Pharmacology Biochemistry and Behavior, Vol. 41, pp. 807–812, 1992

0091-3057/92 $5.00 + .00

Further Evidence for the Mechanisms That May Mediate Nicotine Discrimination

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Received 26 July 1991

SCHECHTER, M. D. AND S. M. MEEHAN. *Further evidence for the mechanisms that may mediate nicotine discrimination.* PHARMACOL BIOCHEM BEHAV **41**(4) 807–812, 1992.—Rats were trained to discriminate the interoceptive stimuli produced by subcutaneously administered 0.4 mg/kg nicotine in a two-lever, food-motivated, operant task. Once criterion performance was attained, dose-response experiments indicated an ED_{50} value of 0.1 mg/kg and subsequent time course experiments showed a maximal effect between 10 and 30 min postadministration with a return to saline-like responding at 2 h. Pretreatment with the presynaptic dopamine release inhibitors CGS 10746B (30 mg/kg), as well as with the dihydropyridine calcium blocker isradipine (15 mg/kg), each produced a significant blockade of nicotine discrimination. In contrast, the 5-hydroxytryptamine (5-HT) receptor 5-HT_3 antagonist ICS-205930 did not produce any effect upon nicotine discrimination. Thus, drugs that interfere with calcium influx, viz., isradipine, or with dopamine release (CGS 10746B) also interfere with nicotine discrimination and these results suggest that calcium influx and dopamine release may be necessary conditions for nicotine discrimination.

Nicotine CGS 10746B Stimulus properties of drugs Isradipine 5-HT_3 receptors Dopamine Rats

NICOTINE is capable of attaining stimulus control of operant conditioning so that rats can be trained to make one response in the presence of the drug and a second response after administration of its vehicle. In the context of this nicotine-vehicle discrimination, a novel drug with actions that may be similar or dissimilar to nicotine can be administered to test if nicotine-like response can be elicited: an agonism study. In addition, a drug may be administered as pretreatment prior to nicotine tests of discrimination and this cotreatment can be observed as to its effect upon nicotine discrimination: an antagonism study. In 1971, one of us (M.D.S.) observed that pretreatment with the brain-accessible ganglionic blocker mecamylamine decreased the ability of rats to discriminate a peripheral dose of nicotine, whereas the quaternary, brain-nonaccessible, drug hexamethonium had no such effect (28). Subsequently, other centrally accessible nicotine antagonists, viz., pempidine (22) and chlorisondamine (9), were also shown to block the nicotine-discriminative stimulus. Over the last 20 years, there have been many drugs that have been tried as pretreatment antagonists to nicotine discrimination with only minimal success (29).

Recent receptor-binding investigations led to the identification of specific ligands for subpopulations of neurochemical receptors, and some of these ligands have been shown to be effective in altering nicotine-induced behavior. For example, the 5-hydroxytryptamine-3 (5-HT_3) antagonists ICS-205930 and MDL 72222 have each been shown to decrease nicotine-conditioned place preference when administered at a dose of 0.03 mg/kg subcutaneously (3).

Reactions of calcium channel blockers upon various stimulants have also been the focus of recent work. One of these, nimodipine, was shown to partially block the discriminative stimulus properties of cocaine (21) and amphetamine (18). It is, therefore, of interest to investigate if the calcium channel blocker isradipine, which at 2.5 mg/kg had previously prevented cocaine-induced dopamine release and motor stimulation (19), would have an effect upon nicotine-controlled discriminative behavior.

Lastly, nicotine has been shown to stimulate dopamine release in in vivo preparations (10,13,15). The ability of the compound CGS 10746B, an atypical antipsychotic agent that reduces the release of dopamine without affecting postsynaptic receptors (1), to alter nicotine-discriminative performance would allow investigation of the dopaminergic mediation of the nicotine-induced discriminative stimulus.

METHOD

Subjects

Ten male Sprague-Dawley rats, purchased from Zivic-Miller Laboratories (Allison Park, PA), weighing 230–250 g at the beginning of the experiments were individually housed

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in a colony room maintained on a 12 L:12 D (0600–1800) cycle and kept at a constant temperature and humidity. Rats were given water ad lib in their home cages in addition to daily rationing of commercial rat chow to maintain them at 85–90% of their free-feeding weights as determined by the growth chart from the supplier.

Apparatus

Twelve standard rodent operant chambers (Lafayette Instruments Corp., Lafayette, IN), each containing two identical levers situated 7 cm apart and 7 cm above a metal grid floor, were used. Equidistant between the levers was a food receptacle that received delivery of 45 mg Noyes food pellets. Each operant chamber was enclosed in a sound-attenuating cubicle with an exhaust fan. Solid-state programming equipment (Med Associates, E. Fairfield, VT) was located in an adjacent room and used to control and record discrimination sessions.

Lever Pressing and Discrimination Training

The food-deprived rats were trained to press one lever under the drug condition and the second lever in the nondrugged (distilled water vehicle) state. Training sessions were conducted once a day, 5 days a week, with one lever in each chamber designated as the "nicotine lever" and the second lever designated as the "vehicle lever." For half the group, the nicotine lever was to the right of the food receptacle, whereas for the other five animals it was to the left. Initially, all animals were trained to respond on the vehicle lever 10 min after SC administration of 1 ml/kg water on a fixed-ratio (FR) schedule of 1, that is, one response resulted in one reinforcement. During 10 consecutive training sessions, the FR schedule was gradually incremented to an FR 10, in which 10 responses on the vehicle lever yielded 1 food reinforcement. The animal was removed from the operant chamber and returned to its home cage after receiving 40 reinforcements on each FR schedule.

Once an FR10 was established on the vehicle lever, training began on the opposite lever (10 min) following the injection (SC) of an equal volume (1 ml/kg) of vehicle containing 0.4 mg/ml nicotine (as the tartrate salt and calculated as base). Rats were subsequently only rewarded for responses upon the nicotine lever and, as with previous vehicle training, the initial reinforcement schedule of FR1 was gradually increased to FR10; this was accomplished over a period of seven daily sessions.

Rats were considered trained to lever press once FR10 responding was established on both levers. Discrimination training began 10 min after the daily administration of either vehicle or 0.4 mg/kg nicotine and rats received vehicle (V) or nicotine (N) according to the following 2-week, repeating, injection schedule: N,V,V,N,N; V,N,N,V,V. The first lever upon which 10 responses were accumulated at the beginning of each session was considered the "selected" lever for that daily session. At the time of the tenth response, presses on both the selected and nonselected lever were recorded. The session was continued, regardless of the correctness of the selected lever, until 400 responses were made on the correct lever for that session and, therefore, 40 reinforcements (on the FR10 schedule) were received. The 0.4-mg/kg training dose of nicotine and vehicle training continued for 5 weeks to allow all animals to attain the criterion set to adjudge them capable of discriminating between nicotine and its vehicle, that is, correctly choosing the lever appropriate for the injection received in 8 of 10 consecutive training sessions, twice. This 80% performance level was required before dose-response testing commenced.

Dose-Response Tests

Once the discriminative criterion was attained by all animals, the discriminative training regimen was limited to every other day to maintain discrimination. On intervening days, rats were tested with two lower doses of nicotine (0.1 and 0.2 mg/kg) with each dose tested twice, once following a drug maintenance session and once following a vehicle session. This counterbalancing was employed to control for any possible residual influences from the previous day's maintenance session. If at any time during testing a rat's maintenance discrimination fell below the 80% criterion, data on that animal was to be dropped from the results. This, however, did not occur throughout the entire experimental procedures reported herein. During all dose-response tests, rats were immediately removed upon pressing 1 lever 10 times without receiving reinforcement. This was done to preclude reinforcement (and possibly training) at a dose different than the 0.4-mg/kg dose used in training and maintenance sessions.

Time Course of Nicotine Action

Subsequent to the dose-response tests, the time course of discriminative action following nicotine administration was investigated. To this end, nicotine (at the training dose of 0.4 mg/kg) was administered SC and rats were returned to their home cages for various postadministration intervals ranging from 5–120 min. At that time, the rat was placed into the operant chamber and once it pressed 1 lever 10 times it was returned to its home cage. Each of the postadministration intervals was employed on two occasions with each preceded by a nicotine maintenance and a vehicle sessions at the 10-min postadministration interval used in training.

Agonism with 0.8 mg/kg d-*Amphetamine*

Nicotine-trained rats were SC administered 0.8 mg/kg *d*-amphetamine (as sulfate with dose calculated as base) and their discriminative performance was tested at either 5 or 30 min postadministration on two occasions each. In all dose-response, time course, agonism and antagonism (below) studies, rats were immediately removed from the experimental chamber upon accumulating 10 responses on either of the 2 levers so as to not reinforce (thus, train) them at a dose/drug/time different than the 0.4 mg/kg nicotine at 10 min postadministration used in their training.

Antagonism Studies

Each of the three putative antagonists, viz., CGS 10746B, isradipine, and ICS-205930 were administered prior to nicotine administration by a route and at a time that, according to the scientific literature, allowed for maximal central efficacy. Thus, CGS 10746B in doses previously shown to block discrimination of other centrally active drugs (25,26) was administered IP 20 min prior to SC nicotine administration and animals were tested, in extinction, 10 min after the second injection. Similarly, isradipine was administered IP and, 60 min later, nicotine was injected SC 10 min before testing.

Lastly, ICS-205930 was administered SC 45 min before nicotine and, therefore, 55 min before testing. As in all testing situations, animals were immediately removed upon pressing 1 of the levers 10 times.

Measurements and Statistics

The data collected in the drug discrimination sessions are expressed as both quantal and quantitative measurements. Each of the individual measurements provides a different indicator of lever preference prior to any reinforcement. The quantal measurement is the percentage of rats that choose the nicotine-appropriate lever as their selected lever, that is, the first lever to accumulate 10 presses. The quantitative measurement is the number of responses on the nicotine lever divided by the total number of responses on both the nicotine and vehicle levers at the time that 10 responses are accumulated on either single lever. This fraction is expressed as a percentage. Unlike the all-or-none quantal measurement, the quantitative measurements allow for responses on both selected and unselected levers to be considered and, thus, provide a relative measurement of the magnitude, as well as the direction, of lever preference. In addition, statistics such as *t*-tests can be performed on the quantitative data.

The Litchfield-Wilcoxon procedure (14), which employs probits vs. log-dose effects, was used to generate ED_{50} values for the nicotine dose-response data. A computerized program for this procedure was employed (33). A $p < 0.05$ was chosen to indicate a significant difference.

RESULTS

The training data after nicotine and vehicle administration for the 5-week period needed for all animals to reach the second session to criterion are reflected in Table 1A. Results indicate that the nicotine-appropriate lever was chosen by 90% of the rats after nicotine administration and by 56% after saline administration in the first 2 weeks after lever-press training. During weeks 3-4, the discrimination of nicotine after nicotine administration increased to 96% and, at the same time, the percentage of (quantal) responses made on the nicotine-appropriate lever after vehicle dropped to 14% or, to look at it a different way, 86% of all first lever selections were on the vehicle-appropriate lever after vehicle. Lastly, Table 1A reflects only three trials with nicotine and three trials with vehicle in week 5 as criterion performance was met during this period by all animals. This is reflected in 90% of first choices made on the nicotine lever after nicotine and 5% on the same lever after vehicle. The sessions to criterion indicates that the first of 10 trials in which eight responses were correctly made was met in a mean of 4.6 sessions and for the second sessions

TABLE 1

(A) LEARNING RATE, (B) DOSE-RESPONSE, AND (C) TIME COURSE OF NICOTINE-DISCRIMINATIVE PERFORMANCE IN RATS (n = 10) TRAINED TO DIFFERENTIATE BETWEEN 0.4 mg/kg NICOTINE (SC) AND ITS VEHICLE

A. Learning Rate (percent responses on nicotine-correct lever)

	Vehicle		Nicotine	
Weeks	Quantal	Quantitative (SD)	Quantal	Quantitative (SD)
1,2	56.0	64.6 (22.6)	90.0	78.1 (7.2)
3,4	14.0	23.1 (7.4)	96.0	85.1 (4.9)
5	5.0	12.5 (4.9)	90.0	80.3 (11.7)
	STC (SD)	1: 4.6 (3.2)	Range: 2-12	
		2: 14.6 (3.2)	12-22	

B. Dose-Response

Dose Nicotine	Quantal	Quantitative (SD)
0.4	85.0	80.4 (1.1)
0.2	70.0	68.9 (26.0)
0.1	50.0	53.1 (9.8)
0.0 (veh)	5.0	12.9 (4.2)
ED_{50} (95% CL)	0.10 (0.06-0.17)	

C. Time Course

Postadministration Time (min)	Quantal	Quantitative (SD)
5	40.0	47.6 (16.3)
10	85.0	79.3 (2.7)
30	85.0	78.2 (0.1)
60	65.0	62.4 (0.4)
120	15.0	23.1 (7.1)

to criterion the mean was attained in 14.6 sessions. Thus, all animals were adjudged capable of discriminating nicotine from its vehicle by the 32nd session.

The dose-response relationship after lower doses of nicotine were tested appears in Table 1B; the results indicate a progressive decrease in discrimination both in the quantal and quantitative measurements. The quantal ED_{50} value generated (with 95% confidence limits) was 0.10 (0.06-0.17) mg/kg. The last part of Table 1C is the time course of nicotine's discriminative stimulus effects from 5-120 min postadministration. At 5 min postadministration, 40% of the first choice lever selections were made on the nicotine-appropriate lever, at 10 min 85% of first selections were on this lever, and this peak effect was maintained through 30 min with a gradual decrease and return to approximately vehicle-like responding by 120 min postadministration.

Results of a pretreatment with doses of CGS 10746B on test days interspersed with the nicotine and saline maintenance days appear in Table 2A. Although 20 mg/kg CGS 10746B was shown to decrease nicotine discrimination, only the 30-mg/kg dose produced a significant decrease ($p < 0.05$) in comparison to the quantitative data of interspersed nicotine maintenance trials. Table 2B represents pretreatment with three doses of israpidine, with the two highest doses (10 and 15 mg/kg) producing a significant ($p < 0.01$) decrease in the quantitative measurement when compared to nicotine maintenance experiments conducted on interspersed sessions. In contrast to these results, Table 2C indicates that pretreatment with ICS-205930 had no significant effect upon nicotine responding and, in fact, the results suggest that ICS-205930 may actually increase discriminability of nicotine. To test this possibility, the highest dose of ICS-205930 (0.4 mg/kg) was administered prior to decreasing doses of nicotine, starting with 0.4 and decreasing through 0.2, 0.1 mg/kg, as well as administering this agent with vehicle. Although there was a slight increase when 0.4 mg/kg ICS-205930 was administered with the 0.2-mg/kg nicotine dose, that is, the quantal measurement went from 70% (Table 1B) to 90% (Table 3B), there were no significant differences in the quantitative measurements between ICS-205930-pretreated animals and nicotine dose-response experiments.

Lastly, Table 3A indicates the effects of 0.8 mg/kg *d*-amphetamine tested at both 5 and 30 min postadministration. At the earliest time, 5% of the quantal responses were made on the nicotine-appropriate lever whereas at 30 min postadministration the number increased to 65%. This latter time effect resulted in a quantitative measurement (62.9 ± 13.9) significantly greater than seen after vehicle (6.3 ± 5.6) but also significantly different than after nicotine maintenance (87.2 ± 7.6).

TABLE 2

EFFECT OF PRETREATMENT WITH (A) CGS 10746B, (B) ISRADIPINE, OR (C) ICS-205930 UPON (0.4 mg/kg) NICOTINE DISCRIMINATION

Drug Dose	Quantal	Quantitative (SD)
(A) CGS 10746B		
30.0	50.0	48.9 (8.9)*
20.0	75.0	70.6 (15.9)
Nicotine	96.7	90.8 (8.7)
Vehicle	3.3	9.8 (5.0)
(B) Isradipine		
15.0	50.0	51.0 (0.3)†
10.0	55.0	54.3 (1.3)†
5.0	80.0	75.2 (0.5)
Nicotine	96.0	88.1 (6.2)
Vehicle	2.0	9.0 (5.2)
(C) ICS-205930		
0.4	100.0	86.2 (15.5)
0.2	100.0	88.5 (1.1)
0.1	95.0	80.2 (11.7)
Nicotine	94.0	86.1 (8.0)
Vehicle	2.0	5.3 (5.8)

*Significant decrease in quantitative measurement when compared to similar measurement during nicotine maintenance sessions conducted during testing: $p < 0.05$, †$p < 0.01$ (Student's *t*-test).

TABLE 3

AGONISM (GENERALIZATION) OF 0.8 mg/kg *d*-AMPHETAMINE AT 5 AND 30 MIN (A) POSTADMINISTRATION AND (B) COTREATMENT OF 0.4 mg/kg ICS-205930 AND 0.1, 0.2, AND 0.4 mg/kg NICOTINE

	Quantal	Quantitative (SD)
(A) 0.8 mg/kg *d*-amphetamine		
5 min	5.0	7.5 (10.5)
30 min	65.0	62.9 (13.9)
(B) 0.4 mg/kg ICS-205930+ nicotine dose		
0.4	100.0	86.2 (15.5)
0.2	90.0	83.2 (7.6)
0.1	55.0	57.1 (5.1)
0.0 (saline)	10.0	12.5 (9.5)
Nicotine	95.0	87.2 (7.6)
Vehicle	1.7	6.3 (5.6)

DISCUSSION

Nicotine at a dose of 0.4 mg/kg was shown to be readily discriminable when injected subcutaneously and trained 10 min postadministration. The first session to criterion was met in a mean of 4.6 sessions and the criterion of 16 correct first lever selections in 20 consecutive sessions was reached by all rats by the 32nd training session (Table 1); this rapid acquisition has been previously reported (23,30). In addition, the testing of decreasing doses of nicotine produced progressively decreasing discriminative performance and allowed for the generation of an ED_{50} value equal to one fourth of the training dose, viz., 0.1 mg/kg. In a previously reported experiment, the effect of different postinjection intervals upon the dose responsiveness of the nicotine-discriminative cue was investigated (31). In groups of rats trained with 0.4 mg/kg nicotine, subcutaneously administered at either 5, 20, or 35 min before the beginning of training, the ED_{50} value in each group was approximately 0.09 mg/kg, remarkably close to the present finding. In fact, when these same investigators used a postinjection interval of 15 min (closest to the present postinjec-

tion interval) they found an ED_{50} value of 0.14 mg/kg (20) and suggested that this ED_{50} value for the cueing effect of nicotine produces an estimated plasma nicotine concentration of 48 ng/ml, a concentration within the range of 4–72 ng/ml found in cigarette smokers who inhale (24). Thus, the dose used in nicotine-discriminative training is one (approximately 145 ng/ml) that is obtainable by human cigarette smokers (24).

The last parametric experimentation performed, before agonism or antagonism studies were commenced, was that of the time course of the nicotine-discriminative effects. At the earliest time tested (5 min), 40% of the animals were seen to choose the nicotine-appropriate lever and the peak effect was observed between 10 and 30 min postadministration, with a return to saline responding at 120 min. This same peak effect and offset of discrimination effects has been previously reported (12,20,27).

The agonism study in which amphetamine was administered at a dose of 0.8 mg/kg and tested at both 5 and 30 min postadministration indicated that the generalization to amphetamine occurs significantly more, $t = 4.486$; $p < 0.05$, at the later time interval. Previous work in which amphetamine generalization was tested in nicotine-trained animals at the same postinjection interval as used in training indicated that amphetamine produced only partial generalization (4,32). There is, however, one recent study in which amphetamine at 0.8 mg/kg produced complete generalization from nicotine-trained animals on a schedule of reinforcement similar to that used in the present experiment (30). Lastly, in three groups of rats each trained to discriminate nicotine at different postinjection intervals the generalization of amphetamine was shown to be greater in those animals trained at 35 min as compared to those animals trained at either 5 and 20 min (31). This would lend support to the notion that the stimulatory effects of nicotine occur approximately 30 min after administration and the amphetamine-like actions are, at best, weak in that the stimulus effects of nicotine are not equivalent to those of the psychostimulant drug amphetamine. Aside from the possibility that the amphetamine-like stimulant effects are responsible for even the partial generalization seen, the possibility remains that amphetamine, which is a releaser of neuronal dopamine, may be active upon the nicotine-trained rats, that is, the (weak) effect after amphetamine may be attributed to its dopamine-releasing properties allowing for a possible link between nicotine and dopamine systems.

This relationship was explored in the experiments in which CGS 10746B was shown to effectively block the nicotine-discriminative cue (Table 2A). Nicotine has been shown to increase synaptic dopamine concentrations by approximately 100% in the nucleus accumbens when administered to rats at 0.6 mg/kg subcutaneously. This nicotine-induced increase in extracellular dopamine was dose related and it was suggested to be caused by the ability of nicotine to stimulate the firing of dopaminergic neurons (7). This ability of dopaminergic activation by nicotine has been shown not to effect serotonergic systems (16) and it has been suggested to be responsible for its ability to stimulate locomotion (5,6). CGS 10746B is novel as to its ability to prevent the release of dopamine. Various antipsychotic agents that block postsynaptic dopamine receptors have been used as pretreatments in animals trained to discriminate nicotine. Thus, haloperidol, which acts on both $dopamine_1$ and $dopamine_2$ receptor sites, as well as Sch 23390, which has selectivity for $dopamine_1$ receptors, have each been shown to significantly weaken discrimination of nicotine (21). In contrast to these results, pimozide and droperidol (drugs with selectivity for $dopamine_2$ receptors) did not significantly effect discrimination of nicotine. These authors conclude that nicotine may facilitate dopamine neurotransmission in the CNS as indicated by its subtle mediation of nicotine-discriminative performance.

Not only was the dopamine release inhibitor CGS 10746B able to attenuate nicotine discrimination, but the centrally active calcium blocker isradipine was similarly able to block nicotine discrimination. Previous work indicated that calcium channel blockers can decrease amphetamine-induced circling (8), as well as the discriminative stimulus effects of both amphetamine (18) and cocaine (2). Although the results of this study indicate that calcium channel blockers can inhibit the behavioral effects of still another centrally active drug, the exact mechanism by which this occurs is presently unknown. Neurochemical evidence indicates that the calcium antagonists nimodipine and isradipine are capable of preventing both the cocaine-induced dopamine release in the striatum, as well as its motor stimulatory effects (19). The possibility thus exists that calcium channel blockade may produce an overall inhibitory effect by interaction with the drug rather than upon the dopaminergic systems that mediates its effects. In contrast, the possibility exists that the inhibitory effects of the class of dihydropyridine calcium blockers depends upon their ability to interfere with calcium influx across L-type voltage-sensitive channels to attenutate the discriminative effects of nicotine.

In contrast to these positive results, the administration of the $5\text{-}HT_3$ receptor antagonist ICS-205930 had no antagonist effect upon nicotine discrimination. Selective ligands for each of the 5-HT receptor subtypes have allowed recognition of the possibility that $5\text{-}HT_3$ receptors are involved in the stimulatory actions of 5-HT upon mesolimbic dopamine neurons. Thus, the increased locomotor activity produced by amphetamine in the nucleus accumbens can be prevented by administration of a $5\text{-}HT_3$ antagonists (11). In fact, the stimulatory effects of various drugs, for example, morphine, nicotine, and ethanol, on a mesolimbic dopamine system disappear when $5\text{-}HT_3$ receptors are blocked (11). The inability of each of four distinct $5\text{-}HT_3$ antagonists, viz., MDL 722222EF, MDL 73147EF, ICS-205930, and ondansetron, to antagonize the ability of rats to discriminate 0.8 mg/kg amphetamine (IP) in a similar discriminative task (17) suggests that $5\text{-}HT_3$ antagonists do not inhibit dopamine release and their interaction with mesolimbic dopamine systems may be at a site prior to the dopamine synapse.

Taken together, the results of this study indicate that dopaminergic systems may be involved in nicotine discrimination. The partial generalization to dopaminergically active amphetamine, as well as the antagonism by the dopamine release inhibitor CGS 10746B, suggest that dopaminergic neurons or release may be stimulated by nicotine, a suggestion previously made by others (10,13,15).

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