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BRIEF COMMUNICATION

CGS 10746B is Able to Attenuate the Effects of Amphetamine: Further Evidence for Dopaminergic Mediation

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SCHECHTER, M. D. AND J. W. BOJA. *CGS 10746B is able to attenuate the effects of amphetamine: Further evidence for dopaminergic mediation.* PHARMACOL BIOCHEM BEHAV **30**(4) 1089–1092, 1988.—Previous results indicate that agents which either decrease synthesis or block postsynaptic dopamine receptors will attenuate the discriminative stimulus produced by *d*-amphetamine. CGS 10746B has been reported to decrease dopamine release without changing its metabolism or occupying its receptors. In the present study, rats successfully trained to discriminate intraperitoneally administered (0.8 mg/kg) *d*-amphetamine in a two-lever, food-motivated operant task were observed to be unable to discriminate amphetamine when pretreated with 30 mg/kg CGS 10746B. This antagonism was shown to be dose-responsive and constitutes a third mechanism, i.e., dopamine release inhibition, that evidences the dopaminergic mediation of amphetamine in the discriminative paradigm. When both cathinone (0.8 mg/kg) and cocaine (10.0 mg/kg) were administered to the amphetamine-trained rats they each were recognized as amphetamine and are, thus, considered to generalize to the amphetamine discriminative stimulus. Coadministration of CGS 10746B and cathinone totally antagonized this generalization, whereas pretreatment with CGS 10746B prior to cocaine significantly reduced cocaine's effects. These results implicate dopamine mechanisms in the discriminative stimulus properties of the psychostimulants amphetamine, cathinone and cocaine.

Drug discrimination Amphetamine Cathinone CGS 10746B Cocaine Dopamine Apomorphine Rats

A great deal of scientific evidence exists suggesting that the mechanism of *d*-amphetamine action, which it is capable of controlling discriminative stimulus behavior in the rat, is centrally-mediated through dopaminergic neurons. A recent extensive review on this subject [7] highlights specific experimentation which indicates that if dopaminergic function is blocked at one of various sites, the discriminative stimulus cue produced after amphetamine administration is attenuated in the rat. Thus, if α-methylparatyrosine which decreases catecholamine production, or if any one of numerous (antipsychotic) drugs that block dopamine postsynaptic receptors, are administered prior to amphetamine the ability of rats to discriminate the effects of amphetamine is effectively abolished. This excellent review [7] also lists 14 drugs that, when given to rats trained to discriminate *d*-amphetamine from its vehicle, will produce an amphetamine-like discriminative effect and, thus, can be considered to generalize to, or transfer from, amphetamine. This list includes apomorphine, cocaine and cathinone; drugs evidenced to have at least a partial effect upon dopaminergic neurons.

CGS 10746B is a recently synthesized benzothiadiazepine which has been reported to decrease dopamine release without either changing dopamine metabolism or occupying dopaminergic receptors [1]. Employing this novel agent allows for a third mechanism, i.e., one differing from inhibition of catecholamine biosynthesis or postsynaptic dopaminergic blockade, to be tested in rats who can discriminate amphetamine. If CGS 10746B does antagonize a rat's ability to differentially respond to amphetamine in a discriminative task, it would be additional evidence as to the dopaminergic mediation of the amphetamine interoceptive cue. In addition, this compound can be used to investigate the dopaminergic mediation of some of those drugs seen to generalize to amphetamine.

METHOD

The eight male rats, as well as the operant experimental apparatus and the procedure used to train them to discriminate 0.8 mg/kg *d*-amphetamine sulfate (as base) from its vehicle (distilled water), have all been detailed in a previous publication [5]. Briefly, the food-deprived rats were trained to press one lever in a two-lever operant chamber for a food reward (45 mg Noyes pellet) following the injection of 0.8 mg/kg *d*-amphetamine and to press the other lever following administration of its vehicle. All injections were made interperitoneally (IP) and training took place 20 min after injection. The rats were required to select the appropriate lever, according to the drug or nondrug state imposed, in 8 of 10 consecutive sessions, twice.

Dose-Response and Generalization Testing

Once this training criterion was achieved by all rats, various doses of amphetamine that differed from the training dose were administered and a dose-response relationship was determined. During this series of experiments, the maintenance of the amphetamine-vehicle discrimination was assured by administering and testing either 0.8 mg/kg amphetamine or vehicle on every second day. Testing with other doses of amphetamine, as well as with other drugs, occurred on interspersed days according to the following schedule: A-T1-V-T2-V-T1, etc., where A=0.8 mg/kg *d*-amphetamine, V=vehicle, T1=one other dose of amphetamine (dose-response) or another drug (generalizations) and T2=second dose of amphetamine or other drug. All doses/drugs were administered IP and tested 20 min after injection with the rat being immediately removed from the experimental chamber, without receiving reinforcement, upon making 10 responses on either of the two levers. This was done so as to preclude reinforcement and/or training at an amphetamine dose or other drug state than that to which the animals were trained. The lever pressed 10 times was designated as the "selected" lever and all amphetamine test doses, and other drugs, were administered in a random order on two occasions with each test session preceded by one 0.8 mg/kg amphetamine session and one vehicle session. The doses of drugs used in generalized test were chosen from the drug discrimination literature.

Antagonism with CGS 10746B

CGS 10746B was administered 10 min prior to the adminstration of either vehicle or 0.8 mg/kg amphetamine and the animals were tested 20 min following the second injection. Thus, the time interval between injection and testing (20 min) was kept constant, whereas the CGS 10746B was employed at a time prior to testing (30 min) similar to that previously shown to produce maximal effects upon dopamine release [1]. As previously, upon making 10 responses the animals were immediately removed and returned to their home cage without receiving reinforcement. Doses of CGS 10746B used were 10, 20, 30 and 40 mg/kg and each of these doses was administered prior to saline, whereas all but 40 mg/kg were coadministered with amphetamine. Once the maximum inhibitory dose of CGS 10746B was found (30 mg/kg) in these experiments with amphetamine, the same dose was administered 10 min prior to the administration of the maximum generalizing dose of cocaine and cathinone. Rats were then placed into the experimental chamber 20 min after the second injection and removed upon pressing either lever 10 times.

TABLE 1

EFFECT OF COCAINE, APOMORPHINE AND CATHINONE IN RATS (n=8) TRAINED TO DISCRIMINATE 0.8 mg/kg *d*-AMPHETAMINE

Treatment	(No. Trials)	Dose (mg/kg)	Quantal	Quantitative (SD)
d-Amphetamine	(24)	0.8	100.0	98.0 (5.6)
Vehicle	(25)	—	2.0	5.9 (4.8)
Cocaine	(2)	10.0	93.8	92.2 (11.1)
	(2)	5.0	75.0	76.3 (16.8)
	(2)	2.5	31.3	38.8 (26.3)
	(2)	1.25	18.8	24.9 (16.8)
Apomorphine	(2)	0.64	50.0	49.6 (0.6)
	(2)	0.32	43.8	43.0 (21.4)
	(2)	0.24	31.3	35.0 (21.9)
	(2)	0.16	18.8	32.5 (0.7)
Cathinone	(2)	0.8	87.5	7.0 (0.7)

Measurements and Statistical Analysis

The percentage of rats selecting the lever appropriate for amphetamine was the quantal measure of discrimination and is presented as percentage of rats making the correct first-choice selection on the amphetamine-correct lever (an all-or-none-effect).The dose response measurements with amphetamine, and any drug that generalized to it, were subjected to analysis by the procedure of Litchfield and Wilcoxon [4] that employs log dose vs. probit measurements and allows for the generation of ED_{50}'s and testing of parallelism between dose-response curves.

The quantitative measurements used represents the total number of lever presses on both levers made prior to completion of 10 presses on either lever, i.e., the number of presses on the amphetamine-correct lever divided by the total responses made (including the 10 on the amphetamine lever) times 100. This measurement was included in order to be able to analyze data on both levers and to be able to incorporate responses upon the "unselected" lever in the statistical analysis. Quantitative measurements were compared with Student's *t*-test with $p<0.05$ set as the level of significance.

RESULTS

As previously indicated [2, 3, 5–7] rats readily learned to discriminate between (0.8 mg/kg) *d*-amphetamine and saline and maintained this discrimination throughout continued testing. Indeed, this dose of amphetamine allowed for 100% of first-choice responses to be on the amphetamine-correct lever, whereas the vehicle produced 98% correct responding on the vehicle-appropriate lever (Table 1). The administration of 10 mg/kg cocaine produced 93.8% correct responding on the amphetamine-lever and decreasing doses of 5, 2.5 and 1.25 mg/kg produced decreasing discriminative performance. Analysis of this dose-response curve indicates an ED_{50} of 2.97 mg/kg for the quantal measurements and a similar 2.68 mg/kg for the quantitative measurements. The administration of four doses of apomorphine (0.16–0.64 mg/kg) produced a maximum of 50% of responses on the amphetamine-appropriate lever and, although there was an increasing

TABLE 2

COADMINISTRATION OF CGS 10746B WITH VEHICLE, AMPHETAMINE, COCAINE AND CATHINONE

Dose CGS 10746B (mg/kg) +	Vehicle	Amphetamine (0.8 mg/kg)	Cocaine (10.0 mg/kg)	Cathinone (0.8 mg/kg)
10	0.0;4.8 (1.6)*	75.0;75.2 (13.8)		
20	12.5;13.2 (13.6)	43.8;49.2 (34.7)		
30	0.0; 5.4 (0.8)	6.3;18.6 (12.0)	37.5;36.2 (2.6)†[a]	0.0;11.4 (6.2)‡
40	18.8;21.5 (7.0)		31.3;39.3 (8.5)†[b]	

*Quantal Measurement=percentage of rats (n=8) that selected the amphetamine-correct lever; quantitative measurements (±SD)=number of amphetamine lever responses/total number of responses prior to 10 responses on either lever ×100, calculated across all rats in any given test session. Coadministrations were conducted with CGS 10746B being injected IP 30 min before testing and the second treatment 20 min prior to testing on two occasions.

†Significant difference (Student's t-test, two-tailed) from quantitative measurement (92.2 ± 11.1) after 10 mg/kg cocaine alone (Table 1) at the [a]$p<0.01$ and [b]$p<0.02$ level.

‡Significant difference (Student t-test; two-tailed) from quantitative measurement (87.0 ± 0.7) after 0.8 mg/kg cathinone alone (Table 1) at the $p<0.002$ level.

trend toward more amphetamine-appropriate responding with the highest-doses, the appearance of behavioral disruption at the highest dose precluded attempts at higher doses. A dose of cathinone (0.8 mg/kg) that had previously been shown [7] to produce generalization in amphetamine-trained animals was observed to elicit 87.5% of quantal responses made on the amphetamine-correct lever.

Pretreatment of animals with CGS 10746B prior to administration of the vehicle produced essentially vehicle-like responding, whereas, the ability of CGS 10746B to antagonize the amphetamine discriminative cue is shown to be dose-responsive in Table 2. Increasing doses of CGS 10746B produced a progressive decrease in the rats' ability to discriminate the training dose of amphetamine. Thus, 10 mg/kg of CGS 10746B decreased the errorless discrimination to 75%, 20 mg/kg decreased it further to 43.8% and the 30 mg/kg dose of CGS 10746B reduced amphetamine discrimination to 6.3%. The IC_{50}, defined as the dose of antagonist to produce a 50% reduction in effect, was calculated to be 16.8 mg/kg.

The effect of pretreatment with 30 mg/kg of CGS 10746B upon the generalizing doses of (10 mg/kg) cocaine and (0.8 mg/kg) cathinone are also presented in Table 2. This dose of the dopamine release inhibitor completely antagonized the cathinone generalization, whereas it significantly reduced that produced by 10 mg/kg cocaine. To indicate if a higher dose of the antagonist would more ably attenuate the cocaine generalization, 40 mg/kg CGS 10746B was administered prior to cocaine and the quantal responses decreased further to 31.3%, whereas the quantitative measurement actually increased. Higher doses of this combination were precluded by the appearance of very long delays between placement into the experimental chamber and initiation of leverpressing.

DISCUSSION

Amphetamine is readily capable of controlling differential responses in a drug discrimination paradigm as recently reviewed [7]. This discriminative stimulus performance can be produced when drugs which work by similar mechanisms are administered to animals trained to discriminate amphetamine and in the present work, both cocaine and cathinone were (once again) shown able to generalize. In contrast, apomorphine, a postsynaptic dopaminergic agonist, was only able to produce a maximum of 50% of amphetamine-like responding.

These observations simply replicate that which is already in the literature. What is a novel observation is that the agent CGS 10746B, which has been reported to decrease dopamine release without either changing its metabolism or affecting its receptors [1], was capable of attenuating the amphetamine-induced interoceptive cue. The observation, thus, becomes the third site/mechanism by which drugs that effect dopaminergic function have been shown to abolish amphetamine's discriminative effects; α-methylparatyrosine being used to decrease the synthesis of dopamine and dopaminergic receptor blockers employed to decrease amphetamine's action [7].

In addition to attenuating the amphetamine stimulus, CGS 10746B was also capable of antagonizing the generalization to two other stimulants, cathinone and cocaine. Cathinone is the active component of Khat, a plant that is widely abused in certain areas of the world outside of the United States, whereas cocaine is abused worldwide. When the ED_{50} of cocaine derived from the generalization data [4] in the present study (2.97 mg/kg for the quantal data) is compared to the ED_{50} of mg/kg with amphetamine previously generated (0.31 mg/kg; [5]), the potency ratio would indicate that amphetamine is 9.6 times as potent as cocaine in animals trained to discriminate 0.8 mg/kg amphetamine. This relative potency is midway between that reported in previous work [2,3]. Although the ability of animals trained to discriminate d-amphetamine has only sometimes been reported to generalize to the direct dopaminergic agonist apomorphine [7], Stolerman and D'Mello [6] showed that apomorphine only produced drug-appropriate responding in animals trained with higher doses of amphetamine. This observation explains the current results that indicate an intermediate effect of apomorphine.

The present observation that a dopamine release inhibitor only partially affects the cocaine generalization in amphetamine-trained animals can best be explained by the possibility that the dopaminergic component of the discriminative stimulus cue produced by cocaine is not as large as it is for amphetamine and cathinone. This suggestion would, furthermore, indicate that cocaine's discriminative stimulus effects are more complex and multidimensional than that seen with amphetamine, e.g., cocaine's effects on noradrenergic systems, as well as its local anesthetic properties, may be small but definite facets of its stimulus cue.

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