ABSTRACT The prototypic nicotinic acetylcholinergic receptor (nAChR) agonist, nicotine, is one of the primary psychoactive ingredients of tobacco. This review examines the effects of nicotine and similar compounds in nicotine discrimination procedures, a paradigm in which the subjective effects of nicotine can be modeled in animals. Results of these studies have shown that the discriminative stimulus effects of nicotine are shared by nicotine analogues and by the novel nicotinic agonists, epibatidine and ABT-418, and can be antagonized by the nAChR channel blocker, mecamylamine. Individual and strain differences in sensitivity to the discriminative stimulus effects of nicotine have been noted, suggesting the possibility of genetic differences in the functioning of nACh receptors or related processes. In human smokers, individual variability is also observed; however, the degree of within smoker (but between situation) variability far exceeds between smoker variability. Further research into the ways in which situational factors affect the discriminative stimulus effects of nicotine is needed. Drug Dev. Res. 38:222-230 © 1996 Wiley-Liss, Inc.

Key words: nicotine; epibatidine; ABT-418; discrimination; review

INTRODUCTION

Nicotine, the prototypic nicotinic acetylcholinergic receptor (nAChR) agonist, is one of the primary psychoactive ingredients of tobacco. Although the carbon monoxide and carcinogens in tar inhaled during cigarette smoking have far worse consequences on human health than does nicotine, this psychoactive drug appears to be the pharmacological agent responsible for the maintenance of human tobacco use [Rosecrans, 1995; Stolerman and Jarvis, 1995]. In addition to the ability of nicotine to induce paradoxical effects on behavior in both rats and humans (either increase or decrease arousal levels contingent on baseline rates of behavior) [Hendry and Rosecrans, 1982], nicotine has putative effects on other centrally mediated processes, including attention, cognition, pain, and motivation [Damaj et al., 1994b; Jarvik, 1991; Levin, 1992; Sahakian et al., 1989; Warburton, 1992]. These effects have caused much attention as to the potential therapeutic uses of nicotine or nicotine-like drugs. A question not yet answered, however, is whether it is possible to develop nAChR agonists that retain the therapeutic effects of nicotine without also incurring its other behavioral and/or autonomic effects or its potential to produce behavioral dependence.

One approach to evaluating potential new nicotine-like (or antagonist) compounds is to test novel...
compounds to determine whether their subjective effects are similar to those of nicotine, particularly at doses at which potential therapeutic effects are produced. The subjective effects of nicotine can be measured using drug discrimination approaches. In this procedure, the subject can be trained to respond when nicotine is received and another response when placebo or vehicle is received (for more details, see next section). Previous research has demonstrated that discrimination with nicotine (and other psychoactive drugs) can be carried out in a variety of species, including humans [Kallman et al., 1982; Perkins et al., 1994], rats [Rosecrans, 1989; Stolerman et al., 1984], monkeys [Takada et al., 1989], and mice [Varvel et al., 1996], although rats are most commonly used. A high degree of concordance between the results of drug discrimination studies with animal subjects and the results of this type of study with human research participants has been observed, suggesting that rat drug discrimination may serve as an animal model of the subjective effects of psychoactive drugs in humans [Kallman et al., 1982; Balster, 1990; Kamiel et al., 1993].

The purposes of the present paper are (1) to review the results of recent studies that have tested novel nicotine-like compounds in nicotine discrimination procedures; and (2) to summarize recent findings that suggest a large degree of individual and strain variability in the discriminative stimulus effects of nicotine. An overview of nicotine discrimination methodology will precede discussion of research results.

NICOTINE DISCRIMINATION METHODOLOGY

The general training procedure for nicotine discrimination studies has used a two-lever operant procedure involving the reinforcement of responses on one lever following administration of a specified (training) dose of nicotine and reinforcing responses on a second lever following administration of vehicle (Fig. 1). With consistent pairing of the type of injection and the position of the reinforced lever, animals can be trained over a number of days to respond reliably on the injection appropriate lever. In a well-trained animal, the percentage of total responses on the nicotine associated lever approaches 90–100% when the animal is given the training dose of nicotine and approaches 5–10% when the animal is injected with vehicle.

After animals have acquired the discrimination, substitution tests may be performed by injecting the animal with different drug or with different doses of nicotine (in addition to the training dose) to determine the sensitivity of the subject to nicotine or to determine the similarities of these novel agents to nicotine. Nicotine antagonism studies are conducted by preadministering receptor antagonists 10–30 min before evaluating the ability of subjects to detect nicotine during nicotine test sessions. Common measures of the degree to which the substituted drug shares discriminative stimulus properties with nicotine are (1) percentage of nicotine lever responding, or (2) percentage of animals that obtain their first reinforcer by pressing the nicotine lever.

The discriminative stimulus properties of nicotine and other drugs serving as discriminative stimuli are mediated by receptors located in the central nervous system [Rosecrans and Glennon, 1979; Rosecrans, 1989; Balster, 1990]. First, peripherally acting drugs that have minimal central effects are less effective in producing stimulus control in drug discrimination experiments [Schechter and Rosecrans, 1972; Balster, 1990]. Second, the discriminative stimulus effects of a drug can be antagonized by central receptor blockers, but not by peripheral receptor blockers [Schechter and Rosecrans, 1971; Stolerman et al., 1983; Martin et al., 1993]. Finally, central administration of a small amount of a drug can produce stimulus control in drug discrimination procedures [Rosecrans and Meltzer, 1981]. Another characteristic of discriminative stimulus effects of drugs is cross-generalization between drugs that act at the same receptors [Rosecrans and Glennon, 1979; Balster, 1990]. Substitution of other drugs of the same class or with different doses of the training drug produces a dose-dependent generalization gradient, similar to those produced by different intensities of an exteroceptive stimulus.

Because the percentage of responding on the nicotine lever is not entirely independent of the total number of responses that occur on either lever, determination of the degree of stimulus generalization of substituted drugs requires consideration of response rate as a secondary factor. For example, high doses of many drugs, including nicotine, produce a decrease in response rate (an unconditioned response). In tests where the substituted drug produces a high percentage of responding on the nicotine lever and a response rate that does not differ from vehicle levels, the conclusion that the substituted drug shares discriminative stimulus properties with nicotine is straightforward; however, in cases in which a high percentage of nicotine-lever responding is accompanied by a significant decrease in overall response rate, the discriminative stimulus and unconditioned stimulus effects of the drug are confounded and either may offer a potential explanation of the observed effects of the drug on responding. In many instances, large decreases in...
Fig. 1. Nicotine discrimination training and testing procedure in rats. (Drawn by Mary Tokarz.)

response rate are accompanied by intermediate levels of drug-lever responding, possibly reflecting a disruption of discrimination. Thus, the degree to which the substituted drug shares discriminative stimulus properties with nicotine cannot be determined with certainty under conditions of low response rates.

MECHANISMS OF THE NICOTINE DS: RECEPTOR AND BRAIN AREA SITES

Before attempting to evaluate the mechanism of action of how nicotine is able to elicit DS control of behavior, it should first be realized that the central cholinergic system is composed of two types of receptors, muscarinic (mACh) and nicotinic (nACh). Unlike the autonomic nervous system, brain receptor and neuron types are not strictly serial in character, but appear to present a parallel arrangement. Thus, scopolamine or atropine (mACh competitive antagonists) are able to block the DS elicited by the mACh agonists such as arecoline or oxotremorine but unable to block the effects of nicotine (Table 1). By contrast, mecamylamine is unable to block the DS generated by mACh agonists. Peripherally acting nACh antagonists such as hexamethonium and mACh agonists such as methylscopolamine and methylatropine are unable to block their respective receptor types in the brain, supporting the notion that the DS properties elicited by cholinergic drugs are contingent upon the existence of central mACh and nACh receptors (Table 1).

Another interesting cholinergic drug is physostigmine which substitutes for the arecoline or oxotremorine, presumably via an increase of acetylcholine at sensitive mAChRs, but does not substitute for nicotine in nicotine-trained rats. These observations led to the conclusion that nicotine was acting on two types of receptors, one sensitive to nicotine and a second not innervated by ACh. While these findings
TABLE 1. Comparison of Discriminative Stimuli Generated by nAChR and mAChR Agonists*  

<table>
<thead>
<tr>
<th>DS parameter</th>
<th>nAChRs</th>
<th>mAChRs</th>
</tr>
</thead>
<tbody>
<tr>
<td>Antagonized by</td>
<td>Mecamylamine</td>
<td>Scopolamine</td>
</tr>
<tr>
<td>Not antagonized by</td>
<td>Scopolamine, atropine, hexamethonium, or 5-HT, DA, and opiate antagonists</td>
<td>Mecamylamine, methylatropine or 5-HT, DA, and opiate antagonists</td>
</tr>
<tr>
<td>Generalize to</td>
<td>3-PMP, (+)-nicotine, ABT-418 Cytisine, epibatidine</td>
<td>Oxotremorine, physostigmine, and other nAChR agonists</td>
</tr>
<tr>
<td>Does not generalize to</td>
<td>nAChR agonists or acetylcholinesterase inhibitors</td>
<td>nAChR agonists</td>
</tr>
<tr>
<td>Brain sites of action</td>
<td>Hippocampus, reticular formation, ventral tegmentum</td>
<td>Few sites sensitive</td>
</tr>
</tbody>
</table>

*Rats were trained to discriminate nicotine (0.4 mg/kg, s.c.) or arecoline (1.0 mg/kg, s.c.) vs. vehicle under a VI-15 sec schedule of food reinforcement. Generalization or antagonism of test compounds was evaluated during 2-min nonreinforced test sessions.

are intriguing, it is misleading to believe that the nicotine is not acting by mimicking ACh (directly or indirectly) at the nAChR. Recent research indicates that physostigmine may have inadvertently induced a receptor desensitization (via an increase of ACh at the nAChR); hence, rats selected the vehicle-associated lever, as they usually do when mecamylamine is administered alone in nicotine generalization studies.

Additionally, recent research indicates that nAChR's may also have a predominant presynaptic neuronal location on central noncholinergic as well cholinergic neurons [Schwartz et al., 1984; Iwamoto, 1989]. Thus, the neuronal arrangements on these neurons have a resemblance to what is observed in the autonomic nervous system (i.e., nicotinic innervation of the adrenal gland). These observations provide a base for select nicotine effects that modulate several amine containing neurons, noradrenergic (NE), dopaminergic (DA), serotonergic (5-HT), and even some opiate (enkephalinergic or β-endorphinergic) receptors. Interestingly, the nicotine-generated DS appears to be predominantly a nACh receptor effect, unmixed with other noncholinergic influences. By contrast, other pharmacological effects such as nicotine-induced cognitive enhancement [Levin et al., 1996] and the positive reinforcing effects of nicotine may be mediated via the release (indirect or direct) of presynaptic or postsynaptic DA [Corrigall et al. 1992].

The central administration of a small amount of a drug can also produce stimulus control in drug discrimination procedures [Rosecrans and Meltzer, 1981]. Areas of importance to the nicotine DS appear to involve nAChR's located in the midbrain reticular formation, dorsal hippocampus, and the ventral tegmentum (Fig. 2). Interestingly, when nicotine is injected into the nucleus accumbens (NAc), a dopamine rich area innervated by cholinergic neurons projecting from the ventral tegmentum (VTA), it is relatively ineffective in eliciting a nicotine-DS. Generalization to the peripheral DS is observed (although relatively weak) when nicotine is injected into the VTA. Important to this discussion is the observation that nicotine is able to elicit dopamine (DA) release in the NAc, but not in the VTA, indicating that dopamine release is contingent upon cholinergic neurons innervating the NAc (J.A. Rosecrans, unpublished observations). Thus, the DA system appears to play a minor role in the DS.

EVALUATION OF NICOTINE-LIKE COMPOUNDS

A number of previous reviews have summarized results of nicotine discrimination studies in rats [Decker et al., 1995; Rosecrans, 1989; Rosecrans and James, 1993; Rosecrans and Villanueva, 1991]. The studies reviewed in these manuscripts have shown that the discriminative stimulus effects of nicotine are reasonably specific to drugs that show high affinity for nicotinic acetylcholine receptors, in that, drugs that interact primarily with other neurotransmitter systems typically do not substitute for nicotine [Rosecrans, 1989; Decker et al., 1995]. (A possible exception is (+)-amphetamine, which consistently produces partial substitution for nicotine [Rosecrans, 1989].) The non-nicotinic cholinergic agonists, physostigmine and arecoline, also do not substitute for nicotine [Rosecrans, 1989]. On the other hand, the discriminative stimulus effects of nicotine are stereoselective; i.e., the (+)-isomer is 8–10 times less potent than the (−)-isomer that is typically used for discrimination training and is the active alkaloid in tobacco [Rosecrans, 1989]. Structural analogues of nicotine, including 3-methylpyridylpyrrolidine (3-PMP), cytisine, and anabasine, fully substitute for nicotine, with varying degrees of potency [Rosecrans, 1989; Rosecrans and James, 1993; Rosecrans and Villanueva, 1991]. Further, the discriminative stimulus effects of nicotine are produced by the drug itself and not by active metabolites, as these metabolites (cotine and noncocaine) produce, at best, only partial substitution or are 30–2,000 times less potent than (−)-nicotine [Decker et al., 1995; Rosecrans, 1989; Rosecrans and James, 1993]. Although the discriminative stimulus effects of nicotine are antagonized by
Fig. 2. Nicotine activates cholinergic neurons in the ventral tegmental area (A10), which causes dopamine release in the nucleus accumbens. These actions on the mesolimbic reward system are believed to contribute to nicotine’s positive reinforcing effects.

Recent nicotine discrimination research has focused on examination of the discriminative stimulus effects of newly discovered compounds with structural similarity to nicotine (Fig. 3). Two novel compounds are noteworthy: epibatidine and ABT-418 [(S)-3-methyl-5-(1-methyl-2-pyrrolidinyl)isoxazole hydrochloride]. Epibatidine is an alkaloid, originally isolated from skin extracts of a tiny Ecuadorian frog, *Epipedobates tricolor*. Subsequently, it was synthesized and is currently available as optical D- and L-isomers as well as in a racemic version. The chemical structure of epibatidine resembles that of nicotine and it possesses a similar pharmacological profile, although it is more potent. As might be expected, epibatidine binds to several known subtypes of nAChR with varying affinities but, interestingly, it also binds to an unidentified nicotinic receptor [Houghtling et al., 1994, 1995]. Damaj et al. [1994a] have tested the optical isomers of epibatidine in a two-lever nicotine discrimination procedure in rats. Their results show that both isomers dose dependently substitute for nicotine with approximately equal potencies (ED50 = 0.93 µg/kg for L and 1.0 µg/kg for D). Racemic epibatidine also substituted for (-)-nicotine in nicotine-trained rats [Sullivan et al., 1994]. The nicotine-like discriminative stimulus effects of epibatidine were
Further research is needed to determine whether the discriminative stimulus effects of ABT-418 are different in newly trained versus experienced nicotine-discriminating rats. As with epibatidine, attempts to train rats to discriminate ABT-418 from vehicle were unsuccessful [Brioni et al., 1995].

INDIVIDUAL AND STRAIN VARIABILITY

Responsivity to nicotine's various behavioral effects exhibits a large degree of individual and strain variability. For example, early studies found that the effect of nicotine on exploratory activity differed depending on initial baseline rates of behavior; that is, rats that showed high activity in a novel environment exhibited decreased activity in a second novel environment, while increased activity was observed for rats that initially showed low activity [Rosecrans, 1971]. Since several previous reviews have described individual and strain variability in responsiveness to nicotine's behavioral effects in rodents [Overstreet, 1995; Rosecrans, 1995; Rosecrans et al., 1995], this section focuses on review of recent studies that have found individual and strain variability in the effects of nicotine in drug discrimination procedures.

Acute tolerance to the discriminative stimulus effects of nicotine is one area in which individual differences have been investigated. James et al. [1994] reported that a single challenge dose of 0.8 mg/kg (s.c.) nicotine administered 15–180 min before the training dose of nicotine (0.4 mg/kg, s.c.) decreased subsequent nicotine-lever responding in some rats, but not in others. The time course of development of acute tolerance to the discriminative stimulus effects of nicotine was variable among the rats that showed the effect. Rats were classified as those that exhibited acute tolerance (desensitzers) and those that did not (nondesensitzers). This classification and interpretation of the results was an extension of previously reported observations that the pharmacological tolerance produced by chronic administration of nicotine was accompanied by nACh receptor up-regulation [Marks et al., 1983] and that this tolerance resulted from desensitization of nACh receptors [Grady et al., 1994; Rowell and Hillebrand, 1994; Wonnacott et al., 1990]. The study conducted by James et al. [1994] provided evidence that desensitization of nACh receptors (and the resulting acute tolerance) may occur following a single bolus injection of nicotine. Further, this effect occurred in only some of the rats, suggesting variability in the functioning of nACh receptors among individual rats of the same strain.

A recent investigation completed in our laboratory has confirmed that different rat strains also show...
differential sensitivity to the discriminative stimulus effects of (-)-nicotine (J.A. Rosecrans, unpublished observations). In this study, three strains of rats were trained to discriminate (-)-nicotine from saline in a standard two-lever nicotine discrimination procedure. Following acquisition, a dose-effect curve for (-)-nicotine was determined in each group of rats. A summary of the results is presented in Table 2. When the ED₅₀ values for nicotine were compared across strains, Long-Evans rats were most sensitive and Fischer-344 rats were least sensitive. In addition, the Fischer-344 rats required a higher training dose of (-)-nicotine in order to acquire the discrimination.

Variability in the discriminative stimulus effects of nicotine has also been observed in selectively bred rodent lines [Overstreet, 1995]. The Flinders line of rats has been selectively bred for sensitivity to disopropyl fluorophosphate, an anticholinesterase agent [Overstreet et al., 1979; Russell and Overstreet, 1987]. The Flinders sensitive line (FSL) exhibit heightened sensitivity to the behavioral effects of muscarinic agonists compared to the Flinders resistant line (FRL). Overstreet [1993] demonstrated that FSL rats are also more sensitive than are FRL rats to the rate suppressing effects of nicotine in an operant task. Although neither line of Flinders rats could be trained to discriminate the usual training doses of nicotine (0.2 and 0.4 mg/kg, s.c.), several FSL rats (n = 4) were trained to discriminate 0.8 mg/kg nicotine from saline (J.A. Rosecrans, unpublished observations). By contrast, only one FRL rat was able to discriminate nicotine.

Differences in sensitivity to the response rate decreasing effects of this dose of nicotine were consistent with the results of Overstreet [1993]; that is, lower response rates occurred in FSL rats compared to FRL rats. Interestingly, consistent with the results of behavioral tests, a decrease in the displacement of [³H]-nicotine binding was observed in FRL rats compared to FSL rats and both lines exhibited decreased nicotine binding compared to outbred Sprague-Dawley rats (Table 3).

Differential sensitivity to the discriminative stimulus effects of nicotine has also been observed in a line of rats selected for sensitivity to a behavioral effect of ethanol [Gordon et al., 1993]. Ethanol-prefering (P) and ethanol-nonpreferring (NP) rats are distinguished by their differing propensities to drink ethanol in an oral self-administration procedure [Lumeng et al., 1977]. When tested in an ethanol discrimination procedure, the ED₅₀ values for ethanol substitution were similar for both rat lines; however, the degree to which nicotine substituted for ethanol was greater in P rats compared to NP rats [Gordon et al., 1993]. While nicotine (0.4–1.2 mg/kg) produced a dose-dependent increase in the number of P rats choosing the ethanol-lever (up to a maximum of 58.3%), it produced exclusively vehicle-lever choices in the NP rats. A higher dose of nicotine (1.6 mg/kg) severely disrupted responding in both rat lines. This correspondence between selectively bred preference for ethanol and sensitivity to ethanol-like discriminative stimulus effects of nicotine suggests that the loci

<table>
<thead>
<tr>
<th>Strain (N)</th>
<th>Training dose (mg/kg)</th>
<th>ED₅₀ (mg/kg)</th>
<th>% nicotine correct responses</th>
<th>Response rate/sec</th>
</tr>
</thead>
<tbody>
<tr>
<td></td>
<td></td>
<td></td>
<td>Nicotine</td>
<td>Vehicle</td>
</tr>
<tr>
<td></td>
<td></td>
<td></td>
<td>Vehicle</td>
<td>Nicotine</td>
</tr>
<tr>
<td>Sprague-Dawley</td>
<td>0.4</td>
<td>0.14</td>
<td>87 ± 2</td>
<td>13 ± 2</td>
</tr>
<tr>
<td>Fischer-344</td>
<td>0.9</td>
<td>0.32</td>
<td>93 ± 3</td>
<td>7 ± 3</td>
</tr>
<tr>
<td>Long-Evans</td>
<td>0.4</td>
<td>0.07</td>
<td>92 ± 3</td>
<td>10 ± 2</td>
</tr>
</tbody>
</table>

<table>
<thead>
<tr>
<th>Strain</th>
<th>α-BG Striatumᵃ</th>
<th>[³H]nicotine Hypothalamusᵃ</th>
<th>[³H]nicotine Thalamusᵃ</th>
</tr>
</thead>
<tbody>
<tr>
<td>Control Sprague-Dawley</td>
<td>17.3 ± 5.1</td>
<td>16.0 ± 3.8</td>
<td>47.2 ± 3.30</td>
</tr>
<tr>
<td>FSL (-20%)</td>
<td>12.9 ± 3.3</td>
<td>12.9 ± 3.3</td>
<td>36.7 ± 6.4</td>
</tr>
<tr>
<td>FRL (-60%)</td>
<td>6.87 ± 0.4</td>
<td>6.12 ± 4.7</td>
<td>29.1 ± 2.3</td>
</tr>
</tbody>
</table>

ᵃValues represent numbers of nAChRs/pM of protein in 3–4 rats from each strain. Rat strains were supplied by Dr. David Overstreet (University of North Carolina) and brain area nicotine binding assays conducted in Dr. Allan Collins’s (University of Colorado) laboratory.

*%, % decrease from control.
for genes that modulate responsiveness to certain behavioral effects of ethanol and nicotine are likely to be in close proximity.

**SUMMARY AND CONCLUSIONS**

In summary, nicotine discrimination represents a method by which the subjective effects of nicotine can be modelled in animals. Results of recent nicotine discrimination studies in rats have shown that, similar to other nicotine analogues, the novel nicotinic agonists, epibatidine and ABT-418, substitute for nicotine. Further, the nicotine-like discriminative stimulus effects of nicotine and its analogues can be antagonized by the nACh channel blocker, mecamylamine. The potency at which mecamylamine blocks the discriminative stimulus effects of nicotine analogues is related to the binding affinity of these compounds at nACh receptors.

Additional research in the nicotine discrimination field has shown that there is a large degree of variability among individuals and between strains of rats. Studies with selectively bred rodent lines suggest that some of this variability may be linked to genetic differences in the functioning of nACh receptors or related processes. In addition, this research has suggested that genetic factors contributing to preference for oral ethanol may be closely associated with those contributing to ability to discriminate the nicotine-like effects of ethanol.

Clinical empirical studies have confirmed that large individual differences in responsiveness to the effects of nicotine, including its discriminative stimulus effects, also occur in humans [e.g., Jones, 1986; Perkins, 1995; Perkins et al., 1994]. In addition, a link between smoking and alcohol consumption has been demonstrated, in that the prevalence of smoking among alcoholics is higher than among nonalcoholics, as is the prevalence among abusers of other drugs [for a review, see Gilbert and Gilbert, 1995].

In conclusion, individual and strain variability in the discriminative stimulus effects of nicotine appear to be related to differences in the functioning of nACh receptors, which may, in turn, be linked with genetic differences; however, as Perkins [1995] notes, the degree of within smoker (but between situation) variability far exceeds between smoker variability. Perhaps the next step in nicotine discrimination research is to delineate major situational factors that affect the way in which nicotine is perceived. Research is also needed to determine the ways in which the discriminative stimulus effects of nicotine may contribute to its self-administration.

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**REFERENCES**


