothman et al., 2001). Administration of (−)ephedrine to the TDIQ-trained animals resulted in partial generalization (i.e., the animals made a maximum of 47% of their responses on the drug-appropriate lever). TDIQ was administered to animals trained to discriminate (−)ephedrine from vehicle, and here, too, partial generalization was observed (i.e., the animals made 57% of their responses on the (−)ephedrine-appropriate lever). It is tempting to speculate that TDIQ displays greater stimulus similarity to (−)ephedrine than to (+)amphetamine because of the greater adrenergic (and/or reduced dopaminergic) character of the former. However, in the absence of complete stimulus generalization, it is difficult to reach a definitive conclusion.

The TDIQ stimulus generalized to cocaine in a dose-related manner (Table 1). Cocaine and MDMA possess serotonergic, adrenergic and/or dopaminergic character (reviewed in Fischman and Haney, 1999; Fischman and Johanson, 1996; Glennon and Higgs, 1992; Schechter, 1989). It is commonly held that the inhibition of dopamine reuptake is the major mechanism underlying the subjective effects of cocaine (Volkow et al., 1997). However, there is also evidence for a modulatory role in cocaine's behavioral effects by serotonergic and adrenergic systems (Cunningham and Callahan, 1991; Johanson and Barrett, 1993; Klevin and Koek, 1998; Snoddy and Tessel, 1985; Terry et al., 1994; Tessel and Barrett, 1986; Tyler and Tessel, 1980). With regard to discriminative stimulus actions, although neither selective serotonin reuptake inhibitors nor norepinephrine reuptake inhibitors substituted for cocaine (but see, for example, Spealman, 1995), both produced leftward shifts in the dose–response curves to cocaine in cocaine-trained animals (e.g., Cunningham and Callahan, 1991; Klevin and Koek, 1998). However, it has been argued that activation of the serotonergic system is neither necessary nor sufficient to evoke cocaine-like stimulus effects (Klevin and Koek, 1998). There may be a more important role for the adrenergic system, and although differences in animal species (rat, mouse, pigeon, monkey) and training dose confound the issue (see Klevin and Koek, 1998; Spealman, 1995; Terry et al., 1994), studies with adrenergic agonists and antagonists and selective reuptake inhibitors variously implicate a role for $\alpha 1$, $\alpha 2$ and β-adrenergic receptors in the stimulus actions of cocaine (e.g., Johanson and Barrett, 1993; Klevin and Koek, 1998; Snoddy and Tessel, 1985; Wood et al., 1985). Clearly, then, in addition to the dopaminergic system, the adrenergic and serotonergic systems are involved to some extent in the actions of cocaine. Consequently, we examined the possible involvement of such mechanisms in the stimulus actions of TDIQ. The serotonin-releasing agent fenfluramine produced a maximum of 33% drug-appropriate responding, whereas the norepinephrine reuptake inhibitor nisoxetine produced 48% drug-appropriate responding. The serotonin (5-HT_{1A}) partial agonist buspirone elicited a maximum of 53% drug-appropriate responding. Due to the high degree of partial generalization elicited by this serotonergic anxiolytic agent, we examined another serotonergic agent (i.e., trazodone) and another mechanistically different anxiolytic agent (i.e., diazepam). Neither trazodone nor diazepam produced >20% drug-appropriate responding.

To further examine a role for serotonin and norepinephrine, we evaluated the nonselective tricyclic antidepressants imipramine and desipramine. Desipramine produced a maximum of 3% drug-appropriate responding but, interestingly, imipramine resulted in partial generalization (59% drug-appropriate responding at 0.3 mg/kg). The significance of the latter finding is unclear because only three of six animals responded at this dose. However, the β-adrenergic agonist clenbuterol produced 59% drug-appropriate responding, which, coupled with the results with nisoxetine, suggest some adrenergic involvement in the actions of TDIQ.

Finally, we examined the dopamine D_2 antagonist haloperidol and the D_1-selective antagonist *R*(+)SCH-23390. Haloperidol can antagonize drug-appropriate responding in animals trained to discriminate (+)amphetamine and substantially attenuates drug-appropriate responding in animals trained to discriminate cocaine from vehicle (reviewed in Woolverton, 1991; Young and Glennon, 1986). SCH-23390 attenuates the stimulus effects of (+)amphetamine and cocaine (reviewed in Goudie, 1991; Woolverton, 1991). In the present investigation, haloperidol partially antagonized the TDIQ stimulus (i.e., reduced TDIQ-appropriate responding to 46%), whereas *R*(+)SCH-23390 was without apparent antagonist action. Even though cocaine engenders >80% TDIQ-appropriate responding, there appears to be some mechanistic differences between the two agents on the basis of results with the dopamine antagonist SCH-23390.

Even though TDIQ is a conformationally restricted phenylalkylamine, it is clear that TDIQ is not a simple amphetamine-like phenylalkylamine stimulant. Indeed, given that TDIQ (a) is not a locomotor stimulant in mice,

(b) shares some properties (i.e., stimulus properties) with cocaine but also displays differences and (c) because TDIQ stimulus generalization occurs with cocaine but not with amphetamine or methylphenidate, it would seem to be deserving of additional evaluation. For example, considerable effort has been directed toward the identification of potential treatments for the management of cocaine abuse (e.g., Fischman and Haney, 1999; Fischman and Johanson, 1996; McCance, 1997). This research typically involves the evaluation of novel agents in bioassays such as drug self-administration, drug discrimination, place conditioning, intracranial self-stimulation and in animal models of "craving" or relapse to cocaine use (Fischman and Haney, 1999; Fischman and Johanson, 1996). Common goals are to identify compounds that would block the effect of cocaine or act as less addictive substitutes for cocaine (McCance, 1997). It would seem reasonable to investigate further the effects of TDIQ in such tests to determine whether or not TDIQ might serve as a potential pharmacotherapy in cocaine dependence. One of the first investigations to be conducted will be administration of TDIQ to cocaine-trained animals.

5. Summary

The present results indicate that animals very rapidly learned to discriminate TDIQ from vehicle and that the stimulus actions of TDIQ are at least 1 h in duration. The TDIQ stimulus failed to generalize to (+)amphetamine or methylphenidate. However, substitution occurred with cocaine. Partial generalization was seen with MDMA and (−)ephedrine and with the adrenergic agents nisoxetine, imipramine and clenbuterol and the serotonergic agents buspirone and fenfluramine. The dopaminergic antagonist haloperidol partially antagonized the TDIQ stimulus. In (−)ephedrine-trained rats, partial generalization occurred to TDIQ. Taken together, it might be tentatively concluded that TDIQ produces its stimulus effects via a mixed mechanism that involves to some extent adrenergic, dopaminergic and perhaps serotonergic systems. It might even be that specific subpopulations of serotonergic (and even adrenergic and/or dopaminergic) receptors are involved in the actions of TDIQ. The actions of central stimulants seem to involve the complex interplay of several neurotransmitter systems, and these systems might not be contributing to the activity of each agent, or to each action, in an identical manner (Fleckenstein et al., 2000). For example, the reinforcing effects of stimulants are thought to be related to their ability to increase mesolimbic synaptic concentrations of dopamine, whereas their ability to produce subjective effects in humans has been shown to correlate better with their potency in releasing norepinephrine (Rothman et al., 2001). Additional mechanistic studies are required in order to evaluate more fully the relative contributions of each neurotransmitter system to the stimulus effects produced by TDIQ. Furthermore, the initial training dose of TDIQ was selected as that dose of TDIQ found to produce the greatest degree of MDMA-appropriate responding in MDMA-trained animals (Malmusi et al., 1996b). A different training dose might qualitatively alter the profile of TDIQ.

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