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Pharmacology, Biochemistry and Behavior 78 (2004) 821–826

PHARMACOLOGY BIOCHEMISTRY AND BEHAVIOR

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Stimulus effects of three sulfur-containing psychoactive agents

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Received 24 November 2003; received in revised form 17 February 2004; accepted 28 May 2004
Available online 23 July 2004

Abstract

Two agents gaining popularity on the illicit drug market are the phenylalkylamines 4-MTA and 2C-T-7 [or 1-(4-methylthiophenyl)-2-aminopropane and 2-(2,5-dimethoxy-4-*n*-propylthiophenyl)-1-aminoethane, respectively]. At this time, there exists a paucity of information on the behavioral actions of these sulfur-containing agents. The present investigation examined these agents, and the *N*-monomethyl analog of 4-MTA (i.e., 4-MTMA), in tests of stimulus generalization (substitution) using a two-lever drug discrimination task with groups of rats trained to discriminate either the hallucinogen DOM [1-(2,5-dimethoxy-4-methylphenyl)-2-aminopropane], the stimulant cocaine, or the empathogen MDMA from vehicle. 4-MTA and its *N*-monomethyl analog 4-MTMA (ED_{50} = 0.8 mg/kg in both cases) substituted only for the MDMA stimulus, whereas 2C-T-7 (ED_{50} = 0.8 mg/kg) substituted only for the DOM stimulus. Thus, at the doses examined, 4-MTA and 4-MTMA appear to be MDMA-like agents, and 2C-T-7 seems best classified as a DOM-like hallucinogen. These results provide additional data that extend the structure–activity relationships of phenylalkylamines and that are consistent with what little is currently known about the action of 4-MTA and 2C-T-7 in humans.

Keywords: Hallucinogens; Stimulants; Empathogens; DOM; Cocaine; MDMA; 2C-T-7; 4-MTA; Drug discrimination

1. Introduction

Historically, sulfur-containing drugs of abuse are considered rarities or oddities. Examples of such substances are known (e.g., Shulgin and Shulgin, 1991), but their human use has not been particularly widespread. However, within the past few years, two sulfur-containing agents have appeared on the clandestine market, and their increasing popularity has resulted in actions to schedule them under the Controlled Substances Act (Federal Register, 2001, 2003, 2004). One is 1-(4-methylthiophenyl)-2-aminopropane, or 4-MTA (known as "*Flatliners*" and "*Golden Eagles*"), and the other is 2-(2,5-dimethoxy-4-*n*-propylthiophenyl)-1-aminoethane, or 2C-T-7 (known variously as "*T7*", "*Blue Mystic*", and "*Tripstasy*"). Both of these substances are substituted phenylalkylamines (see Fig. 1 for chemical structures).

Abused phenylalkylamines can be broadly classified either as hallucinogens or stimulants; there is third category of agents, empathogens [typified by *N*-methyl-1-(3,4-methylenedioxyphenyl)-2-aminopropane, or MDMA], whose actions can be differentiated from those of the hallucinogens or stimulants (Glennon, 2002). The stimulus actions of many phenylalkylamines can be differentiated and classified using animals trained to discriminate either a hallucinogen [e.g., DOM, or 1-(2,5-dimethoxy-4-methylphenyl)-2-aminopropane], a stimulant (e.g., amphetamine or cocaine) or MDMA from saline vehicle. Various phenylalkylamines have been demonstrated to produce one or more of several distinct stimuli in rats using drug discrimination procedures (e.g., Glennon and Young, 2002).

Anecdotal information on the human actions of 4-MTA and 2C-T-7 is available on the Internet, as are some limited descriptions of their effects in individual subjects (e.g., Shulgin and Shulgin, 1991; Winstock et al., 2002). However, detailed clinical studies are lacking. On the basis of what little information is available, 4-MTA is purportedly an MDMA-like agent (Winstock et al., 2002). 2C-T-7 might most aptly be classified as a hallucinogen and, at total doses of 10 to 30 mg, reportedly produces visual hallucinations that have been loosely compared with those produced by the classical hallucinogens 2-CB (i.e., 4-bromo-2,5-dimethoxyphenethylamine) and mescaline (Federal Register, 2003; Shulgin and Shulgin, 1991).

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doi:10.1016/j.pbb.2004.05.021

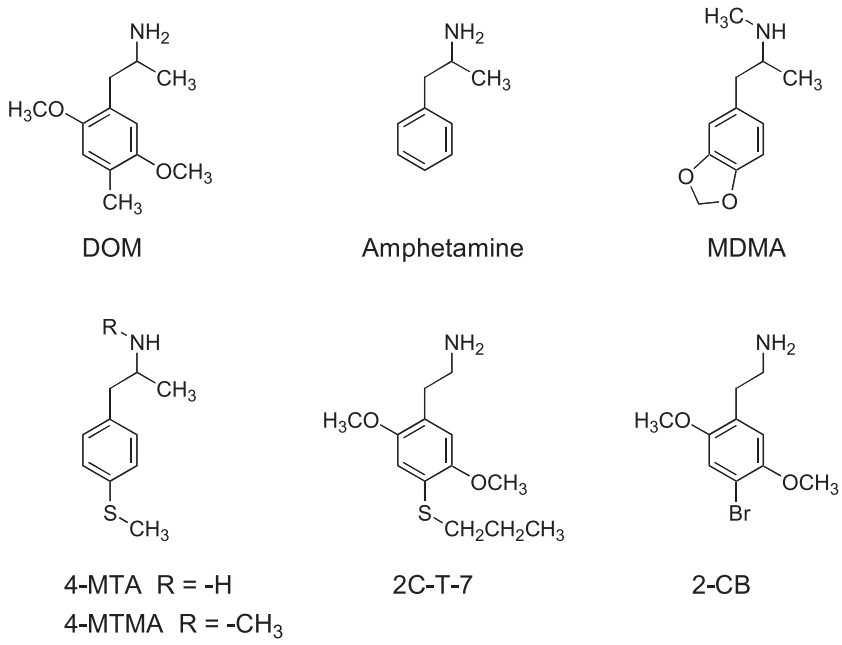

Fig. 1. Chemical structures showing the structural similarity of the agents discussed.

In the present investigation, it was of interest to determine if the stimulus actions of 4-MTA and 2C-T-7 are consistent with what is known, scant as it may be, about their actions in human subjects. To this end, 4-MTA and 2C-T-7 were examined in three groups of animals using a two-lever drug discrimination task. The *N*-monomethyl analog of 4-MTA (i.e., 4-MTMA) was also examined because of its closer structural analogy to MDMA (see Fig. 1). All three agents were evaluated in groups of rats trained to discriminate either the hallucinogen DOM, or the stimulant cocaine, or the empathogen MDMA from saline vehicle. The evaluation of these sulfur-containing agents should provide new information on their stimulus actions and will provide additional data to extend the established structure–activity relationships of phenylalkylamines.

2. Materials and methods

2.1. Drug discrimination studies

Twenty male Sprague–Dawley rats (Charles River Laboratories), weighing 250–300 g at the beginning of the study, were trained to discriminate (15-min presession injection interval) either DOM (1.0 mg/kg, $n=8$), cocaine (8.0 mg/kg, $n=6$), or MDMA (1.5 mg/kg, $n=6$) 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 (Dukat et al., 2002; Young and Glennon, 1997). The animals' body weights were maintained at 80% of their free-feeding weights by food restriction; animals had free access to water in their individual home cages. The animals used in these studies were maintained in accordance with the U.S. Public Health Service Policy on Humane Care and Use of Laboratory Animals, and studies were conducted under an approved Institutional Animal Care and Use Committee protocol.

In brief, daily training sessions were conducted with the training dose of the training drugs or saline. Learning was assessed every fifth day during an initial 2.5-min nonreinforced (extinction) session, followed by a 12.5-min training session. Data collected during the extinction session included response rate (i.e., responses per minute) and the number of responses on the drug-appropriate lever (expressed as a percent of total responses). The animals were not used in the subsequent stimulus generalization studies until they made $\geq 80\%$ of their responses on the drug-appropriate lever after the administration of the training drug and $\leq 20\%$ of their responses on the same drug-appropriate lever after the administration of saline, for three consecutive weeks. During the stimulus generalization (i.e., substitution) phase of the study, maintenance of the training-drug/saline discrimination was insured by the continuation of the training sessions on a daily basis (except on a generalization test day). On one of the 2 days before a generalization test, half 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 training criteria during the extinction session were excluded from the subsequent 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 nonrein-

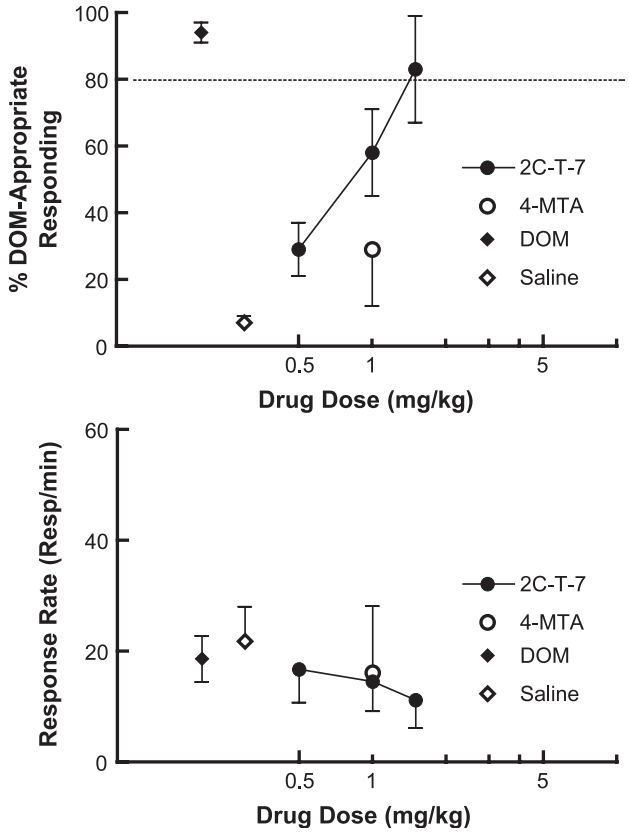

Fig. 2. Mean drug-appropriate responding (±S.E.M.) of rats trained to discriminate DOM (1.0 mg/kg) from saline vehicle (upper panel). The data points for DOM and saline reflect the effect of 1.0 mg/kg of DOM and 1.0 ml/kg of saline vehicle, respectively. Doses of 4-MTA >1.0 mg/kg disrupted the animals' lever-pressing behavior. Response rates are shown in the lower panel.

forcement conditions. An odd number of training sessions (usually five) separated any two generalization test sessions. Doses of test drugs were administered in a random sequence, using a 15-min presession injection interval, to the three groups of rats. Stimulus generalization was considered to have occurred when the animals, after a given dose of drug, made ≥80% of their responses (group mean) on the training drug-appropriate lever. Animals making ≤5 total responses during the 2.5-min extinction session were considered as being behaviorally disrupted. When more than half of the animals produced ≤5 total responses following a given drug dose, the results were not plotted. Response rate data refer only to animals making ≥5 responses during the extinction session. Where stimulus generalization occurred, an ED_{50} dose was calculated by the method of Finney (1952). The ED_{50} dose represents the drug dose where animals would be expected to make 50% of their responses on the drug-appropriate lever.

2.2. Drugs

Racemic 1-(2,5-dimethoxy-4-methylphenyl)-2-aminopropane HCl (DOM) and racemic 3,4-methylenedioxymethamphetamine HCl (MDMA) were obtained as gifts from NIDA. 2-(2,5-Dimethoxy-4-*n*-propylthiophenyl)-1-aminoethane HCl (2C-T-7) was provided by the College on Problems of Drug Dependence (CPDD). Cocaine HCl was purchased from Sigma-Aldrich (St. Louis, MO) and (+)amphetamine sulfate was available as the result of earlier studies. Both 1-(4-methylthiophenyl)-2-aminopropane (4-MTA) and *N*-monomethyl-1-(4-methylthiophenyl)-2-aminopropane (4-MTMA) were prepared as their HCl salts as previously reported (Dukat et al., 2002). Drug doses refer to the weight of the salts. All solutions were prepared fresh daily in sterile 0.9% saline.

3. Results

In the DOM-trained animals (Fig. 2), 4-MTA produced 29% DOM-appropriate responding at 1.0 mg/kg, with three of five animals making ≥5 responses during the extinction session. Tested at 3.0 mg/kg, 4-MTA disrupted the lever-pressing behavior of all the animals; that is, none of five animals made ≥5 responses. For this reason (and because

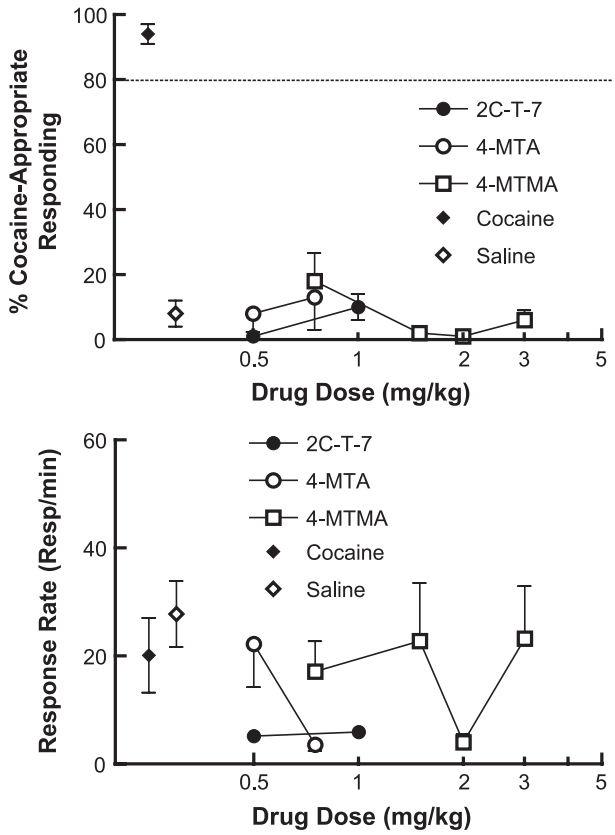

Fig. 3. Mean drug-appropriate responding (±S.E.M.) of rats trained to discriminate cocaine (8.0 mg/kg) from saline vehicle (upper panel). The data points for cocaine and saline reflect the effect of 8.0 mg/kg of cocaine and 1.0 ml/kg of saline vehicle, respectively. Doses of 4-MTA ≥1.0 mg/kg disrupted the animals' lever-pressing behavior, as did 4-MTMA at doses ≥3.75 mg/kg and 2C-T-7 at doses ≥1.5 mg/kg. Response rates are shown in the lower panel.

of limited supplies), 4-MTMA was not evaluated in the DOM-trained animals. The administration of the doses of 2C-T-7 resulted in dose-related substitution in the DOM-trained animals (ED_{50} = 0.8 mg/kg; 95% CL = 0.5–1.3 mg/kg). At 1.5 mg/kg, 2C-T-7 elicited 83% DOM-appropriate responding; 2.0 mg/kg of 2C-T-7 disrupted the animals' lever-pressing behavior; that is, one animal made no responses on either lever, and four animals responded only on the DOM-appropriate lever (response rate = 0.4 to 1.2 responses/min) but failed to meet the ≥5 total response threshold. The animals' response rates (Fig. 2) were not substantially different following 2C-T-7 doses of 0.5 to 1.5 mg/kg or the training dose of DOM (i.e., 18.6 responses/min).

Administration of 4-MTA and 4-MTMA to rats trained to discriminate cocaine from vehicle resulted in the animals making <20% of their responses on the drug-appropriate lever (Fig. 3). A dose of 0.75 mg/kg of 4-MTA had a rate suppressing effect on the animals' response rate, and doses of 1.0 and 1.5 mg/kg of 4-MTA, and 3.75 and 4.5 mg/kg of 4-MTMA, disrupted the animals' lever-pressing behavior. Likewise, 2C-T-7 produced saline-appropriate responding at 0.5 and 1.0 mg/kg, whereas doses of 1.5 and 2.0 mg/kg were behaviorally disruptive.

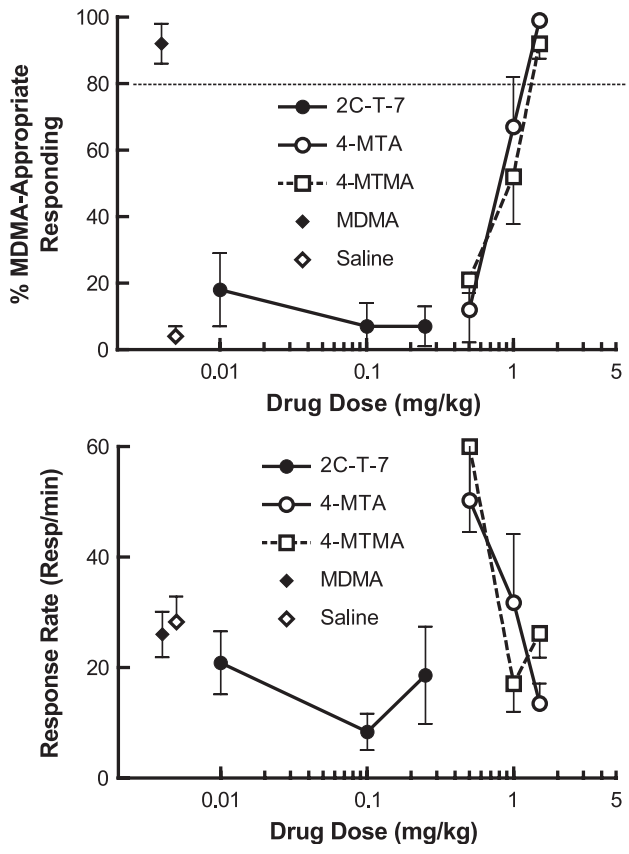

Fig. 4. Mean drug-appropriate responding (± S.E.M.) of rats trained to discriminate MDMA (1.5 mg/kg) from saline vehicle (upper panel). The data points for MDMA and saline reflect the effect of 1.5 mg/kg of MDMA and 1.0 ml/kg of saline vehicle, respectively. A dose of 0.5 mg/kg of 2C-T-7 disrupted the animals' lever-pressing behavior. Response rates are shown in the lower panel.

4-MTA (ED_{50} = 0.8 mg/kg; 95% CL = 0.5–1.2 mg/kg) and 4-MTMA (ED_{50} = 0.8 mg/kg; 95% CL = 0.5–1.3 mg/kg), but not 2C-T-7, substituted for the MDMA stimulus (Fig. 4). At 0.5 mg/kg, both 4-MTA and 4-MTMA elicited vehicle-appropriate responding; at this dose, the animals' response rate (approximately 50–60 responses/min) was about twice that following training drug (26.0 responses/min) or saline vehicle (28.3 responses/min) (Fig. 4). Higher drug doses resulted in increased drug-appropriate responding, with a decrease in response rates to near control rates. At the drug dose that produced ≥80% MDMA-appropriate responding, the animals' response rate following 4-MTMA administration was similar to that following the administration of MDMA, whereas that following 4-MTA administration was depressed by about 50%. The administration of 0.5 mg/kg of 2C-T-7 to the MDMA-trained animals resulted in a disruption of behavior, necessitating the evaluation of lower drug doses. At the 0.5 mg/kg dose, only two of six animals made ≥5 responses during the entire extinction session—one animal making 74% of their responses on the MDMA-appropriate lever (12.4 responses/min) and the other, 56% MDMA-appropriate responding (3.6 responses/min); of the remaining four animals, none made responses on either lever during the extinction session. At three other doses examined (0.01, 0.1, and 0.25 mg/kg), 2C-T-7 produced saline-appropriate responding.

4. Discussion

The present results indicate that the stimulus effects of two sulfur-containing phenylalkylamines, 4-MTA and 2C-T-7, are clearly different from one another and that they are consistent with claims of their actions in humans. For example, 4-MTA has been sold on the illicit market as "*flatliners*" and has been misrepresented as MDMA. In a survey of >1000 dance-drug users, 10% had reportedly used 4-MTA; although 4-MTA supposedly produces MDMA-like empathogenic effects, the degree of similarity of this effect to the effects of MDMA was not clearly established (Winstock et al., 2002). In the present investigation, 4-MTA substituted for MDMA but did not substitute either for the hallucinogen DOM or the stimulant cocaine at the doses evaluated. Huang et al. (1992) have previously reported stimulus generalization between 4-MTA and MDMA, and the results provided here support the results of the earlier investigation. There are no reports of the abuse of 4-MTMA. However, because 4-MTMA possesses an *N*-methyl group (as does MDMA), it was of interest to determine its actions and potency relative to 4-MTA. 4-MTMA was found to be similar to 4-MTA; that is, 4-MTMA substituted for MDMA and was equipotent with 4-MTA, but did not substitute for cocaine.

Although the substitution results are fairly clear-cut, it seems that the MDMA-trained animals were particularly susceptible to the rate-suppressing effects of 0.5 mg/kg of 2C-T-7. This might be due to the DOM-like nature of the agent, or to some other action of 2C-T-7 not considered in the present investigation. In any event, it was not possible to evaluate higher doses of this agent in the MDMA-trained animals. In contrast, the administration of 0.5 mg/kg of 4-MTA and 4-MTMA resulted in rate enhancement; curiously, the animals' response rates returned to near-control levels upon the administration of higher drug doses. This might be taken as an indication that these two agents produce an effect at low doses that is somewhat different than that observed at higher doses.

It has been reported that 4-MTA produces, among its effects, sleeplessness in humans (Winstock et al., 2002). One explanation for this action might be that 4-MTA produces stimulant-like effects. In the present study, 4-MTA (and 4-MTMA) failed to produce a cocaine-like effect (Fig. 3), and Huang et al. (1992) have previously reported that 4-MTA did not substitute in animals trained to discriminate (+)amphetamine from vehicle (although specific data were not provided). More recently, however, 4-MTA and 4-MTMA were shown to produce a maximum of 36% and 59% (+)amphetamine-appropriate responding, respectively, in (+)amphetamine-trained rats (Dukat et al., 2002). In addition, Coop (2000) found that 4-MTA substituted for (+)amphetamine in two of four monkeys when administered via the intramuscular route but did not substitute when administered orally. It is also interesting to note in the present investigation that a relatively low dose of 4-MTA or 4-MTMA displayed a rate-enhancing effect on lever responding when administered to MDMA-trained rats (Fig. 4). Taken together, these seemingly inconclusive results might reflect the influence(s) of differences in species, metabolism, and/or routes of administration. They also raise the possibility that 4-MTA exerts some degree of CNS stimulation, as part of a more complex pharmacological effect, that results in the reports of sleeplessness. Indeed, it is tempting to speculate that such stimulant-like effects of racemic 4-MTA and/or 4-MTMA are embedded in one of its optical isomers (or their individual metabolites). Future studies should address this issue.

4-MTA and 4-MTMA, like MDMA, target the 5-HT signaling system, interact with the 5-HT transporter, and can cause release of 5-HT (Cozzi and Foley, 2003; Gobbi et al., 2002; Huang et al., 1992; Winstock et al., 2002). 4-MTA is a more potent inhibitor than is 4-MTMA or MDMA of [^{3}H]5-HT re-uptake, but MDMA is the most potent of the three in causing the release of 5-HT (Huang et al., 1992; Murphy et al., 2002). It has been concluded that the methylenedioxy substituent of MDMA has a greater impact than does a thiomethyl group on the release of 5-HT, whereas *N*-methylation decreases the potency of these agents as re-uptake inhibitors; in this regard, 4-MTA and 4-MTMA released 5-HT to a similar extent (Murphy et al., 2002). An additional consideration that should be taken into account is that these agents might produce effects at other neurotransmitter systems. A recent study, for example, demonstrated that 4-MTA is not without catecholaminergic effects, and that its actions in the production of hyperthermia might involve serotonergic and catecholaminergic components (Carmo et al., 2003). Moreover, it has been recently demonstrated that (+)4-MTA is 18 times more potent than (−)4-MTA is as an inhibitor of monoamine oxidase (Hurtado-Guzman et al., 2003), and some evidence exists for species differences in 4-MTA metabolism (Carmo et al., 2004). In any case, it seems clear that 4-MTA (Huang et al., 1992 and present findings) and 4-MTMA (Fig. 4) share greater stimulus similarities with MDMA than with the hallucinogen DOM or stimulants cocaine and amphetamine, and it will be of interest to note if additional sulfur-containing drugs appear on the illicit market in the future.

In the three groups of animals examined, 2C-T-7 substituted only for DOM, and this finding indicates that the effects of 2C-T-7 might be primarily of a hallucinogen-like nature. It has been suggested that the effects of 2C-T-7 are similar to the structurally related phenylethylamine hallucinogens mescaline and 2-CB (see Introduction). In support of this, previous studies have demonstrated that a DOM stimulus generalizes to these latter two agents (Glennon et al., 1983, 1988). Thus, all three agents have now been established to produce a DOM-like stimulus effect in animals. Likewise, 4-MTA substituted only in the MDMA-trained animals, indicating that 4-MTA might be primarily MDMA-like in nature. Like MDMA, 4-MTA also substituted in animals trained to discriminate the structurally related agent PMMA [*N*-methyl-1-(4-methoxyphenyl)-2-aminopropane] from saline vehicle (Dukat et al., 2002; Glennon and Young, 2002). Regardless of what other effects these agents might produce (vide supra), the findings described herein indicate that the stimulus properties of 2C-T-7 and 4-MTA are readily distinguishable. Overall, therefore, the present results are not inconsistent with what little is known about the human actions of 4-MTA and 2C-T-7. That is, 4-MTA is primarily an MDMA-like agent—compatible with its being sold as a substitute for MDMA, whereas 2C-T-7 is likely more of a DOM-like (hallucinogenic) agent. The results further indicate that 4-MTMA is also an MDMA-like agent.

Acknowledgements

This work was supported in part by USPHS award DA 01642 and by the Drug Enforcement Administration.

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