Nicotine and Arecoline as Discriminative Stimuli: Involvement of a Non-Cholinergic Mechanism for Nicotine[1]

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MELTZER, L T AND J A ROSECRANS *Nicotine and arecoline as discriminative stimuli Involvement of a non-cholinergic mechanism for nicotine* PHARMACOL BIOCHEM BEHAV 29(3) 587–593, 1988 —The cholinergic innervation of central muscarinic (M-Ch) and nicotinic (N-Ch) receptors was evaluated by studying the interaction of physostigmine with the discriminative stimulus (DS) effects of arecoline and nicotine Rats were trained to discriminate either arecoline (1 74 mg/kg) or nicotine (1 14 mg/kg) from saline using a two-lever, milk reinforced, operant task Physostigmine (0 125 mg/kg) pretreatment potentiated, and when administered alone (0 25 mg/kg), generalized with the DS induced by arecoline In contrast, physostigmine, at the same dose, neither potentiated nor generalized with the DS effects of nicotine These findings provide evidence that central muscarinic receptors are cholinergically innervated (physiologic) while central nicotinic receptors are not cholinergically innervated but are cholinoceptive (pharmacological)

Nicotine Arecoline Discriminative stimulus Muscarinic Nicotinic

SEVERAL studies have characterized the central sites and mechanisms of action by which nicotine elicits discriminative stimulus (DS) control of behavior [10,12]. In addition, the DS properties of arecoline, a central muscarinic receptor agonist, have been evaluated providing data concerning the psychopharmacology of both muscarinic and nicotinic cholinergic systems [6]. These studies have demonstrated that the nicotine-induced DS is extremely specific and mediated by an agonist action at a distinct population of central nicotinic-cholinergic receptors. In addition, the nicotine DS did not generalize to arecoline in nicotine-trained rats, nor did arecoline generalize to nicotine in arecoline-trained rats. Furthermore, mecamylamine (a central nicotinic receptor antagonist), but not atropine (a central muscarinic cholinergic receptor antagonist) will antagonize the DS elicited by nicotine in a dose-related manner [5,17] Conversely, atropine but not mecamylamine will antagonize the arecoline-induced DS [11] Finally, preliminary data indicate that cholinergic receptors mediating the DS effects of arecoline or nicotine may be located in different brain regions [7]

A major assumption in this research has been that regardless of the receptor acted upon, each cholinergic agonist (nicotine and arecoline) is acting at cholinergic receptors sensitive to acetylcholine (ACh) [4, 8, 15] However, more recent studies by Abood *et al* [1] and Sershen *et al* [14] suggest that central nicotinic receptors are noncholinergic and ACh may not be the endogenous ligand at nicotine sensitive binding sites As pointed out by Karczmar [2,3], the cholinoceptive response of a neuron to nicotine's agonist effect cannot be accepted as proof that it has a cholinergic innervation unless further pharmacological and physiological data are available. Moreover, a neuron that is not cholinergically innervated but is cholinoceptive, may be affected by the exogenous administration of cholinergic drugs.

If the receptors which mediate the DS effects of arecoline and nicotine are innervated by neurons that release ACh, then the DS effects of arecoline and nicotine would be mimicked and/or potentiated by increasing central ACh levels via cholinesterase inhibition [11] This question was examined by studying the interaction of the cholinesterase inhibitor physostigmine with the DS effects of arecoline and nicotine In addition, these interactions were also carried out in rats in which either central muscarinic or nicotinic receptors were the only sites available for stimulation by the physostigmine-induced elevation of brain ACh (Table 1) For

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TABLE 1

AN EXPERIMENTAL APPROACH TO THE SELECTIVE STIMULATION OF NICOTINIC OR MUSCARINIC RECEPTORS BY ACETYLCHOLINE (ACh) VIA ACETYLCHOLINESTERASE INHIBITION BY PHYSOSTIGMINE

In Vivo Isolation of Nicotinic Receptors (N-Ch)	*In Vivo* Isolation of Muscarinic Receptors (M-Ch)
A NICOTINE trained rats pretreated with	A ARECOLINE-trained rats pretreated with
ATROPINE—Blocks both peripheral/central M-Ch receptors	MECAMYLAMINE—Blocks both peripheral/central N-Ch receptors
and	and
HEXAMETHONIUM—Blocks peripheral N-Ch receptors	METHYL ATROPINE—Blocks peripheral M-Ch receptors
B PHYSOSTIGMINE—Injected after A to increase ACh levels at available central N-Ch receptors	B PHYSOSTIGMINE—Injected after A to increase ACh levels at available central M-Ch receptors

this purpose, central muscarinic receptors were pharmacologically confined by pretreatment with both methylatropine (peripheral muscarinic receptor antagonist) plus mecamylamine, central nicotinic receptors were pharmacologically confined by pretreatment with both atropine plus hexamethonium (peripheral nicotinic receptor antagonist).

METHOD

Subjects

Male Sprague-Dawley rats (175–200 g) without previous drug or experimental experience were purchased from Flow Research Animals, Dublin, Virginia, and used in all experiments. These rats were individually housed in a temperature-controlled environment under 12-hour light/dark cycle. Initially, food (Purina Rodent Chow) and water were available ad lib. After allowing two to four weeks for acclimation, rats were reduced to 80% of their expected free-feeding weight by restricted feeding. For the remainder of the study, water was freely available in the home cages and adjusted amounts of rodent chow were offered after each experimental session to maintain the animals at 80% of their expected free-feeding weight.

Apparatus

The experimental space was a standard operant test chamber (Lehigh Valley Electronics, Model 1417 or Coulbourn Model E10-10). One wall of the chamber contained two levers with a dipper centered between them for delivery of liquid reinforcement. Except where noted, both levers remained in the chamber. Above the dipper was a white house light that was lit for the entire session. The experimental chamber was located in a larger sound-insulated and light-proof isolation cubicle. Solid-state and electrochemical programming equipment was used to control sessions. Data were recorded automatically in the form of response and reinforcement totals. The reinforcement consisted of equal parts of sugar and non-fat powdered milk (Land O Lakes, Inc.) mixed in tap water and delivered by the dipper (0.01 ml).

Experimental Procedures

Initial training. One lever in chamber. Fourteen Sprague-Dawley rats, reduced to approximately 80% of their normal body weight by restricted feeding, were trained to press one lever in a two-lever operant chamber using milk reinforcement. This lever was designated as the saline lever. After three to four days of responding on a continuous reinforcement schedule rats were trained to respond on the second (drug) lever. Rats were injected (SC) with either 1.14 mg/kg nicotine (n=7), ten minutes prior to, or 1.74 mg/kg arecoline (n=7) five minutes prior to being placed in the operant chamber, with only the drug lever present. Rats usually spontaneously initiated responding on the lever prior to drug exposure, some rats were shaped by hand if necessary. Session durations were 15 minutes. After two or three days of CRF on the drug lever, training under saline and drug conditions were alternated. Saline was administered for two consecutive days, arecoline or nicotine for two to four consecutive days, with only the state (drug or saline) appropriate lever in the chamber. At this time, a VI schedule of reinforcement was instated. The schedule was slowly increased from a VI-3 sec until rats attained a VI-12 sec on both levers, discrimination training begun at this point. For three or four of the rats in each group, the left lever was the saline correct lever, and right lever was the drug correct lever. The conditions were reversed for the remaining rats. These doses of drug were chosen since they proved optimal for discrimination learning in prior experiments.

Discrimination training. Both levers in chambers. Rats were injected with drug or saline five or ten minutes (depending on the drug) before being placed in the operant chamber. Both levers were in the chamber. Responses on the state correct lever were reinforced on a VI-12 second schedule. Responses on the incorrect lever had no scheduled consequence. Saline and drug injections were administered on a double-alternation procedure (d, d, s, s, etc.). Responses on each lever as well as total reinforcements received were automatically recorded. Discrimination was assessed during a two-minute non-reinforced period that began the first day of each alternation. Once discrimination had stabilized (10–15 double alternations) experiments investigating the interaction of physostigmine with the DS properties of arecoline and nicotine were conducted.

Specific Experiments

Experiment A. Interaction of physostigmine with discriminative stimulus elicited by arecoline and nicotine. Nicotine and arecoline dose-response relationships, with and without physostigmine pretreatment, were carried out in animals trained to discriminate either arecoline or nicotine. Physostigmine or saline was administered (SC) 25 minutes prior to testing. Arecoline and nicotine were administered five and ten minutes prior to testing, respectively. The different test conditions were presented in a counter-balanced sequence. Previous studies had demonstrated that ACh levels in rat brain were maximally elevated 25 minutes after physostigmine administration [11].

The dose of physostigmine used (0.125 mg/kg) was selected from pilot studies as one that did not completely disrupt responding. The interaction of neostigmine with the DS effects of arecoline was assessed after Experiment B was completed. The dose of neostigmine used (0.10 mg/kg) was equimolar with the dose of physostigmine (0.125 mg/kg) used. Neostigmine was administered (SC) 25 minutes prior to testing. Arecoline (0.58 mg/kg) was administered five minutes prior to testing. Discrimination was assessed during nonreinforced sessions. Responding animals were removed from the chambers after 2 minutes or after five responses were emitted if animals took longer than 2 minutes to respond. Test sessions for nonresponding subjects were extended to maximum of 15 minutes, after which the rat was removed and considered disrupted. The data from these rats were not included in any statistical analysis.

Experiment B. Generalization of nicotine and/or arecoline to physostigmine in rats pretreated with specific cholinergic antagonists. The generalization of physostigmine, administered alone, and with different antagonist combinations in rats trained to discriminate arecoline or nicotine, was assessed. For a description of the approach used, see Table 1. Pilot experiments demonstrated that when administered to rats trained to discriminate arecoline or nicotine, physostigmine (0.25 mg/kg) completely disrupted the responding of most animals. Thus, in the present experiments, nicotine-trained rats were pretreated with hexamethonium (1.0 mg/kg) and either atropine sulfate (4.0 mg/kg) or atropine methylnitrate (2.0 mg/kg) in an attempt to antagonize the peripheral nicotinic and central and peripheral muscarinic effects of physostigmine. Arecoline-trained rats were pretreated with atropine methylnitrate (2.0 mg/kg) and mecamylamine (1.0 mg/kg) in an attempt to antagonize the peripheral muscarinic central and nicotinic effects of physostigmine. The most noticeable peripheral effects produced by physostigmine were salivation, diarrhea (muscarinic stimulation) and muscle fasiculation (nicotinic stimulation). Drugs were administered using the same time parameters as described in the previous section.

Drugs Used in These Studies

The following drugs were used in these experiments. Arecoline hydrobromide (Chemical Dynamic Co., Plainfield, NJ), atropine methylnitrate, atropine sulfate, and hexamethonium chloride (Sigma Chemical Co., St. Louis, MO), mecamylamine hydrochloride (Merck, Sharp, and Dohme, West Point, PA), and optically pure (−)-nicotine di-l-tartrate (synthesized and kindly supplied by Dr. Everette L. May), were obtained as the salt. Neostigmine methylsulfate (Hoffman La Roche, Nutley, NJ) and physostigmine salicylate (O'Neal, Jones, and Feldman, St. Louis, MO) were obtained in aqueous solution from the hospital pharmacy in injection vials. All drugs were diluted with 0.9% saline to a concentration that resulted in an injection volume of 1 ml/kg body weight. All injections were made SC and all drugs were administered as the salt.

Free base equivalents of the salt (mg/kg) of the drugs used in the present study are as follows: arecoline HBr (1.74=1.14), nicotine bitartrate (1.14=0.40), neostigmine methylsulfate (0.10=0.07), physostigmine salicylate (0.125=0.08), atropine methylnitrate (4.0=1.58), and atropine sulfate (4.0=3.3). These values are provided for comparisons to other studies which presented data as free base [10].

Data Analysis

The discrimination data derived from the nonreinforced test periods were presented as percent drug bar responding (%DBR) which is calculated as the responses on the drug correct lever/total responses. Response rate data was presented as responses/minute (RPM). Data was analyzed using either paired Student's t-test or treatment-by-treatment by subjects analysis of variance. The mean of test replications for each animal was used to determine the group mean ± S.E.M.

RESULTS

Effects of Physostigmine on the DS Effects of Arecoline and Nicotine

The results of experiments attempting to alter the DS effects of arecoline and/or nicotine via physostigmine (0.125 mg/kg) pretreatment appear in Fig. 1. At this dose physostigmine, by itself, did not alter %DBR in either arecoline or nicotine trained rats. Two doses of nicotine and arecoline were evaluated following physostigmine pretreatment, one which produced <20% DBR, and one which approximated an ED50 dose in each drug discrimination group. Physostigmine potentiated the arecoline-induced DS (2–5 fold), while the nicotine-induced DS was unchanged.

The interactions of physostigmine with the arecoline and nicotine dose-effect relationship was analyzed by a treatment-by-treatment-by-subjects analysis for each training drug. For both analyses, the factors analyzed were dose (of nicotine or arecoline) and pretreatment condition (saline or physostigmine). For the nicotine-physostigmine interaction, there was a significant dose effect, $F(1,6)=4.45$, $p<0.05$, indicating a dose response relationship. However, the pretreatment condition, $F(1,6)=1.0$, $p<0.2$, and the treatment by dose interaction, $F(1,6)=1.22$, $p<0.2$, were nonsignificant. These results indicated that neither saline nor physostigmine pretreatment affected the nicotine dose-response relationship.

The arecoline-physostigmine interaction resulted in a significant effect of dose, $F(1,6)=15.1$, $p<0.001$, and pretreatment factors, $F(1,6)=32.4$, $p>0.001$, indicating a significant facilitation of the arecoline dose-response relationship by physostigmine. The pretreatment by dose interaction was nonsignificant ($F<1$, $p>0.2$), indicating that the dose-effect relationship was not different between the two pretreatments.

After the completion of the above experiment, the interaction of neostigmine with the DS effect of arecoline was assessed in six rats. This was carried out to determine if physostigmine was producing its effects through the inhibition of the metabolism of arecoline. Administration of 0.1 mg/kg neostigmine methylsulfate, a peripheral cholinesterase inhibitor (the dose is equimolar to the dose of physostigmine used) 25 minutes prior to administration of 0.58 mg/kg arecoline, produced a 25.6±15.9% DBR. This is similar to the %DBR observed in these rats after 0.58 mg/kg arecoline alone (9.0±4.5%) and with physostigmine pretreatment (51.3±14.8%).

Generalization of the Arecoline-Induced DS to Physostigmine

The generalization of physostigmine to the discriminative stimulus effects of areocline is presented in Table 2. Administration of physostigmine (0.125 mg/kg) after pretreatment

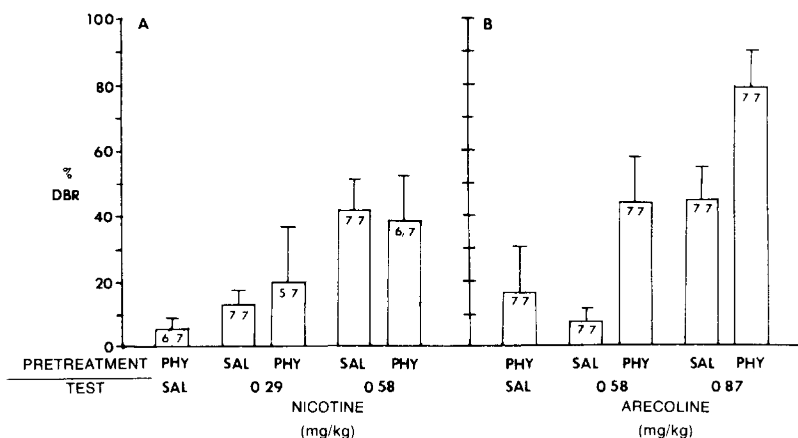

FIG 1 Interactions of physostigmine with the Discriminative Stimulus properties of nicotine (left panel) and arecoline (right panel) Numbers inside the bars indicate the number of rats completing response requirement/number tested Each value is the group mean±SEM of one drug administration in each rat

TABLE 2

GENERALIZATION OF THE DISCRIMINATIVE STIMULUS PROPERTIES OF ARECOLINE TO PHYSOSTIGMINE AND THE ANTAGONISM OF THIS GENERALIZATION BY ATROPINE

Cholinergic Antagonist* (mg/kg)	Cholinergic Agonist (mg/kg)	N†	R‡	RPM§ ±SEM	% DBR§ ±SEM
Saline	Sal (1 ml)	6/6	2	15 3 ± 3 8	2 9 ± 1 7
	Are (1 7)	6/6	2	3 9 ± 1 4	92 0 ± 4 3
MeAt (2 0)	Sal (1 ml)	6/6	1	27 0 ± 10 8	0 ± 0
+ Mec (1 0)	Are (1 7)	6/6	2	4 8 ± 2 3	76 6 ± 7 6
	Phy (0 25)	4/6	2–4	3 8 ± 2 1	66 8 ± 12 6¶
At (4 0)	Are (1 7)	5/6	1	1 9 ± 0 3	25 8 ± 10 2
+ Mec (1 0)	Phy (0 25)	6/6	2	4 0 ± 1 6	22 7 ± 7 6¶

*Cholinergic antagonists were administered 10 min prior to physostigmine (Phy) or 30 minutes prior to the training drugs arecoline (Are) or saline (Sal), Phy was administered 25 min prior to being tested during a 2 min test session Doses of specific agonists and antagonists [methyl atropine (MeAt), atropine (At) and mecamylamine (Mec)] appear in parentheses

†N=number of rats completing responses required/number tested

‡R=replications of each experiment

§Data are presented as % drug-correct responding (% DBR) All data are presented as mean ± standard error of the mean RPM=responses/min

¶Significantly different from each other, $p<0\ 01$

with atropine methylnitrate and mecamylamine produced 29% DBR. The effects of 0 25 mg/kg physostigmine were assessed after pretreatment with atropine methylnitrate (2 0 mg/kg) and mecamylamine (1 0 mg/kg) When tested 45 and 25 minutes after physostigmine administration, the percent DBR was approximately 40% and 67% respectively. Increasing the dose of physostigmine to 0 5 mg/kg completely disrupted the responding of all rats Pretreatment of rats with atropine sulfate (4 0 mg/kg) and mecamylamine (1 0 mg/kg) significantly decreased the percent DBR produced by physostigmine (0 25 mg/kg) Pretreatment with atropine methylnitrate and mecamylamine did not affect the percent DBR after saline, but did decrease the percent DBR after the training dose of arecoline

After atropine methylnitrate and mecamylamine pretreatment, the percent DBR for physostigmine (0 25 mg/kg) and arecoline (1 74 mg/kg) were similar (approximately 70% DBR), although response rates were below the baseline discrimination rates for arecoline In addition, injection of atropine sulfate (4 0 mg/kg) and mecamylamine (1 0 mg/kg) antagonized the discrimination produced by physostigmine and arecoline to a similar extent (approximately 25% DBR)

Nicotine as a DS Lack of Generalization to the Physostigmine-Induced DS

Experiments designed to evaluate the possible generalization of nicotine to physostigmine are presented in Table 3 When administered alone, physostigmine (0 125 mg/kg)

TABLE 3

LACK OF GENERALIZATION OF NICOTINE TO THE DISCRIMINATIVE STIMULUS EFFECTS OF PHYSOSTIGMINE

Cholinergic Antagonist (mg/kg)	Cholinergic Agonist* (mg/kg)	N†	R‡	RPM§ ±SEM	% DBR§ ±SEM
Saline	Sal (1 ml)	7/7	2	15 1 ± 2 9	1 0 ± 0 6
	Nic (1 14)	7/7	2	22 6 ± 7 3	90 7 ± 5 2
Hex (1 0)	Sal (1 ml)	7/7	1	16 7 ± 5 0	0 ± 0
+ At (4 0)	Nic (1 14)	7/7	1	22 2 ± 6 8	92 8 ± 3 2
	Phy (0 25)	7/7	3	4 1 ± 0 7	29 1 ± 12 4
	Phy (0 50)	7/7	2	7 1 ± 3 4	30 0 ± 8 0
Mec (1 0)	Nic (1 14)	7/7	1	3 8 ± 1 6	13 3 ± 8 6
+ At (4 0)	Phy (0 25)	7/7	2	2 0 ± 0 3	18 2 ± 9 6

*Cholinergic antagonists were given 10 min prior to physostigmine (Phy) or 25 minutes prior to the training drugs nicotine (Nic) or saline (Sal), Phy was administered 25 min prior to being tested for a 2 min test session Doses of specific agonists and antagonists, hexamethonium (Hex), mecamylamine (Mec), and atropine (At), appear in parentheses

†N=Number of rats completing responses required/number tested

‡R=Replications of each experiment

§Data are presented as % drug-correct lever responding (% DBR) All data are presented as mean ± standard error of the mean RPM=responses/min

produced approximately 5% DBR and only slightly decreased response rates compared to saline Administration of 0 25 mg/kg physostigmine by itself (not presented), completely, disrupted the responding of three out of four rats tested, and was not tested further. Thus, rats trained to discriminate nicotine were pretreated with hexamethonium (1.0 mg/kg) and either atropine methylnitrate (2 0 mg/kg) or atropine sulfate (4.0 mg/kg) in an attempt to partially block some of the peripheral nicotinic and peripheral and central muscarinic effects of physostigmine Pretreatment with atropine methylnitrate and hexamethonium prior to physostigmine administration did not block the disruptive effects of 0 25 mg/kg physostigmine (three out of seven rats responded) indicating a central action for the rate suppressant effect of physostigmine All rats pretreated with either 4 0 or 8 0 mg/kg atropine sulfate and 1 mg/kg hexamethonium prior to physostigmine (0 25 mg/kg) responded, but response rates were still depressed Due to the observed group variability on percent DBR with physostigmine, some antagonist-physostigmine interactions were replicated two or three times in each animal A mean value was calculated for each animal and were averaged to derive the group mean and standard error of the mean Approximately 30% DBR was observed with the atropine, hexamethonium, and 0.25 mg/kg physostigmine treatment. No change in percent drug bar responding was observed when physostigmine (0 25 mg/kg) was administered 45 minutes prior to testing Increasing the dose of physostigmine to 0 5 mg/kg did not increase the percent DBR Pretreatment with atropine sulfate and the central nicotinic antagonist mecamylamine did not affect the percent DBR produced by physostigmine administration Neither the discrimination level nor response rates after saline and nicotine (1 14 mg/kg) were affected by pretreatment with atropine and hexamethonium Pretreatment with atropine and mecamylamine antagonized the % DBR produced by nicotine administration, demonstrating that this antagonist combination can block a centrally mediated nicotine effect

DISCUSSION

The results of the present series of experiments demonstrate that the DS properties of arecoline, but not nicotine, can be potentiated by and generalize to physostigmine The ability of physostigmine to potentiate the DS effect of low doses of arecoline (Fig 1) is thought to be due mainly to the inhibition of degradation of ACh by acetylcholinesterase Thus, the ACh which is protected from hydrolysis can then interact with the central muscarinic receptors at which arecoline is acting, producing a response summation [11] The potentiation of the DS effect of arecoline by neostigmine (Experiment A, data not shown), although not as great as physostigmine, indicates that peripheral cholinesterase inhibition may also be involved in this interaction Arecoline has a carboxylic ester group that may be susceptible to hydrolysis by esterases Inhibition of the metabolism of arecoline may therefore be a factor in the potentiation of the DS effect of arecoline by cholinesterase inhibitors However, no studies have investigated if (1) arecoline is hydrolyzed by esterases, or (2) cholinesterase inhibitors can affect the hydrolysis of arecoline

The ability of physostigmine, administered after peripheral muscarinic and central and peripheral nicotinic antagonists, to generalize (approximately 70% DBR) to the arecoline DS, provides additional evidence for a cholinergic innervation of the muscarinic receptors that mediate the effects of arecoline (Tables 1 and 2) The cholinergic specificity of this interaction was further demonstrated by the antagonism of the arecoline-like DS effects of physostigmine by atropine sulfate

The failure of physostigmine to potentiate or generalize with the DS effects induced by nicotine indicates that this action of nicotine is not mediated through the release of ACh The data also indicate that there may be a lack of a cholinergic innervation to the receptors that mediate the DS effect of nicotine An alternate explanation is that the

nicotinic cholinergic system has a low level of spontaneous activity. The ability of physostigmine to enhance the action of ACh is dependent on ACh release and hence neuronal activity Thus, if the nicotinic cholinergic system has a low level of spontaneous activity, then physostigmine would not be able to greatly potentiate or mimic stimulation of the system by exogenous nicotinic agents However, under the confines of these experiments, arecoline and nicotine appear to be acting as agonists on different receptor populations in which only one (arecoline sensitive sites) is cholinergic in nature

In support of these conclusions, Rosecrans *et al* [11] have demonstrated that atropine, but not methyl atropine, completely antagonized the physostigmine-induced disruption of avoidance behavior (dose and time parameters were similar to those used here) without affecting the increase in brain ACh via cholinesterase inhibition Thus, arecoline stimulus generalization to physostigmine is probably mediated via ACh at a common cholinergic receptor Furthermore, the fact that atropine completely antagonized physostigmine's effects on avoidance behavior provides additional support for the idea that these increases in ACh may result in only a muscarinic cholinergic stimulation If nicotinic receptors were also involved, then one might anticipate only a partial antagonism by atropine Consistent with these latter findings, other investigators examining the interaction of selective cholinergic antagonists with physostigmine have demonstrated that the central effects of cholinesterase inhibition were mediated through muscarinic, but not by nicotinic receptors, these effects were antagonized by atropine or scopolamine, not by mecamylamine [8,18] In retrospect then, there seems to be little *in vivo* evidence that ACh can elicit an affect at nicotine receptors, and thus, it is not surprising to find that the nicotine-induced DS may not be mediated via a cholinergic receptor

The notion that nicotinic receptors may not be non-cholinergic in nature, however, is not new Abood *et al* [1] have made a similar suggestion based upon the failure of nicotinic receptor antagonists to compete with the binding of ^{3}H(−)-nicotine to brain tissue mecamylamine did not reduce ^{3}H(−)-nicotine binding, but did attenuate its behavioral effects as demonstrated here (Table 2) Sershen *et al* [14] have come to a similar conclusion In contrast, Romano and Goldstein [9] provided data that stereospecific nicotine binding can be identified centrally which appears to be cholinergic in nature These workers find that nicotine binding can be displaced by ganglionic nicotinic agonists, but like Abood *et al* [1] and Sershen *et al* [14], found nicotinic antagonists unable to specifically compete for those same binding sites Schwartz *et al* [13] using an analogous (but *in vitro*) strategy to that employed in this investigation, studied ^{3}H-ACh binding in rat brain tissue incubated with physostigmine and atropine, thus, nicotinic receptors were the only binding sites available to ^{3}H-ACh The findings of these latter workers are quite compatible with the drug discrimination studies reported here, and with those of Abood *et al* [1] and Sershen *et al* [14] Two observations by Schwartz *et al* [13] are important to understanding the interactions of nicotine centrally First, nicotine does appear to compete with ACh at some similar binding sites in equivalent concentrations (Ki values for ACh and (−)-nicotine were 7 6 nM and 6 4 nM, respectively) Secondly, mecamylamine was unable to effectively compete with ^{3}H-ACh (822,000 nM) When taken together, these findings suggest that nicotine may be acting at two cholinoceptic receptors, one with a cholinergic innervation (N_1-Ch receptor), and one which is non-cholinergic in nature (N_2-Ch receptor)

The lack of generalization of the nicotine-induced DS to ACh increases, mediated via cholinesterase inhibition (Table 3), suggests that the nicotine-mediated DS may occur at sites which are non-cholinergic in nature This is supported further by the inability of mecamylamine to compete with either ^{3}H(−)-nicotine and/or ^{3}H-ACh binding sites, which may also suggest that the antagonism of the effects of nicotine could be occurring at some site involving a second neuron Nicotine, therefore may still be ultimately acting at a cholinergic synapse, but one which is innervated via a non-cholinergic interneuron sensitive to mecamylamine As pointed out earlier in this discussion, the turnover of ACh presynaptically may be too slow at nicotinic-cholinergic receptors to permit a significant nicotine-like DS effect via physostigmine, and thus, it cannot be concluded that nicotine is completely devoid of any cholinergic effect centrally An alternative hypothesis has been put forward by Stolerman [16] which suggests that these nicotinic receptors may reside on ion channels regulated via two distinct sites, one sensitive to mecamylamine but ACh insensitive, the other sensitive to both nicotine and ACh The present investigation, while providing data that nicotine may be acting at a non-cholinergic neuron, also points out the need for additional research in this area before these issues can be clarified

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