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5-HT_3 Agonist 2-Methylserotonin as a Training Drug in Drug Discrimination Studies

RICHARD A. GLENNON,[1] RICHARD YOUNG AND MALGORZATA DUKAT

Department of Medicinal Chemistry, School of Pharmacy Medical College of Virginia, Virginia Commonwealth University, Richmond, VA 23298-0540

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GLENNON, R. A., R. YOUNG AND M. DUKAT. *5-HT_3 agonist 2-methylserotonin as a training drug in drug discrimination studies.* PHARMACOL BIOCHEM BEHAV **41**(2) 361-364, 1992.—Using a standard two-lever operant procedure, rats were trained to discriminate 5 mg/kg of the 5-HT_3 agonist 2-methylserotonin (2-Me 5-HT; ED_{50} = 2.6 mg/kg) from saline using a VI 15-s schedule of reinforcement. The 2-Me 5-HT stimulus did not generalize to the 5-HT_1/5-HT_2 agonist 5-methoxy-*N*,*N*-dimethyltryptamine, but did generalize to the new 5-HT_3 agonist 1-(*m*-chlorophenyl)biguanide (ED_{50} = 1.6 mg kg). The 5-HT_3 antagonist ICS 205-930 potently antagonized the 2-Me 5-HT stimulus (ID_{50} = 0.001 mg/kg), whereas its quaternary amine analog, which does not readily penetrate the blood-brain barrier, failed to completely antagonize the 2-Me 5-HT stimulus at a 10,000-fold higher dose. The results of the present investigation show that 2-Me 5-HT serves as a discriminative stimulus in rats when paired with saline and suggest that its stimulus properties are likely mediated via a central 5-HT_3 mechanism. As such, this is the first demonstration that a 5-HT_3 agonist can be used as a training drug in drug discrimination studies.

2-Methylserotonin 5-HT_3 Drug discrimination ICS 205-930 Q-ICS 205-930
1-(*m*-Chlorophenyl)biguanide

CENTRAL serotonin (5-HT) receptors have been divided into several major populations: 5-HT_1, 5-HT_2, and 5-HT_3. 5-HT_3 receptors are distinct from other 5-HT receptors (G-protein coupled) in that they are ligand-gated ion channel receptors (14). Until relatively recently, it was uncertain whether 5-HT_3 receptors existed in the central nervous system; however, the results of numerous radioligand binding studies and other pharmacological investigations have provided ample supporting evidence that they do [see Kilpatrick et al. (14) and Costall et al. (4) for recent reviews]. 5-HT_3 receptors have attracted widespread interest lately because of their potential involvement in chemotherapy-induced emesis, migraine, and various mental disorders [e.g., (4,14)]. It also seems that 5-HT_3 antagonists might be useful in relieving symptoms associated with withdrawal from various drugs of abuse (4). To date, there are relatively few agents that might be considered truly selective for one population of 5-HT receptors vs. another (11); nevertheless, the drug discrimination paradigm has proven highly successful for the investigation and classification of such agents. Various 5-HT_1 and 5-HT_2 agonists have been used as training drugs in drug discrimination studies [see (10) for a recent review], and the most widely used family of 5-HT_2 agonists, that is, 1-(2,5-dimethoxy-4*X*-phenyl)-2-aminopropanes (such as DOM, DOB, and DOI where *X* = methyl, bromo, and iodo, respectively), was first identified on the basis of their discriminative stimulus properties (10,12). Missing from the list of serotonergic agents that have served as training drugs are examples of 5-HT_3 agonists. Perhaps one reason for this is a lack of 5-HT_3-selective agonists. The most widely used 5-HT_3-selective agonist is 2-methylserotonin (2-Me 5-HT) (4,14). In various isolated tissue and other pharmacologic procedures, 2-Me 5-HT has been shown to be an effective 5-HT_3 agonist with a potency slightly less than that of the nonselective 5-HT (1,8,14).

Most training drugs are generally thought to produce their stimulus effects via a central mechanism (3). Although 5-HT does not readily penetrate the blood-brain barrier, there is some evidence that rats can be trained to discriminate 5-HT from vehicle; Colpaert (personal communication) found that acquisition of a 5-HT stimulus was difficult and that the animals' performance was unreliable. It was hoped that the presence of the 2-methyl group of 2-Me 5-HT would sufficiently enhance lipid solubility just enough that it might more readily penetrate the blood-brain barrier and serve as a training drug. Consequently, we attempted to train a group of rats to discriminate the 5-HT_3 agonist 2-Me 5-HT from vehicle in a standard two-lever operant procedure. We demonstrate in the present study that rats can be trained to discriminate 5 mg/kg

[1] Requests for reprints should be addressed to Richard A. Glennon, Department of Medicinal Chemistry, School of Pharmacy, Medical College of Virginia, Virginia Commonwealth University, Richmond, VA 23298-0540.

2-Me 5-HT from saline and provide evidence that the stimulus effects of 2-Me 5-HT may be centrally mediated and involve a 5-HT_3 mechanism.

METHOD

Animals used in this study were 10 male Sprague-Dawley rats maintained at approximately 80% of their free-feeding body weight by partial food deprivation. Behavioral testing was conducted in standard two-lever operant chambers (Coulbourn Instruments model E10-10) housed within light- and sound-attenuating outer chambers. All rats were trained to respond on both levers for sweetened milk under a variable interval 15-s schedule of reinforcement. Once lever pressing was established, animals were trained to respond on one lever when administered 0.9% sterile saline (1 ml/kg) and on the other lever when administered 2-Me 5-HT by reinforcing the appropriate responses. A presession injection interval of 15 min was employed and training sessions were of 15 min duration. For half the animals, the right lever was the drug-appropriate lever, whereas the situation was reversed for the other half. Saline or 2-Me 5-HT was administered on a double-alternation schedule (i.e., 2 days saline, 2 days drug). On every fifth day, discrimination learning was assessed during an initial 2.5-min nonreinforced (extinction) session. Data collected during this period included total responses made on each lever and response rate (i.e., mean responses per min). The extinction session was followed by a 12.5-min training session. Over 7 months of training, animals did not learn to make their responses on the appropriate lever after administration of 1.0, 2.0, or 3.0 mg/kg 2-Me 5-HT. The final training dose of 2-Me 5-HT was 5 mg/kg. After 5 months of training sessions, animals consistently made >80% of their total responses on the drug-appropriate lever after administration of 5.0 mg/kg 2-Me 5-HT and <20% of their responses on the same lever after administration of saline.

Stimulus Generalization Studies

Maintenance of the 2-Me 5-HT/saline discrimination was ensured in all animals by continuing the training sessions throughout this portion of the study. Training sessions were conducted on the 4 days prior to a stimulus generalization test session. On one of these days, half the animals received 5 mg/kg 2-Me 5-HT and the other half received saline; after a 2.5-min extinction period, training was continued for an additional 12.5 min as described above. Animals not meeting the above (i.e., 80–20%) criteria were not used in that week's stimulus generalization test. The stimulus generalization test session was conducted every fifth day by administration of a challenge drug or a dose of 2-Me 5-HT(<5 mg/kg); animals were allowed 2.5 min to respond under nonreinforced conditions and were then returned to their individual home cages. Doses of these agents were administered in a random sequence 15-min prior to testing. Stimulus generalization was said to have occurred when the animals made greater than 80% of their responses on the drug-appropriate lever. Animals making less than five total responses during the entire 2.5-min extinction session were reported as being disrupted. Where generalization occurred, ED_{50} values were calculated by the method of Finney (6).

Stimulus Antagonism Studies

Stimulus antagonism studies were conducted as described for the stimulus generalization studies except that doses of potential antagonists were administered 15 min prior to an injection of 5 mg/kg 2-Me 5-HT; 15 min later, animals were placed in the operant chamber for a 2.5-min extinction session.

Drugs

5-Methoxy-*N*,*N*-dimethyltryptamine hydrogen oxalate (5-OMe DMT) and 2-methylserotonin maleate (2-Me 5-HT) were synthesized in our laboratory following a literature procedure (13). 1-(*m*-Chlorophenyl)biguanide hydrochloride was synthesized according to the general method of Curd and Rose (5); the melting point was 194–195°C after recrystallization from 95% ethanol [literature (16) melting point: 195–196°C]. ICS 205-930 (3-tropanyl indole-3-carboxylate) was purchased from Research Biochemicals (Natick, MA); its quaternary salt 3-tropanyl indole-3-carboxylate methiodide (Q-ICS 205-930) was obtained as a gift from the same source. All solutions were made fresh daily in 0.9% saline and all injections were via the intraperitoneal route.

RESULTS AND DISCUSSION

The investigation began with 10 animals and a 2-Me 5-HT training dose of 1 mg/kg. Over a period of about 7 months, the training dose was gradually increased from 1 to 5 mg/kg. During this time, four animals died (to causes most likely unrelated to the training drug). After an additional 5 months, four animals were reliably trained to discriminate 5 mg/kg 2-Me 5-HT from saline. Two additional animals did not learn to consistently respond on the drug-appropriate lever after administration of 5.0 mg/kg 2-Me 5-HT.

Administration of 2-Me 5-HT doses lower than that of the training dose elicited decreased percent responding on the drug-appropriate lever (Table 1); response rates were not different from those observed under control conditions (i.e., 1 ml/kg 0.9% saline or 5 mg/kg 2-Me 5-HT). 5-OMe DMT is considered a high-affinity, nonselective 5-HT agonist; however, although not required for affinity (13), a free hydroxyl group seems to be necessary for 5-HT_3 agonist activity and tryptamine analogs without this hydroxyl group lack activity as 5-HT_3 agonists [for a review of structure-activity relationships, see Richardson and Buchheit (18)]. Due to its nonselective nature, stimulus generalization occurs between 5-OMe DMT and numerous other serotonergic agents regardless of which is used as training drug (10). Due to its lack of 5-HT_3 agonist properties, stimulus generalization would not be expected to occur in the present study if the 2-Me 5-HT stimulus is 5-HT_3 mediated. On the other hand, stimulus generalization might be observed if the 2-Me 5-HT stimulus is mediated by some other population(s) of 5-HT receptors. The results in Table 1 show that 5-OMe DMT produces saline-appropriate responding at doses of up to 0.75 mg/kg and disruption of behavior at 1 mg/kg [i.e., a dose that has been demonstrated to produce stimulus effects of its own (10)]. While these studies were in progress, Kilpatrick et al. (15) reported that 1-(*m*-chlorophenyl)biguanide (mCPB) constitutes a new 5-HT_3-selective agent that behaves as an agonist both in vitro and in vivo. In the present investigation, it is shown that the 2-Me 5-HT-stimulus generalizes to mCPB in a dose-related manner (ED_{50} = 1.6 mg/kg relative to 2.6 mg/kg for 2-Me 5-HT).

In contrast to the lack of 5-HT_3 agonists, several antagonists are available (9,14); one of the most widely used 5-HT_3 antagonists is ICS 205-930. 5-HT_3 antagonists are typically characterized by their extreme potency, and sub-mg/kg doses are usually sufficient to antagonize 5-HT_3-mediated effects in animals [e.g., (2,7,14)] and in humans [e.g., (20)]. Table 1 shows that ICS 205-930 is a potent antagonist (ID_{50} = 0.001

TABLE 1
RESULTS OF DRUG DISCRIMINATION STUDIES WITH 2-Me 5-HT AS TRAINING DRUG

Agent	Dose (mg/kg)	Na*	Drug-Appropriate Responding†	Responses/min‡
2-Me 5 HT	1.25	4/4	19% (4)	12.7 (1.8)
	2.50	4/4	37% (13)	11.3 (4.5)
	3.75	4/4	52% (22)	11.8 (2.7)
	4.25	4/4	78% (12)	10.9 (1.3)
	5.00	4/4	93% (5)	13.4 (1.9)
		ED_{50} = 2.6 (1.6–4.2)§ mg/kg		
Saline (1ml/kg)		4/4	12% (2)	13.3 (1.9)
mCPBG	0.25	4/4	0%	12.1 (1.5)
	1.0	4/4	29% (9)	12.6 (1.9)
	1.5	4/4	54% (17)	15.7 (3.7)
	2.5	4/4	68% (13)	13.5 (2.7)
	5.0	4/4	88% (7)	9.5 (1.1)
		ED_{50} = 1.6 (0.7–3.4)§ mg/kg		
5-OMe-DMT	0.5	3/3	12% (7)	10.9 (1.3)
	0.75	2/3	18% (7)	5.3 (1.2)
	1.0	0/3	¶	
ICS 205-930#	0.0001	3/3	92% (4)	13.1 (1.8)
	0.0005	3/3	66% (12)	12.7 (1.2)
	0.001	3/3	31% (7)	12.1 (1.0)
	0.01	3/3	25% (4)	11.8 (1.2)
	0.1	3/3	24% (8)	12.7 (1.3)
		ID_{50} = 0.001 (0.0002–0.009)§ mg/kg		
Q-ICS 205-930#	0.1	4/4	87% (5)	12.9 (1.7)
	1.0	4/4	66% (14)	13.6 (1.0)
	10	4/4	62% (11)	11.7 (1.8)

*Number of animals responding/number receiving drug.

†Percent (of total) responses on the drug-appropriate lever. Data were collected during a 2.5-min extinction session and ± SEM are given in parentheses.

‡Mean responses per min. Data were collected during a 2.5-min extinction session and ± SEM are given in parentheses.

§ED_{50} value followed by 95% confidence limits in parentheses.

¶Disruption of behavior; none of the animals made more than a total of five responses during the 2.5-min extinction session.

#Drug was administered 15 min prior to administration of 2-Me 5-HT (5 mg/kg).

mg/kg) of the 2-Me 5-HT stimulus when administered in combination with the training dose of 2-Me 5-HT. The quaternary amine analog of ICS 205-930 (i.e., Q-ICS 205-930) is an example of a high-affinity 5-HT_3 antagonist [e.g., (17,21)] that does not readily penetrate the blood-brain barrier. In some studies, Q-ICS 205-930 binds with several times the affinity of the parent compound (19). As shown in Table 1, 10 mg/kg Q-ICS 205-930 (a dose 10,000 times the ID_{50} dose of ICS 205-930) failed to completely block the 2-Me 5-HT stimulus. To obtain a direct dose comparison, we evaluated the effect of 0.1 mg/kg Q-ICS 205-930 (a dose not included in the original stimulus antagonism study) and 0.1 mg/kg Q-ICS 205-930 in combination with the training dose of 2-Me 5-HT. It can be seen (Table 1) that the quaternary amine is without effect on percent drug-appropriate responding or response rate, whereas the parent ICS 205-930 reduces the effect of 2-Me 5-HT to essentially saline-like levels. These results suggest that the stimulus effects of 2-Me 5-HT are probably central in origin. The small degree of antagonism noted with the quaternary antagonist might indicate that the 2-Me 5-HT stimulus involves a minor peripheral component or, more likely, that at the extraordinarily high doses employed a small amount of quaternary amine is penetrating the blood-brain barrier.

In summary, at a dose of 5 mg/kg 2-Me 5-HT serves as a training drug in rats. The 2-Me 5-HT stimulus does not generalize to the 5-HT_1/5-HT_2 agonist 5-OMe DMT but does generalize to the new 5-HT_3-selective agonist mCPB. The stimulus effects of 2-Me 5-HT are potently antagonized by the 5-HT_3 antagonist ICS 205-930 but are not completely antagonized by its quaternary amine analog, an agent that does not penetrate the blood-brain barrier. As in other pharmacological assays, ICS 205-930 exhibits extreme potency and is, in fact, one of the most potent agents ever examined in a drug discrimination procedure. The use of the drug discrimination paradigm with animals trained to discriminate 2-Me 5-HT might constitute a novel method for the identification and characterization of new 5-HT_3 agonists and antagonists with potential therapeutic benefit. Now that an effective training dose has been established, it should be possible to train animals more quickly than demonstrated in the present study; furthermore, it is entirely possible that significantly lower doses of training drug can be used if it is administered by intracerebroventricular injection.

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