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A Preliminary Investigation of the Psychoactive Agent 4-Bromo-2,5-Dimethoxyphenethylamine: A Potential Drug of Abuse

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GLENNON, R. A., M. TITELER AND R. A. LYON. *A preliminary investigation of the psychoactive agent 4-bromo-2,5-dimethoxyphenethylamine: A potential drug of abuse.* PHARMACOL BIOCHEM BEHAV **30**(3) 597–601, 1988.—4-Bromo-2,5-dimethoxyphenethylamine (α-desMe DOB) is a psychoactive agent that may possess significant abuse potential. Because of its structural similarity to the established hallucinogen 1-(4-bromo-2,5-dimethoxyphenyl)-2-aminopropane (DOB), and because almost no pharmacological data are available on this agent, we undertook this preliminary investigation. α-DesMe DOB (K_i=1 nM), like DOB itself (K_i=0.79 nM), displays a high affinity for [^{3}H]DOB-labeled central 5-HT_2 serotonin receptors. However, unlike DOB, the α-desmethyl derivative also binds with significant affinity to 5-HT_{1A}, 5-HT_{1B}, and 5-HT_{1C} serotonin receptors and, as such, is less selective than DOB. In drug discrimination studies using rats trained to discriminate either DOM (i.e., the 4-methyl analog of DOB) or R(−)DOB from saline, stimulus generalization occurred in both groups of animals. However, stimulus generalization was associated with extensive disruption of behavior, α-DesMe DOB may produce stimulus effects similar, but not identical, to those of DOM and R(−)DOB; in addition, this agent may be capable of producing other, as yet undefined, central effects at comparable doses. These other effects may be reflective of the lack of selectivity of α-desMe DOB for 5-HT_2 serotonin receptors. Because other hallucinogenic agents display high affinity for 5-HT_2 serotonin receptors and result in stimulus generalization in DOM- and/or DOB-trained animals, it is tentatively concluded that α-desMe DOB is a psychoactive agent with at least some hallucinogenic or DOB-like properties.

5-HT_1 5-HT_2 DOB DOM Discrimination Drug abuse α-desMe DOB

4-BROMO-2,5-DIMETHOXYPHENETHYLAMINE or 2-(4-bromo-2,5-dimethoxyphenyl)-1-aminoethane (BDMPEA, 2-CB) is closely related in structure to the phenylisopropylamine hallucinogen 1-(4-bromo-2,5-dimethoxyphenyl)-2-aminopropane (DOB) and, as such, is also referred to as alpha-desmethyl DOB or α-desMe DOB. α-desMe DOB is a psychoactive substance which produces, in human subjects, euphoria and perceptual enhancement (i.e., increased receptiveness of visual, auditory, olfactory, and tactile sensation) [12]. At least one case study has already been reported in which accidental ingestion of this agent by several students resulted in hallucinatory behavior [10], and there is a real concern regarding its abuse potential [11]. There is currently available almost no pharmacological data on this agent; this prompted us to undertake a preliminary investigation of α-desMe DOB.

Phenalkylamine hallucinogens, such as DOB and its 4-methyl analog 1-(2,5-dimethoxy-4-methylphenyl)-2-aminopropane (DOM), display high affinity and selectivity for central 5-HT_2 receptors as determined by radioligand binding [3,4]. Indeed, [^{3}H]DOB can be used to label the agonist high-affinity state of 5-HT_2 receptors [8,13]. In addition, phenalkylamine hallucinogens (e.g., DOM, DOB) serve as effective training drugs in drug discrimination studies [2,4] and the potencies of various related hallucinogens in tests of stimulus generalization using DOM-trained animals is significantly correlated with their affinities (K_i values) for 5-HT_2 receptors [3]. It has been proposed that 5-HT_2 receptors might be involved in the mechanism of action of the classical hallucinogens [3,4]. In the present investigation, we determined the affinity of α-desMe DOB for 5-HT_{1A}, 5-HT_{1B}, 5-HT_{1C}, and both [^{3}H]ketanserin-labeled and [^{3}H]DOB-labeled 5-HT_2 serotonin receptors. In addition, we evaluated the stimulus properties of this agent in groups of rats trained to discriminate either DOM or R(−)DOB from saline in order to determine if it would produce similar stimulus effects.

TABLE 2

RESULTS OF STIMULUS GENERALIZATION STUDIES USING RATS TRAINED TO DISCRIMINATE R(−)DOB (0.2 mg/kg) OR DOM (1.0 mg/kg) FROM SALINE

Agent	Dose (mg/kg)	N*	PSII†	Percent Drug-Appropriate Responding (SEM)	Mean Resp Rate/Min (SEM)
			(A) R(−)DOB-Trained Animals		
R(−)DOB	0.2	6/6	15	92% (4)	10.6 (1.4)
			ED_{50}=0.05 mg/kg‡		
Saline (1 ml/kg)		6/6	15	12% (6)	9.4 (1.6)
α-desMe DOB	0.1	4/6	15	13% (8)	11.4 (1.8)
	0.2	3/6	15	16% (2)	8.4 (1.1)
	0.3	2/6	15	—§	
	0.4	5/6	15	11% (2)	4.2 (0.9)
	0.5	1/6	15	—	
	0.6	2/6	15	—	
	0.7	2/5	15	—	
	0.7	4/4	5	25% (6)	13.2 (2.8)
	0.7	1/4	30	—	
	0.7	3/5	60	70% (14)	5.8 (0.6)
	0.2	4/6	60	4% (4)	7.2 (2.1)
	0.5	3/5	60	41% (16)	6.7 (0.8)
	0.6	3/6	60	73% (24)	5.0 (1.3)
	0.7	3/5	60	70% (14)	5.8 (0.6)
	0.75	3/6	60	82% (8)	4.4 (1.0)
	0.8	3/6	60	96% (2)	2.8 (0.4)
			ED_{50}=0.48 (0.33–0.69) mg/kg¶		
(±)DOM	0.1	4/4	15	8% (3)	11.4 (2.2)
	0.2	3/3	15	28% (7)	12.3 (4.5)
	0.25	6/6	15	48% (13)	10.4 (2.7)
	0.3	3/3	15	84% (10)	6.5 (1.3)
	0.5	5/5	15	91% (4)	6.6 (1.7)
			ED_{50}=0.24 (0.16–0.36) mg/kg¶		
Quipazine	0.05	6/6	15	1% (1)	8.2 (1.0)
	0.1	6/6	15	8% (2)	6.0 (0.6)
	0.15	0/4	15	—	
	0.2	1/5	15	—	
	0.4	1/3	15	—	
Pirenperone#+ Saline	0.005	3/3	30+15	13% (3)	9.0 (3.0)
	0.01	3/3	30+15	2% (1)	14.0 (2.2)
	0.04	4/4	30+15	9% (3)	6.9 (2.0)
	0.05	3/3	30+15	16% (6)	9.1 (3.5)
Pirenperone#+ R(−)DOB	0.005	5/5	30+15	81% (8)	9.2 (1.8)
	0.01	3/3	30+15	42% (14)	12.5 (1.4)
	0.04	4/4	30+15	31% (9)	13.5 (4.7)
	0.05	4/5	30+15	4% (1)	9.3 (3.0)
			(B) DOM-Trained Animals		
DOM	1.0	5/5	15	87% (4)	10.9 (2.0)
			ED_{50}=0.44 mg/kg**		
Saline (1 ml/kg)		5/5	15	12% (4)	10.1 (1.7)

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TABLE 2
(Continued)

Agent	Dose (mg/kg)	N*	PSII†	Percent Drug-Appropriate Responding (SEM)	Mean Resp Rate/Min (SEM)
α-desMe DOB	0.3	5/5	15	11% (7)	12.6 (2.4)
	0.5	4/4	15	42% (15)	10.4 (2.4)
	0.75	5/5	15	59% (12)	8.1 (1.9)
	0.9	3/4	15	68% (9)	4.9 (0.5)
	1.2	4/4	15	77% (12)	5.9 (1.4)
	1.5	3/4	15	83% (3)	7.3 (2.4)
	ED_{50}=0.67 (0.40–1.14) mg/kg				

*Number of animals responding/number receiving drug. †PSII=presession injection interval (min). ‡ED_{50} previously reported [6]; included for comparative purposes. §Disruption of behavior (i.e., no responding by majority of animals). ¶ED_{50} value followed by 95% confidence limits. #Pirenperone was administered 30 min prior to administration of either 0.2 mg/kg of R(−)DOB or, in the control studies, 1.0 ml/kg of saline; animals were tested 15 min later. **ED_{50} value previously reported [7]; included for comparative purposes.

tors were labeled by antagonist and agonist radioligands [8,13]. [^{3}H]Ketanserin (0.4 nM, 90.4 Ci/mmole, NEN), a 5-HT_2 receptor antagonist, was used to label these receptors in 4 mg wet weight of rat frontal cortex. The agonist high affinity state of the 5-HT_2 receptors were labeled with 0.4 nM [^{3}H]DOB (40 Ci/mmole, NEN) in 20 mg wet weight of rat frontal cortex. In both cases, nonspecific binding was determined with 1 μM cinanserin, 5-HT_1 and 5-HT_2 receptor assays were incubated at 37°C for 15 and 20 min, respectively. Incubation was terminated by rapid filtration over glass filters (Schleicher and Schuell) followed by washing with 10 ml of ice-cold assay buffer. Individual filters were equilibrated with scintillation fluid (LiquiScint) for 6 hr before counting (Beckman 3801) at an efficiency of 45%. K_i and Hill values were determined using the program EBDA [9].

Drugs

R(−)DOB, racemic DOM, and α-desMe DOB, as their hydrochloride salts, were obtained from NIDA. Pirenperone hydrochloride was a gift from Janssen Pharmaceutica (Beerse, Belgium). In the drug discrimination studies, all solutions were made fresh daily in sterile saline and all injections were via the intraperitoneal route 15 min (unless otherwise stated) prior to testing.

RESULTS

As shown in Table 1, α-desMe DOB, like DOB, possesses a high affinity for 5-HT_2 receptors; that is, removal of the α-methyl group of DOB has essentially no effect on 5-HT_2 receptor affinity. The affinity of α-desMe DOB for 5-HT_{1A}, 5-HT_{1B} and 5-HT_{1C} sites is, respectively, 10, 30, and 2 times that of DOB (Table 1). In the drug discrimination studies, the DOM-stimulus generalized to α-desMe DOB in a dose-related manner (Table2). With an injection-to-test time (presession injection interval, PSII) of 15 min, the R(−)DOB stimulus did not generalize to α-desMe DOB. However, the two animals that responded at 0.6 and 0.7 mg/kg made greater than 85% of their responses on the R(−)DOB-appropriate lever. Using a dose of 0.7 mg/kg of α-desMe DOB, the presession injection interval was varied from 5 to 60 min; at 60 min, 3 of 5 animals made 70% of their responses on the R(−)DOB-appropriate lever. As a consequence, α-desMe DOB was reevaluated using a 60-min presession injection interval. Under these conditions, the R(−)DOB stimulus generalized to α-desMe DOB; however, at the doses where stimulus generalization occurred (i.e., 0.75 and 0.8 mg/kg; Table 2), only half of the six animals responded and response rates were significantly depressed relative to control values. In the R(−)DOB-trained animals, quipazine produced saline-appropriate responding at doses of up to 0.1 mg/kg and disruption of behavior (i.e., no responding) at higher doses. Although we have previously reported that the R(−)DOB stimulus generalizes to DOM (ED_{50}=0.24 mg/kg) and that it can be antagonized by pretreatment of the animals with the 5-HT_2 antagonist pirenperone [2], no data were presented at that time; these data can be found in Table 2.

DISCUSSION

α-DesMe DOB binds with high affinity to 5-HT_2 receptors regardless of whether [^{3}H]ketanserin or [^{3}H]DOB is used as the radioligand (Table 1). In general, serotonin agonists display a higher affinity for 5-HT_2 receptors labeled by the tritiated agonist DOB than when labeled by the tritiated antagonist ketanserin, whereas serotonin antagonists display a nearly identical affinity regardless of which of the two radioligands is used to label the 5-HT_2 receptors [8]. These results would suggest that α-desMe DOB is a potential 5-HT_2 agonist. Interestingly, the affinity of α-desMe DOB for the three 5-HT_1 subpopulations of 5-HT receptors is generally higher than that of DOB (Table 1). In this regard, α-desMe DOB is less selective than DOB for 5-HT_2 receptors.

Stimulus generalization occurs to certain hallucinogenic agents and 5-HT_2 agonists in animals trained to discriminate a 5-HT_2 agonist (e.g., DOM) from saline [2]. The DOM-stimulus generalized to α-desMe DOB (Table 2); this is consistent with the results of the binding studies which suggest

TABLE 1

RESULTS OF RADIOLIGAND BINDING STUDIES*

	α-desMe DOB		DOB	
Receptor:	K_i (nM)	N_H	K_i (nM)	N_H
5-$HT1_A$	320 (±40)	0.99 (±0.01)	3770 (±120)	0.94 (±0.03)
5-$HT1_B$	25 (±5)	0.74 (±0.02)	830 (±40)	0.92 (±0.02)
5-$HT1_C$	36 (±3)	0.64 (±0.02)	70 (±15)	0.42 (±0.05)
5-HT_2 (KET)	32 (±2)	0.64 (±0.04)	40 (±5)	0.85 (±0.03)
5-HT_2 (DOB)	1 (±0.2)	0.90 (±0.13)	0.79	

*K_i values (affinity constants) and N_H (Hill coefficients) represent the mean (±SEM) of three separate experiments each performed in triplicate. The K_i value for the binding of DOB at [^{3}H]DOB-labeled sites was previously reported [6].

KET=[^{3}H]ketanserin-labeled 5-HT_2 receptors; DOB=[^{3}H]DOB-labeled receptors.

METHOD

Drug Discrimination

Two groups of rats were used in the present studies; the first group was trained to discriminate R(−)DOB from saline (n=6) and the second group was trained to discriminate racemic DOM from saline (n=5). The training of these animals was previously reported [2, 5, 6]. Briefly, male Sprague-Dawley rats (200–300 g) were trained to press both levers of standard two-lever operant chamber (Coulbourn Instruments Model E10-10) for food (sweetened powdered milk) reward. Once the task was learned, the animals were trained to discriminate intraperitoneal (IP) injections of either 0.2 mg/kg of R(−)DOB or 1.0 mg/kg racemic DOM from 1.0 ml/kg of 0.9% saline (administered 15 min prior to testing) using a variable interval 15-sec schedule of reinforcement such that the animals eventually made >80% of their responses on the drug-appropriate lever after administration of training drug and <20% of their responses on the same lever after administration of saline. The training sessions were of 15-min duration and discrimination learning was assessed, under both the drug and saline condition, once a week during a 2.5 min extinction session (followed by a 12.5-min training session). In the stimulus generalization studies (during which maintenance of the original R(−)DOB/saline or DOM/saline discrimination was insured by continuation of training throughout this portion of the studies), the animals were administered a dose of test compound 15 min prior to testing and were allowed 2.5 min to respond under extinction conditions; after this 2.5-min session, the animals were returned to their individual home cages. Animals not meeting the original training criteria were not used in the immediately subsequent generalization test session. Animals received no more than one injection of test compound during any given week. Data collected during the extinction session included total responses on the drug appropriate lever (as a percent of total responses) and response rate (mean responses per min). Animals making fewer than 5 total responses during the entire 2.5-min test session were reported as disrupted. Where stimulus generalization (i.e., >80% drug-appropriate responding) occurred, ED_{50} values (i.e., doses where the animals would be expected to make 50% of their responses on the drug-appropriate lever) were calculated by the method of Finney [1]. The stimulus antagonism studies were conducted in essentially the same manner using similar criteria. Doses of pirenperone were administered 30 min prior to 0.2 mg/kg of R(−)DOB (or, in the control studies, 15 min prior to 1.0 ml/kg of 0.9% saline) and, 15 min later, the animals were allowed 2.5 min to respond under extinction conditions.

Binding

The tissue preparation and radioligand binding studies were performed as previously described [8]. Briefly, Taconic Farms Sprague-Dawley rats (ca. 220 g) were sacrificed by decapitation and the brains were removed over ice. The frontal cortices, striata, and hippocampi were dissected and placed in ice-cold 0.9% saline until the tissues were prepared. Pig brains were obtained fresh from a local slaughterhouse. After dissection, the cortices were placed in ice-cold saline as described above. Following blot-drying and weighing, tissue samples were homogenzied in an ice-cold buffer containing 50 mM Tris HCl, 0.5 nM Na_2EDTA and 10 mM $MgSO_4$ (pH 7.7 at 25°C). Tissue homogenates were then centrifuged at 14,000 rpm for 15 min followed by resuspension in buffer and a 15-min incubation period at 37°C. The pellets were resuspended in buffer and centrifuged twice more before use. These pellets were either used immediately or were stored at −30°C until required.

Radioligand binding assays were conducted in a 2.0 ml volume to which membranes were added last. Eleven concentrations of nonradioactive drug (10^{-5} to 10^{-10} M) were made fresh daily in the assay buffer which contained 50 mM Tris HCl, 10 mM $MgSO_4$, 0.5 mM Na_2EDTA, 10 μM pargyline, and 0.1% ascorbic acid (pH 7.4 at 37°C). For competition experiments the nonradioactive drug was co-incubated with radiolabel and the membranes. 5-HT_{1A} receptors were labeled with 0.1 nM [^{3}H]8-OH DPAT (120 Ci/mmole, NEN) in 6 mg wet weight of rat hippocampus. Nonspecific binding was determined with 1 μM 8-OH DPAT. 5-HT_{1B} receptors were labeled with 2.0 nM [^{3}H]5-HT (26 Ci/mmole, NEN) in the presence of 100 nM 8-OH DPAT and 100 nM mesulergine (to block 5-HT_{1A} and 5-HT_{1C} receptors, respectively) in 8 mg wet weight of rat striatum. Nonspecific binding was determined with 10 μM 5-HT. 5-HT_{1C} receptors are labeled with 2.0 nM [^{3}H]mesulergine (85 Ci/mmole, Amersham) in the presence of 20 nM spiperone (to block 5-HT_2 receptors) in 20 mg wet weight of pig cortex. Nonspecific binding was determined with 10 μM 5-HT. The 5-HT_2 recep-

that α-desMe DOB may be a 5-HT_2 agonist. To this extent, R(−)DOB (ED_{50}=0.1 mg/kg) [7] is about seven times more potent than α-desMe DOB (ED_{50}=0.67 mg/kg). DOB binds with high affinity to 5-HT_2 receptors (Table 1). Also, the R(−)DOB-stimulus generalizes to the 5-HT_2 agonist DOM and is potently antagonized by the 5-HT_2-selective antagonist pirenperone (Table 2) suggesting that a 5-HT_2 mechanism is involved in the stimulus effects of this agent. In our initial studies (using a 15-min presession injection interval), the R(−)DOB-stimulus failed to generalize to α-desMe DOB at low doses and higher doses of α-desMe DOB resulted in disruption of behavior. Nevertheless, at these high doses, the few animals that did respond responded primarily on the R(−)DOB-appropriate lever. There are at least two possible explanations for these results: selectivity and pharmacokinetic properties. Because R(−)DOB is somewhat more selective than DOM for 5-HT_2 receptors, the R(−)DOB stimulus might be more selective than the DOM stimulus [2]. Secondly, because α-desMe DOB is less protected than DOB to oxidative deamination in vivo, and because phenethylamines usually do not penetrate the blood-brain barrier as readily as α-methyl phenethylamines (i.e., phenylisopropylamines), distribution might be a problem. Stimulus generalization occurred in the DOM-trained animals, but not in the R(−)DOB-trained animals, even though the time course of effect of DOM and R(−)DOB are quite similar [2]. Although the pharmacokinetic properties of α-desMe DOB are unknown, and though they may certainly contribute to the observed effects, it would seem unlikely, then, that they can, by themselves, account for the present results. On the other hand, the R(−)DOB-stimulus, unlike the DOM-stimulus, did not generalize to the less selective 5-HT_2 agonist quipazine. These results, coupled with the binding data presented in Table 1, would suggest that α-desMe DOB may produce stimulus effects that are less selective than those produced by R(−)DOB.

Thus, we must tentatively conclude that α-desMe DOB is capable of producing stimulus effects that are similar, but not identical, to those of DOM and R(−)DOB, and, due to the decrease in response rates and/or disruption of behavior seen in the stimulus generalization studies, that α-desMe DOB may also be capable of producing other, as yet undefined, central effects at approximately the same doses. These other effects may reflect the different binding profile that is observed for R(−)DOB as compared with α-desMe DOB or may involve pharmacokinetic factors. The present results are not, however, inconsistent with the possibility that α-desMe DOB may be psychoactive agent with abuse potential.

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