Psychopharmacologia (Berl.) 40, 109—120 (1974)

Studies on the Time Course and the Effect of Cholinergic and Adrenergic Receptor Blockers on the Stimulus Effect of Nicotine *

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Received April 11, 1974; Final Version July 10, 1974

Abstract. This study investigated the stimulus property of nicotine in the rat. The primary objectives of the study were 1. to determine the time course of the nicotine stimulus and its relationship to brain levels of the drug and 2. to determine whether the nicotine stimulus is dependent upon the integrity of specific neurotransmitter systems. A lever choice discrimination was used. After injection of nicotine, depression of one lever in an operant test chamber resulted in food reinforcement according to a variable interval schedule of 15 sec. When saline was administered, the opposite lever was reinforced. A high degree of discriminated responding was observed when either 400 μg/kg or 200 μg/kg of nicotine was used as a discriminative stimulus. The degree of discrimination decreased as the length of the time period between the injection of nicotine and the test of discrimination was increased. This decline in discrimination was similar to the decline in brain levels of nicotine suggesting that nicotine discrimination is directly related to the concentration of nicotine in the brain. Atropine, mecamylamine, dibenamine, propranolol and α-methyl-para-tyrosine (AMPT) were all tested, in a range of doses, for effects upon nicotine discrimination. Of these, only mecamylamine antagonized the nicotine stimulus. These results indicate that the stimulus effect of nicotine is mediated specifically through nicotinic-cholinergic receptors and not muscarinic-cholinergic or adrenergic receptors.

Key words: Drug Discriminations — Nicotine — Adrenergic — Cholinergic.

Nicotine has no therapeutic use, but is widely self-administered as a constituent of tobacco. There is much evidence which suggests that many people who voluntarily ingest tobacco products do so to attain the pharmacological effects of nicotine. Deneau and Inoki (1967) have been able to train monkeys to self-administer nicotine intravenously. This indicates that nicotine, *per se*, can have reinforcing properties. In addition, when human subjects receive an intravenous injection of nicotine, they reduce their consumption of cigarettes in a kind of self-titration of nicotine administration (Lucchesi *et al.*, 1967).

Which of the pharmacological actions of nicotine might be sought by the tobacco user is not known, but prominent among nicotine's central

* A preliminary report of this investigation appeared in the Pharmacologist **15**, 452 (1973).

nervous system actions are effects which are perceptual and subjective in nature. It is not possible to directly assess this kind of drug effect in animals. In humans, these effects are generally measured by directly questioning the subject. However, the observation that certain drugs can serve as controlling or discriminative stimuli (Overton, 1968, 1971; Barry, 1968; Winter, 1973) indicates that these drugs produce effects which animals can distinguish from the non-drug condition. Furthermore, the bulk of the published data on this subject suggests that drug stimuli are highly specific. Kubena and Barry (1969), for example, have reported that the stimulus characteristics of alcohol generalize to appropriate doses of other drugs which are classified as general central nervous system depressants such as pentobarbital and chlordiazepoxide, but not to drugs of other pharmacological classes like chlorpromazine or *d*-amphetamine. Similarly, the stimulus properties of mescaline are similar to those of lysergic acid diethylamide (LSD-25), but different from those of barbital (Hirschhorn and Winter, 1971a, b).

Morrison and Stephenson (1969) and Schechter and Rosecrans (1971) have demonstrated that nicotine may serve as a discriminative stimulus in the rat. The present study investigated further the stimulus property of nicotine. One segment of the investigation sought to determine the time course of the nicotine stimulus and its relationship to brain levels of the drug. Another segment of the investigation attempted to determine whether the nicotine stimulus is dependent upon the integrity of specific central neurotransmitter systems. This was accomplished by the administration, prior to nicotine, of agents known to compete for cholinergic or adrenergic receptor sites.

Methods

Subjects. Male Sprague-Dawley rats with no previous drug or experimental experience (Flow Research Animals, Dublin, Va.) were housed in individual home cages and exposed to a 12 h light-dark cycle. Water was freely available in home cages and adjusted amounts of commercial rat chow were offered after each experimental session to maintain the animals at 70—80% of their expected free feeding weight.

Chemical Methods. Brain nicotine levels. Nicotine (methyl-)-^{14}C)-HCl, with a specific activity of 9.2 mCi/mM, was diluted with cold nicotine hydrogen (±)tartrate to make solutions of 400 μg/ml and 200 μg/ml of nicotine. At various times after the administration of ^{14}C nicotine, rats were sacrificed by decapitation. Four rats were used at each time interval. The brains were quickly removed, dissected into telencephalon, diencephalon, and brainstem, and quickly frozen for future assay.

Nicotine concentrations in each brain area were determined by the methods of Hucker, Gillette, and Brodie (1960). Brain tissue was homogenized in 0.1 N NaOH and nicotine was extracted into 15 ml of heptane containing 1.5% isoamyl alcohol. Extracted ^{14}C nicotine was returned to 0.1 N NaOH. The radioactivity of ^{41}C nicotine levels was determined by the procedures of Weiss (1968). A Nuclear-Chicago

planchet counter (series 1042) was used for the counting. Brain area nicotine levels (mμ mol/g of brain) were determined from a comparison of tissue radioactivity counts with ^{14}C nicotine internal and external standards.

Behavioral Methods. The experimental space was a standard operant test chamber (Lehigh Valley Electronics Model 1417). One wall of the chamber contained two levers with a dipper for delivery of liquid reinforcement centered between them. A force of approximately 15 g was necessary to depress the lever. Sweetened condensed milk diluted 2:1 with tap water and delivered by a dipper (0.1 ml) was the reinforcer. The experimental space housed in a larger sound insulated and light proof box equipped with an exhaust fan. Solid state and electro-mechanical programming equipment were used to control and record the sessions.

Discrimination training was similar to that previously described (Hirschhorn and Winter, 1971a). At approximately 10 weeks of age the subjects were trained to press first one, then the other lever. Discrimination training began with 4 preliminary training sessions of 15 min duration in which each correct bar press was reinforced. Subsequent sessions started with a 2.5 min period during which no responses were reinforced and a variable interval of 15 sec (VI-15 sec) schedule was in effect for the remaining 12.5 min. Every session was preceded by 5 min with a subcutaneous injection of either nicotine or saline. During the four preliminary training sessions, nicotine and saline injections were alternated daily; thereafter, 2 days of one treatment were followed by 2 days of the other. By means of this double alternation schedule of drug administration, each treatment was preceded equally often by a session with the same and the opposite treatment. One lever was reinforced after the injection of nicotine and the opposite lever was reinforced following saline. For $^1/_2$ of the subjects, the right lever was rewarded after nicotine and the left lever was rewarded after saline. These conditions were reversed for the remaining animals.

Twelve rats were used in the behavioral study. Six of these received 400 μg/kg of nicotine and saline as the 2 stimuli. The other 6 were trained with 200 μg/kg of nicotine and saline.

After 40 discrimination training sessions, responding was relatively stable. The same animals continued to receive either 400 μg/kg of 200 μg/kg of nicotine and saline according to a double alternation sequence. However, test sessions were interposed, 2.5 min in duration during which no responses were reinforced. An odd number of training sessions, usually either 1 or 3, separated 2 successive test sessions.

Drugs. Doses of nicotine hydrogen (±)tartrate, mecamylamine HCl, atropine sulfate and α-methyl-para-tyrosine (AMPT) were calculated as the free base. Propranolol HCl and dibenamine HCl were calculated as salts. All drugs with the exception of AMPT were dissolved in 0.9% saline solution and injected in a volume of 1 ml/kg. For oral administration, AMPT was suspended in the same sweetened condensed milk and water mixture which served as the reinforcement in the operant test chamber and was administered in a volume of 5 ml/kg. For intraperitoneal injection, AMPT was suspended in water with Tween 80 and injected in a volume of 1 ml/kg. Nicotine hydrogen (±)tartrate was purchased from Gallard-Schlesinger Chemical Corp., Carle Place, New York; nicotine (methyl-C-14), 2HCl from Amersham/Searle, Arlington Heights, Ill.; Mecamylamine HCl from Merck, Sharp and Dohme, West Pt., Pa.; atropine sulphate from Mann Research Labs, New York, N.Y.; D-L-α-methyltyrosine from Aldrich Chemical Corp., Milwaukee, Wis.; dibenamine HCl from K and K Labs, Plainview, N.Y.; propranolol HCl from Ayerst Labs, Montreal, Quebec, Canada.

Results

Discrimination Training. One rat from the group receiving 200 μg/kg of nicotine stopped pressing one bar early in the experiment. Retraining efforts failed and this animal was eliminated from the study. The results obtained when either 200 μg/kg or 400 μg/kg of nicotine was paired with saline as discriminative stimuli are represented by Fig. 1. During the first few sessions, little difference in lever choice pattern between sessions preceded by nicotine and those preceded by saline was observed. However, beginning with session block 2 and continuing for the remainder of the sessions, the rats responded differentially after receiving nicotine or saline. When given nicotine, these animals made a majority of their responses on the nicotine-correct bar; after saline they pressed predominantly the saline-correct lever (a low percentage of nicotine-correct responses).

The magnitude of the differences between responding after nicotine and saline increased with successive training sessions. During the 40 sessions shown, the animals which received 400 μg/kg of nicotine and saline

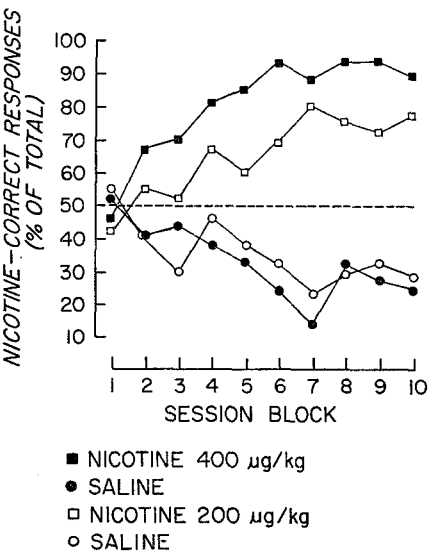

Fig. 1. Discriminated responding following the administration of nicotine and saline. One group of 6 rats received either 400 μg/kg of nicotine or saline 5 min before the session. A second group of 5 rats received 200 μg/kg of nicotine or saline. Each point is the mean of 2 determinations in each animal. On any given day, one-half of the subjects of each group were given nicotine and the remaining animals received saline. Ordinate: number of responses on the nicotine-correct lever in the first 2.5 min of the session expressed as a percentage of total responses. Abscissa: successive blocks of 4 sessions each

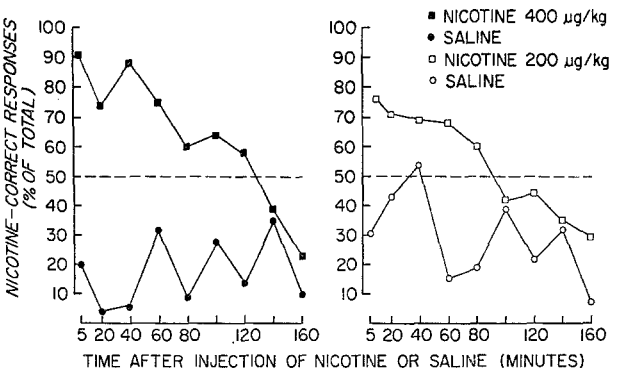

Fig. 2. Time course of nicotine and saline discrimination. Two and one-half min test sessions, during which no responses were reinforced, were interposed among discrimination training sessions subsequent to those represented by Fig. 1. Nicotine was injected at varying times before the test sessions. The experiment was then repeated with saline injected at varying times before the test session. Each point is the mean of one determination in each of 6 animals for the nicotine 400 µg/kg group. Ordinate: number of responses on the nicotine-correct lever expressed as a percentage of total responses. Abscissa: time between injection of nicotine or saline and test sessions

made 81% of their total responses on the nicotine-correct bar when given nicotine and only 33% of their responses on the same bar after saline ($P < 0.01$, paired t-test, two-tail, $df = 5$). The subjects receiving 200 µg/kg of nicotine, made 65% and 35% of their total responses on the nicotine-correct bar after nicotine and saline, respectively ($P < 0.01$ paired t-test, two-tail, $df = 4$). These data are in agreement with those of Schechter and Rosecrans (1971) and Morrison and Stephenson (1969) who reported that nicotine can serve as a discriminative stimulus in the rat. If discriminated responding after the administration of nicotine or saline is a result of a pharmacological effect of nicotine, the degree of discrimination should vary directly with the dose of nicotine. The data of Fig. 1 provide evidence for such a dose-response relationship; a greater difference between responding after nicotine and saline is observed in the animals which received 400 µg/kg of nicotine than in those which received 200 µg/kg ($P < 0.01$, Wilcoxon's signed-ranks test, two-tail).

Time Course of Cue. In test sessions (see Methods) subsequent to the sessions shown in Fig. 1, the time interval between the injection of nicotine and the experimental session was varied in 20-min increments to 160 min. Each subject was tested at every time interval according to a randomized schedule. In a subsequent series of sessions, the time interval between the injection of saline and the experimental session was varied in the same way. The results are shown in Fig. 2. Intervals of up to 60 min

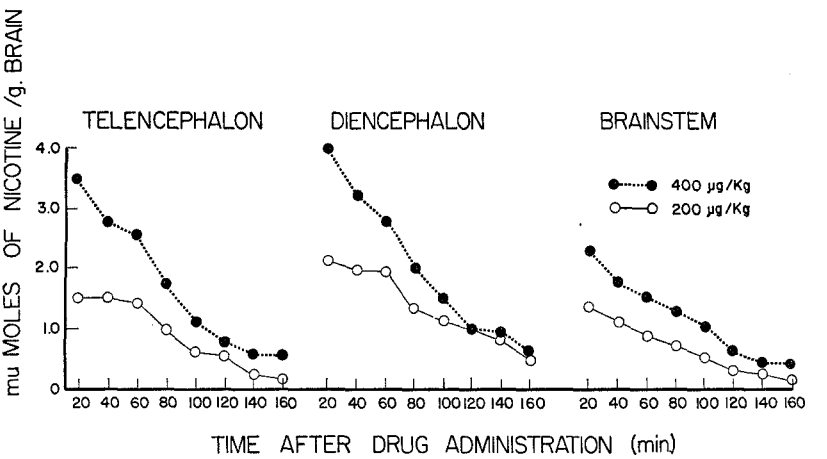

Fig. 3. Brain levels of ^{14}C nicotine at various times after a single injection. Each point is the mean of 4—6 determinations. Ordinate: mμ moles of nicotine/g of brain (1 mμ mole = 162 ng). Abscissa: time after injection of ^{14}C nicotine

after nicotine resulted in a high percentage of nicotine-correct responses. This gradually declined as the interval was lengthened and at 160 min responding was appropriate to saline administration. The animals which received 200 μg/kg of nicotine continued to make a majority of their responses on the nicotine-correct bar up to 80 min after the nicotine injection. The animals receiving 400 μg/kg of nicotine maintained discrimination somewhat longer; at 120 min after the nicotine injection, they still made 58% of their total bar presses on the nicotine-correct lever. No orderly, time-dependent effect upon saline discrimination was observed. The instability of responding during the saline time course experiment, especially in the animals which received 200 μg/kg of nicotine may indicate poor response control by saline as compared with nicotine.

The time course of the concentration of nicotine in the brain after the injection of either 400 μg/kg or 200 μg/kg of nicotine was studied to determine whether this was similar to the time course of the nicotine stimulus. Three separate brain regions were investigated to determine whether the time course of the cue was more closely correlated with one particular brain area than the others. The results are shown in Fig. 3. In each brain area, a higher concentration of nicotine was initially present after administration of 400 μg/kg than of 200 μg/kg of nicotine. The diencephalon had the greatest concentration of nicotine at 20 min and the brain stem had

the lowest. In all brain areas, after administration of either nicotine dose, the drug concentration declined with time. In each area after both doses, the level of nicotine declined to approximately 50% at 90 min. Although it is difficult to relate these data to the data of the nicotine cue, the time-course of these two measures are seen to be similar. There is no apparent difference among brain areas in their relative importance for the nicotine cue.

Drug Interactions. Atropine is believed to combine with muscarinic-cholinergic receptors in the peripheral and the central nervous system and to protect these receptors from muscarinic-cholinergic drugs (Goodman and Gilman, 1970). The results obtained when atropine sulphate was injected, s.c., 10 min before nicotine are presented in Table 1. In the absence of atropine treatment, rats made 93% of their responses on the nicotine-correct lever after receiving 400 μg/kg of nicotine and 75% of their responses on the nicotine lever after 200 μg/kg of nicotine. Pretreatment with 0.5 mg/kg of atropine had no effect on the nicotine response, nor did any of the other doses tested. Doses of atropine greater than

Table 1. Effect of drug pretreatments on nicotine discrimination

Treatment[a] (mg/kg)	Replications	Nicotine-correct[b] responses (% of total ± S.E.M.)		Rate[b] (responses/min)	
		400 μg/kg nicotine	200 μg/kg nicotine	400 μg/kg nicotine	200 μg/kg nicotine
None	6	92.9 ± 2.2	75.3 ± 9.0	5.6	7.5
Atropine (0.5)	2	94.1 ± 3.1	74.1 ± 7.6	6.6	7.0
Atropine (1.0)	2	96.5 ± 3.5	67.7 ± 9.7	4.2	5.2
Atropine (2.0	2	95.1 ± 2.0	69.4 ± 9.0	7.5	5.0
None	4	90.7 ± 4.1	81.5 ± 4.9	7.2	8.8
Dibenamine (10)	2	93.3 ± 2.7	80.1 ± 4.7	8.1	7.6
Dibenamine (20)	1	91.1 ± 5.9	79.2 ± 9.1	7.2	7.4
None	4	94.0 ± 2.9	71.0 ± 8.0	6.7	5.5
Propranolol (1)	1	81.7 ± 8.9	74.7 ± 9.8	8.9	7.7
Propranolol (2)	1	93.1 ± 4.5	74.0 ± 11.2	4.2	4.5
Propranolol (4)	1	78.7 ± 11.5	70.8 ± 15.3	3.4	3.3
None	1	99.0 ± 1.0	86.7 ± 6.4	9.0	11.3
AMPT (200, p.o.)	1	100.0 ± 0	80.7 ± 10.4	1.4	9.1
None	1	100.0 ± 0	90.5 ± 5.3	3.9	17.3
AMPT (3×50, i.p.)	1	100.0 ± 0	93.6 ± 6.4	1.4	11.4

[a] For route of administration and time of injection relative to testing, see Results.

[b] During first 2.5 min of session (unreinforced).

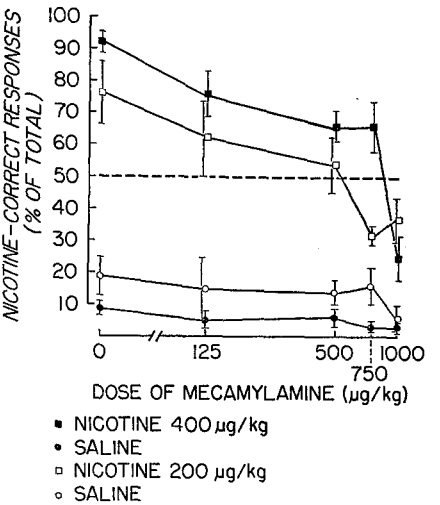

Fig. 4. Effect of mecamylamine on nicotine and saline discrimination. Mecamylamine HCl was injected 10 min before nicotine or saline, which was 15 min before the test session. Each point is the mean of at least 2 determinations in each of 6 animals for the nicotine 400 μg/kg group and 5 animals for the nicotine 200 μg/kg group. Ordinate: number of responses on the nicotine-correct lever expressed as a percentage of total responses. Abscissa: dose of mecamylamine HCl plotted on a log scale. All other details are as in Fig. 4

2 mg/kg could not be tested since this dose already produced an extreme hyperactivity which made the animals very difficult to handle.

The nicotine-cholinergic antagonist, mecamylamine (Goodman and Gilman, 1970) was also given s.c., 10 min before nicotine in a range of doses. Fig. 4 shows the results. Again, a high degree of discrimination was observed in the absence of drug pretreatment, 92% nicotine-correct responses after 400 μg/kg of nicotine and 76% nicotine-correct responses after 200 μg/kg of nicotine. However, some decrease in nicotine discrimination was observed when 125 μg/kg of mecamylamine was given prior to nicotine. This decrease in nicotine discrimination continued with successive increases in the dose of mecamylamine. The highest dose of mecamylamine, 1000 μg/kg, completely blocked the nicotine cue, i.e., subjects pressed predominantly the saline-correct lever. One might reasonably argue that this decrease in nicotine discrimination may be the result of a non-specific effect of mecamylamine on the subjects' ability to discriminate. The observation that the highest dose of mecamylamine produced responding appropriate to saline treatment rather than random responding is evidence against this. In order to test this possibility, the

effect of mecamylamine upon responding following saline was investigated. The data of Fig. 4 indicate that mecamylamine had no effect upon saline discrimination.

It is apparent from Table 1 that neither dibenamine, an α-adrenergic antagonist (i.p., 30 min before nicotine), nor propranolol, a β-adrenergic antagonist (i.p., 45 min before nicotine), altered responding after nicotine. α-Methyl-para-tyrosine (AMPT) was given by two different routes of administration (Rech, Borys, and Moore, 1966). The data of Table 1 indicate that neither 200 mg/kg of AMPT given orally 8 hrs prior to nicotine nor 3 50 mg/kg i.p. injections with the first injection 12 hrs before nicotine altered the response to nicotine. The test sessions were run at times after AMPT administration that correspond with the time of maximum depletion of norepinephrine and dopamine as reported by Rech *et al.* (1966).

The effects of the various pretreatments on the rates of responding are shown in Tables 1 and 2. Response rates are seen to be slightly variable with most of the pretreatments lacking an orderly, dose-related effect. The only marked depression of responding occurred when AMPT was administered prior to 400 μg/kg of nicotine. The same doses of AMPT did not greatly reduce the rate of responding when given prior to 200 μg/kg of nicotine. Therefore, this depression of responding is apparently not an effect of AMPT alone but an additive effect of AMPT and nicotine.

Table 2. Effect of mecamylamine on response rate

Dose of mecamylamine (μg/kg)	Replications	Rate[a] (responses/min)	
		400 μg/kg nicotine	200 μg/kg nicotine
0	6	6.1	7.4
125	2	11.1	8.6
500	3	7.3	6.8
750	2	3.5	6.7
1000	2	4.4	4.8
		Saline	Saline
0	6	7.9	6.0
125	1	3.9	6.3
500	1	8.4	4.8
750	1	10.3	4.9
1000	1	4.7	6.5

[a] During first 2.5 min of session (unreinforced).

Discussion

The present experiments confirm in a third test system, that nicotine can serve as a discriminative stimulus in the rat (Fig. 1). Overton (1971) has suggested that the effectiveness of a drug as a discriminative stimulus in animals is proportional to the potential for abuse by man. Although the data available at present provide an insufficient basis for thorough evaluation of this suggestion, the results with nicotine are consistent with it. Nicotine is one of the pharmacological agents most frequently self-administered by man and it is also one of the most efficacious drug stimuli. The similarity between the decline in nicotine concentration in the brain (Figs. 2 and 3) and the decline in nicotine-appropriate responding suggests that the strength of the nicotine stimulus is directly proportional to the concentration of nicotine in the brain. The greater degree of discrimination observed with 400 μg/kg nicotine than with 200 μg/kg (Fig. 1) is similarly indicative of a dose-effect relationship. Our data do not, however, permit determination of the importance of one brain area relative to another for the nicotine cue.

Some of our results differ from those previously reported by Schechter and Rosecrans (1971, 1972). These investigators reported that the nicotine stimulus was present only until 20 min after administration of 400 μg/kg of nicotine, while in our investigation, subjects continued to make responses appropriate to nicotine up to 120 min. The explanation for this discrepancy may lie in one or more of several procedural differences between the 2 studies. Schechter and Rosecrans used a T shaped maze in which correct arm choices were rewarded with sweetened milk and incorrect choices were punished with electrical shock. The experimenter had to place the rat into the maze at the beginning of each of the daily trials. The test of discriminated responding was a one-trial test and was, therefore, limited to only a few seconds in duration. Because it was a one-trial test, each subject's response on any given day could be recorded either as correct or incorrect for the drug treatment of that particular day. In contrast, the procedures used in the present investigations permitted each measurement of differential responding to be prolonged for 2.5 min. An isolated experimental chamber with automatic programming and recording ensured maximal efficiency and minimal experimenter bias. Demonstration of discriminated responding with procedures utilizing escape from aversive stimuli and one-trial discrimination tests typically requires relatively high doses of drug (Kubena and Barry, 1969; Hirschhorn, 1971). A higher dose requirement for discrimination in the T maze situation than in the lever-choice procedure might explain the time duration discrepancy because the threshold dose for discrimination in the T maze would be reached at a higher dose of nicotine or an earlier time after nicotine injection than in the operant procedure. These pro-

cedural differences are compounded by sex and strain differences. Whereas Schechter and Rosecrans used female rats of CD strain, the subjects in the present study were male Sprague-Dawleys from a different supplier.

If the nicotine cue is dependent upon the integrity of specific central neurotransmitter systems, then it should be possible to block the stimulus effect of nicotine by the administration of agents known to disrupt the integrity of these systems. Schechter and Rosecrans (1972) have reported that a depletion of 5-hydroxytryptamine (5HT) by para-chloro-phenylalanine does not alter the nicotine cue. However, these authors also found that the administration of alpha-methyl-paratyrosine (AMPT), an event which inhibits the synthesis of catecholamines, markedly reduced nicotine discrimination. In the present study AMPT did not affect the nicotine stimulus, nor did the alpha-adrenergic antagonist, dibenamine or the beta-adrenergic antagonist, propranolol (Table 1). Besides the procedural and subject differences discussed above, there are several other possible explanations for the contradictory results between the two studies. These serve to illustrate some of the problems of interpretation which are inherent in experiments in which drugs with known actions upon neurotransmitter systems are utilized in an attempt to elucidate the mechanism of action of another drug. First, an action of AMPT other than the depletion of catecholamines could be responsible for the blockade of the nicotine stimulus under certain conditions. A second possible explanation is that, as a result of presently unknown factors, AMPT may produce a catecholamine depletion which is not always qualitatively consistent (or maybe is consistent within subjects of the same sex and strain but varies among strains and between sexes). It is conceivable, for example, that catecholamines were depleted at a certain critical site in the Schechter and Rosecrans experiment and that they were not depleted sufficiently at that site in the present study. This kind of site-inconsistant depletion could occur even if the amount of depletion, as measured in large brain areas, is comparable. Finally, the neurons in the brain are interconnected in a complex manner. An alteration of a neurotransmitter system which is not directly required for the mediation of the nicotine cue, but which has a modulatory influence upon it, might be just as effective in blocking the nicotine stimulus as a disruption of the neurotransmitter system which is directly involved.

When the problems discussed above are considered, it is in fact, almost surprising that any specific and consistent block of the nicotine cue is observed. That a consistent antagonism has been observed with mecamylamine, a specific nicotine antagonist, but not with muscarinic blockers or adrenergic depleters and blockers is stiking, and suggests that the nicotine stimulus is mediated specifically through nicotinic-cholinergic receptors. This is consistent with Domino's proposal (1967) that specific central

nervous system nicotinic receptors are the site of action for many of nicotine's behavioral actions. It is interesting to note that mecamylamine, when given to human subjects, causes an increased rate of cigarette smoking (Stolerman *et al.*, 1973). This suggests that the reinforcing effect of nicotine in man is also mediated through nicotinic-cholinergic receptors.

Acknowledgments. The authors thank Mr. Ronald Hayes for his excellent technical assistance. This investigation was supported in part by grants from the AMA Education and Research Foundation. I.D.H. was supported as an AMA postdoctoral fellow.

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