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Characterization of Central Nicotinic Receptors by Studies on the Nicotine Cue and Conditioned Taste Aversion in Rats

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STOLERMAN, I. P. *Characterization of central nicotinic receptors by studies on the nicotine cue and conditioned taste aversion in rats.* PHARMACOL BIOCHEM BEHAV **30**(1) 235–242, 1988.—Some recent studies on the discriminative stimulus (cue) and conditioned taste aversion (CTA) effects of nicotine are reviewed. The characteristics of the nicotine cue correlate well with those of high affinity nicotine binding in studies comparing different nicotinic agonists. The dose of nicotine used for training a discrimination is an important variable determining patterns of generalization. The effects of antagonists on the nicotine cue are also compatible with ligand-binding studies although the lack of competitive antagonists generates unsolved problems for investigators. The CTA produced by nicotine has pharmacological characteristics like the nicotine cue. Both effects are produced at CNS sites that resemble to a certain extent the cholinoceptive sites in autonomic ganglia. The small differences in the degree of stereoselectivity of the two effects or in their sensitivity to antagonists do not constitute substantive evidence for mediation by different receptors. The major differences between the procedures lies in their general psychopharmacological characteristics rather than in any special qualities of the response to nicotine. For example, the nicotine cue is not produced by agents from other pharmacological classes whereas a wide range of different drugs can produce CTA. The concept of multiple types of CNS nicotinic receptors, as supported by certain biochemical studies, requires further evaluation in behavioural systems.

Nicotine Mecamylamine Chlorisondamine Hexamethonium Pentolinium Apomorphine
Anabasine Cytisine Nornicotine Quipazine Drug discrimination Conditioned taste aversion

NICOTINE is the main psychoactive constituent in tobacco smoke and its central effects provide the biological basis for tobacco addiction. In order to effectively combat this addiction, it is essential to understand the mechanisms through which nicotine brings about behavioural changes. Studies of the stimulus properties of nicotine can directly address this question; it is the ability of a drug to generate effects that can be identified by an individual, and that can lead to reinforcement or punishment of behaviour, that serves as the interface between actions at the receptor level and perceptions of its effects that lead to repeated use. This paper compares the discriminative stimulus (cue) and conditioned taste aversion (CTA) effects of nicotine with respect to the types of receptors involved. Recent general reviews of work in these areas have been provided by Henningfield and Goldberg [10] and Stolerman [32].

Romano and Goldstein [23] provided evidence for a high-affinity nicotine binding site in brain tissue, with characteristics resembling those expected for a functional receptor. These observations have been confirmed and considerably extended by several other groups. The regional distribution of the binding sites has been studied in detail by means of autoradiographic techniques [4]. Nevertheless, questions have been raised as to the functional significance of these sites. The main, persisting, causes for concern have been the results with antagonists, and the extremely high affinity of the sites. Ganglion-blocking drugs such as mecamylamine, which reliably block most central effects of nicotine, are very low in potency as inhibitors of nicotine binding. This objection makes the questionable assumption that the antagonists act competitively. The difficulty with the affinity of the binding sites arises because it implies that they are saturated at concentrations of nicotine in the nanomolar range, whereas "smoking" doses of nicotine are associated with micromolar brain concentrations. The K_D for nicotine binding in rat brain is typically about 5 nM, whereas the plasma concentrations of nicotine in cigarette smokers who inhale is typically around 200 nM [27], and the drug is further concentrated in brain. This discrepancy cannot be due to species differences since plasma concentrations of nicotine

around 200 nM seem to be necessary to produce marked stimulus effects in rats. However, these arguments make the assumption that the kinetic parameters of nicotine binding *in vivo* would be like those found in the published studies carried out entirely *in vitro*.

Early studies showed convincingly that nicotine could serve as a discriminative stimulus in both T-maze and operant conditioning paradigms [18,19]. Subsequently, Rosecrans and his colleagues clearly showed that the discriminative effects of nicotine were different from those produced by muscarinic-cholinergic agonists, and they systematically studied the pharmacological basis for nicotine discrimination [25,26]. A crucial finding was that the nicotine discriminative stimulus was fully and specifically blocked by ganglion-blocking drugs that penetrated well into the brain. The work clearly pointed to mediation by CNS receptors that were different from those for any of the classical neurotransmitters except acetylcholine. However, this work was mainly carried out before studies of the binding of nicotinic ligands had provided many testable hypotheses about the nature of brain nicotinic receptors, and there were very few structural analogues of nicotine available for comparative study. This paper outlines more recent experiments on the nicotine cue that have been directed at possible correlations with the binding characteristics of tritiated nicotine to rat brain membranes *in vitro*.

Like other psychoactive drugs, nicotine can serve several stimulus functions. Any aversive effects that it has might serve to limit the total exposure of tobacco users to the drug. The possibility exists of capitalising on these aversive effects to develop new therapeutic regimens. Thus, it becomes essential to know whether the receptor sites and brain mechanisms involved in the aversive effects are similar to or different from those mediating the positive reinforcing effects. Nicotine has aversive properties in squirrel monkeys, as shown in punishment and negative reinforcement paradigms [9,30]. This paper considers its effects in conditioned taste aversion (CTA) procedures in rats, which provide another putative index of aversive action. Traditionally, CTA has been interpreted as evidence for the formation of associations between flavour stimuli and noxious effects of drugs (e.g., nausea or gastrointestinal disturbance). Studies with emetic drugs such as nicotine might therefore help to clarify the role of emetic mechanisms in CTA [11,13].

METHOD

Subjects

Male hooded rats initially weighing 220–320 grams were used in all experiments. They were housed individually in rooms maintained at 20–22°C and a regular light-dark cycle was employed (light from 8 a.m. to 8 p.m.).

Discriminative Stimulus Experiments

Standard experimental chambers containing two response bars and a device for presenting food pellets were used. Access to food was restricted to limit the weights of the rats to 80% of those under free-feeding conditions. After preliminary training to establish a baseline of responding, discrimination training began. Rats were trained to discriminate nicotine (0.1 or 0.4 mg/kg) from saline. All injections were given subcutaneously, 15 min prior to 15-min sessions. Half of the rats received food for pressing the left bar after nicotine injections and the remaining rats received food for pressing the right bar after nicotine injections. Responses on the opposite bar produced food after saline injections. Drug and saline training sessions took place in randomised sequences, 5 days a week, with one session per day, to a total of 40–60 sessions. The schedule of reinforcement was tandem VI-1 FR-10; in this schedule, the tenth consecutive press on the correct bar produced food after a variable interval of time averaging 1 min. Rats solve this task by using the presence or absence of nicotine as a cue indicating which of the two bars will produce food in a given session.

When training was complete, tests of responses to nicotine or other drugs were carried out twice weekly in groups of 6–8 rats. On test days, rats were tested for 5 min, with no food presented regardless of which bar was pressed (extinction tests). Training continued on intervening days to maintain the baseline of discriminative performance. Dose-response studies were carried out with each drug dose or vehicle tested once in each rat, with different treatments normally being administered in random order. Full details of the training and testing procedures have been published previously [21,33].

Results are presented as the number of responses on the bar appropriate for the training drug, expressed as a percentage of the total number of responses on both bars. The total number of responses serves as an index of response rate. These scores are analysed by means of repeated measure analyses of variance and by *t*-tests for multiple comparisons with a control group.

Conditioned Taste Aversions

Experiments were carried out in the rats' living cages, after a period of adaptation during which access to water was restricted to 1 hr per day. One of two flavoured solutions (sodium saccharin 0.1% or sodium chloride 0.9%) was presented for 15 min on every second day. The two flavoured solutions were presented alternately, and thus each flavour was presented to a given rat on every fourth day. Immediately afterwards, the rats were injected subcutaneously with drug or saline (flavour-injection "pairing"). For half of the rats in which a given dose was tested, one flavour was repeatedly paired with that dose whereas the other flavour was paired with vehicle. These arrangements were reversed in the remaining rats to balance out effects due to the inherent palatabilities of the flavours. After completion of the conditioning phase of the experiments, comprising a total of 2–8 flavour-injection pairings, the drug- and vehicle-paired flavoured solutions were presented simultaneously for 15 min (two-stimulus test). Two-stimulus tests provided the main measure of CTA since they were known from previous studies to be more sensitive than one-stimulus tests. The index calculated is the intake of the drug-paired flavoured solution expressed as a percentage of total fluid intake, and CTA is shown by scores significantly below 50%. Fuller details of procedures have been published previously [13].

CHARACTERISTICS OF THE NICOTINE DISCRIMINATIVE STIMULUS

Generalisation Tests With Nicotinic Agonists

Following administration of the dose of nicotine used for training, the number of responses on the nicotine-appropriate bar was typically about 85%, as compared with 5% after saline. The response to nicotine was strongly related to dose, and the threshold for an effect was typically about one-tenth of the dose used for training. In early exper-

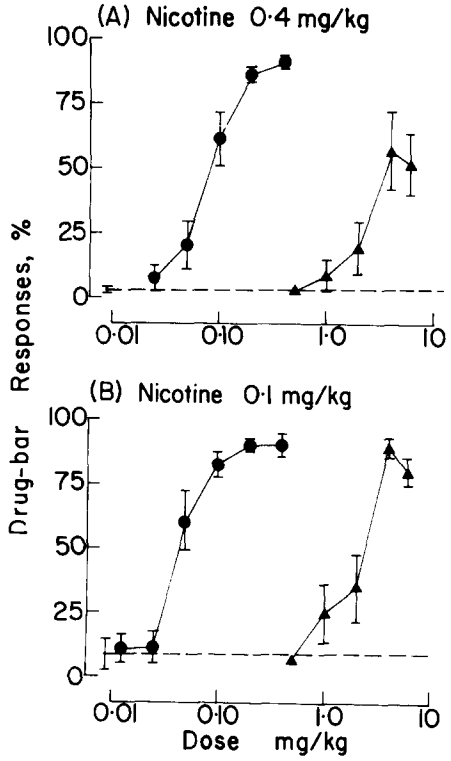

FIG. 1. Dose-response curves for nicotine (●) and anabasine (▲) in rats trained to discriminate 0.4 mg/kg (A) or 0.1 mg/kg (B) of nicotine from saline [33]. Results for control tests after saline injections are indicated by the horizontal dashed lines. All data obtained during 5-min extinction tests (n=8). Vertical bars in this and subsequent figures show ±s.e.m.; overlapping bars and those shorter than diameters of symbols are omitted.

iments, a 0.4 mg/kg dose of nicotine was used for training, and Fig. 1A shows a dose-response curve typical of those obtained under such conditions. These powerful discriminative effects of nicotine are accompanied by barely detectable reductions in the total numbers of responses [21,33].

Certain structural analogues of nicotine potently inhibit the high-affinity binding of nicotine to rat brain, and included among these drugs are anabasine (a tobacco alkaloid) and cytisine (laburnum alkaloid). In rats trained to discriminate a 0.4 mg/kg dose of nicotine, these alkaloids produced no more than 60% drug-appropriate responding [21]. This was an unsatisfactory outcome since mean scores close to 50% are difficult to interpret and may indicate either a weak nicotine-like effect or random responding. However, it was known from work with other classes of drugs that the characteristics of drug-produced cues could be highly dependent on the dose used for training and, therefore, direct comparisons were made in rats trained on different doses of nicotine [33]. Figure 1A shows the partial generalisation to anabasine in rats trained on nicotine (0.4 mg/kg), in contrast to the full generalisation when the training dose of nicotine was reduced to 0.1 mg/kg (Fig. 1B). Similar results were obtained with cytisine, as shown in Fig. 2 [33].

The small, 0.1 mg/kg training dose of nicotine was, therefore, associated with a more convincing correlation with the ligand-binding studies than was the previous standard training dose of 0.4 mg/kg. Pratt *et al.* [21] studied the relationship between the peak plasma concentration of nicotine and the

TABLE 1

CHARACTERISTICS OF THE NICOTINE DISCRIMINATIVE STIMULUS: DRUGS WHICH CAN BE GENERALIZED WITH NICOTINE

Reference	Test Drugs
Chance *et al.* [2]	(3)-Pyridyl-methylpyrollodine
Garcha *et al.* [6]	(−)- and (+)-nornicotine, (+)-nicotine
Meltzer *et al.* [17]	(+)-Nicotine
Romano *et al.* [24]	Anabasine, (+)-nicotine
Stolerman *et al.* [33]	(−)-Anabasine, cytisine

administered dose in rats of the same sex, strain and age as those in the behavioural studies. The 0.4 mg/kg training dose produced plasma concentrations around 140 ng/ml, whereas 0.1 mg/kg of nicotine produced concentrations of 35 ng/ml. In cigarette smokers who inhaled, plasma nicotine concentrations ranged from 4–72 ng/ml with a mean concentration in the regions of 30 ng/ml [27]. These values are of similar magnitude to those produced by the 0.1 mg/kg training dose of nicotine. According to this criterion, 0.4 mg/kg may be too large a dose for effects equivalent to those sought by smokers; for this reason, and because of the clearer correlation with ligand-binding studies, a 0.1 mg/kg dose of nicotine was the standard for training in subsequent discrimination studies.

Under these conditions, full generalisation has been obtained with several additional structural analogues of nicotine. These compounds include the stereoisomer (+)-nicotine, and both (−)- and (+)-nornicotine [6]. The ratio of potencies of the (−)- and (+)-isomers of nicotine was 14:1. These drugs have not been directly compared for generalisation across a wide range of different training doses of nicotine, although training dose seems not to be an important variable for generalisation to (+)-nicotine [17]. Table 1 provides a list of drugs found to be generalised with (−)-nicotine to date. Nearly all these compounds have been shown to potently inhibit the binding of tritiated (−)-nicotine, and there is a fairly good correlation between relative potency in the biochemical and behavioural procedures [6,12]. All analogues have been generalised at doses below those which non-specifically reduced overall rates of responding.

Generalisation Tests With Other (Non-Nicotinic) Drugs

The results with agonists that are structurally related to nicotine are meaningful only if similar findings cannot be obtained with pharmacologically different drugs. A large number of drugs from different pharmacological classes have been tested for generalisation in rats trained to discriminate nicotine. None of these drugs has yet generalised fully, and in most cases scores have not been increased significantly above those for saline. These drugs are listed in Table 2, which includes compounds with agonist or antagonist activity at receptors for muscarinic-cholinergic, serotonergic, opioid, benzodiazepine, adrenergic and dopaminergic agents. All these drugs were tested up to doses that reduced overall rates of responding or were themselves discriminable when used for training. There is also no generalisation to nicotine methiodide, a quaternary analogue of nicotine that does not penetrate easily into the CNS. The only non-nicotinic drug that has consistently produced marked (al-

TABLE 2

DRUGS WHICH ARE NOT GENERALIZED WITH NICOTINE*

Present Technique [21,32]	
Amphetamine	l-Phenyl-isopropyl-adenosine
Apomorphine	Mecamylamine
Atropine	Midazolam
Chlordiazepoxide	Morphine
Chlorisondamine	Oxotremorine
Clenbuterol	Physostigmine
Cocaine	Picrotoxin
Droperidol	Pimozide
Fenfluramine	Quipazine
Haloperidol	Sch 23390
Hexamethonium	Metergoline
Related Discriminative Methods	
Adrenaline	Lobeline
Arecoline	Nicotine methiodide
Caffeine	2- and 4-Nicotine
Dimenhydrinate	Pentobarbitone
Gallamine	Piperidine
	Pyrilamine

*From references in [32,33] and unpublished data.

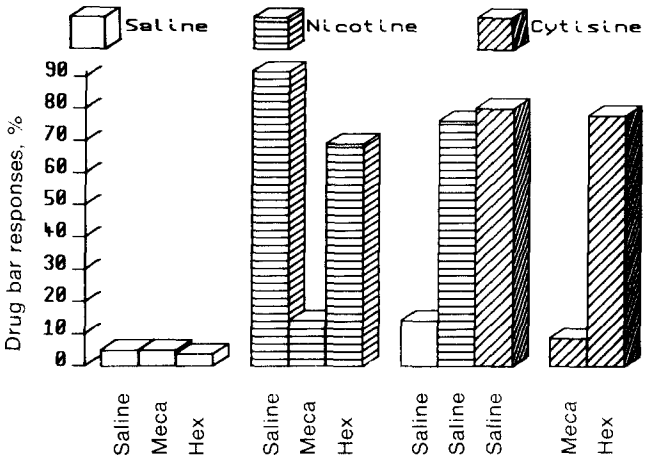

FIG. 2. Effects of mecamylamine (0.5 mg/kg SC) or hexamethonium (5.0 mg/kg SC) in rats trained to discriminate nicotine (0.1 mg/kg SC) from saline (n=8). Mecamylamine (meca) but not hexamethonium (hex) blocked the response to nicotine and generalization to cytisine (2.0 mg/kg SC).

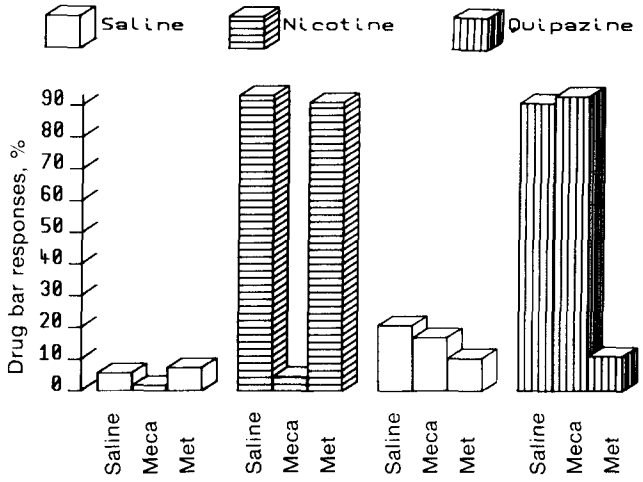

FIG. 3. Effects of mecamylamine (0.75 mg/kg SC) and metergoline (0.25 mg/kg SC) on discrimination of nicotine (0.4 mg/kg) or quipazine (0.35–0.50 mg/kg SC). Mecamylamine (meca) blocked nicotine but not quipazine, whereas metergoline (met) blocked quipazine but not nicotine (n=7–8).

though incomplete) generalisation is (+)-amphetamine [3,33], which elevates mean scores to 60–70%. The mechanism of this effect requires further investigation.

Studies With Antagonists

The discriminative effects of nicotine can be blocked reliably by certain drugs which also act as antagonists at cholinoceptive sites in autonomic ganglia. After systemic administration, the non-quaternary agents mecamylamine and pempidine penetrate into the CNS and completely block the nicotine cue [18, 24, 25]. Figure 2 illustrates this finding for mecamylamine. It appears that this block cannot be overcome by four- to eight-fold increases in the dose of nicotine [35], suggesting its possible non-competitive nature. Systemic administration of mecamylamine also blocks generalisation to the nicotine analogue cytisine (Fig. 2).

Quaternary ganglion-blockers such as hexamethonium, chlorisondamine and pentolinium do not penetrate well into the CNS when given by systemic injection; these compounds do not block the nicotine cue or generalisation to cytisine under such conditions (Fig. 2). Upon intraventricular injection, small 5 μg doses of chlorisondamine block the nicotine cue, although even large doses of hexamethonium and pentolinium fail to do so [7,14].

The blocking action of systemically administered mecamylamine on responses to nicotine has a considerable degree of specificity. Mecamylamine fails to attenuate discriminative responses to several non-nicotinic psychoactive drugs [32,35]. For example, Fig. 3 shows that small doses of mecamylamine that completely blocked the nicotine cue had no effect on discrimination of the 5-hydroxytryptamine (5-HT) agonist quipazine. Conversely, the 5-HT antagonist metergoline completely blocked responses to quipazine in doses that had no effect on the nicotine cue. These results suggest that the nicotine cue is not mediated through 5-HT receptors that are blocked by metergoline [35].

In view of reports of nicotinic receptors located presynaptically on dopamine nerve terminals [8] and the partial generalisation to amphetamine mentioned above, the effects of neuroleptics were determined. Haloperidol and Sch 23390 attenuated the response to nicotine, but mean scores did not fall reliably below about 50% ([34], and unpublished data). These results provide limited evidence for a role of dopamine receptors of the D_1 type in mediating the response to nicotine. The doses of neuroleptics that attenuated responses to nicotine were not themselves generalised, but they drastically reduced overall response rates. Haloperidol also attenuated discrimination of morphine, suggesting that it may have impaired discriminative control of behaviour in a non-specific manner [34].

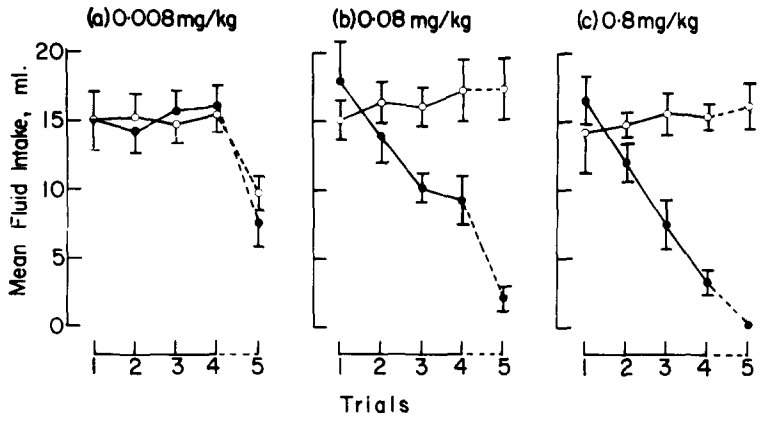

FIG. 4. Conditioned taste aversions to nicotine-paired flavoured solutions in three groups of rats (●, n=8). In the same rats, intakes of saline-paired flavoured solutions were not suppressed (○). Trials 1–4 show conditioning sessions. Trial 5 was a test with simultaneous presentation of both flavoured solutions, hence the fall apparent in consumption of each solution in (a). From Kumar *et al.* [13].

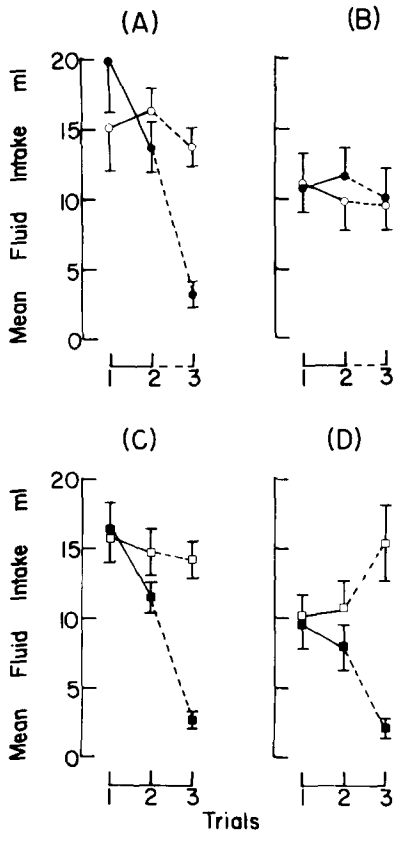

FIG. 5. Conditioned taste aversions in four groups of rats (n=6–8). In (A) nicotine produced CTA which in (B) was blocked by mecamylamine (2 mg/kg SC). In (C) apomorphine produced CTA which in (D) was *not* blocked by same dose of mecamylamine. Doses of nicotine (●) and apomorphine (■) were 0.4 mg/kg (SC). Intakes of control flavoured solutions for each group of rats are also shown (○ and □). In these experiments, mecamylamine or saline was administered 15 min before presentations of flavoured solutions for trials 1 and 2 [13].

CHARACTERISTICS OF CONDITIONED TASTE AVERSIONS PRODUCED BY NICOTINE

Studies With the Stereoisomers of Nicotine

Conditioned taste aversions produced by three doses of (−)-nicotine are shown in Fig. 4. At the intermediate (0.08 mg/kg) dose of nicotine, the intake of drug-paired flavoured solutions fell steadily over successive conditioning sessions. The intake of saline-paired flavoured solutions by the same rats remained relatively constant (Fig. 4b, trials 1–4). The development of CTA was confirmed in the two-stimulus tests, where both the nicotine- and the saline-paired flavoured solutions were presented simultaneously (trial 5). Figure 4a shows that nicotine did not produce a detectable degree of CTA at a dose of 0.008 mg/kg, and Fig. 4c shows the rather greater magnitude of CTA at 0.8 mg/kg of nicotine.

In the four-trial CTA procedure for which results are shown in Fig. 4, the ED_{50} value [13] for nicotine (with 95% confidence limits) was 0.046 (0.022–0.075) mg/kg. The ED_{50} for (+)-amphetamine was 0.22 (0.13–0.34) mg/kg; thus, nicotine was one of the most potent of the many drugs found to produce CTA [13]. The larger the number of conditioning trials, the smaller the dose of nicotine needed to produce a given degree of CTA. After only a single conditioning trial, the ED_{50} for nicotine was 0.66 (0.46–1.54) mg/kg, as compared with 0.15 (0.07–0.29) mg/kg after two trials. For subsequent studies, the two-trial procedure was used since it provided a practical balance between sensitivity and time needed to complete a study.

The stereoisomer (+)-nicotine was tested at several doses using the two-trial procedure. This compound produced CTA when given in doses of 0.7 mg/kg or more [13]. The patterns of fluid intake resembled those shown for (−)-nicotine in Fig. 4. The ED_{50} value for (+)-nicotine was 0.67 (0.41–0.97) mg/kg and thus, the ratio of potencies for the (−)- and (+)-isomers was 4.5:1.

Studies With Antagonists

In rats receiving mecamylamine (2 mg/kg) 15 min *before* each conditioning trial, nicotine (0.4 mg/kg) did not produce

a detectable degree of CTA (Fig. 5B). Control rats receiving saline injections whenever experimental animals received mecamylamine showed clear CTA (Fig. 5A). Mecamylamine also slightly reduced the amounts consumed of the flavoured solutions from trial 1, but did not itself produce a CTA in these experiments [13]. Doses of mecamylamine from 0.1–1.0 mg/kg attenuated the CTA in a dose-related manner but did not block it completely. Hexamethonium in doses of 1.0–3.2 mg/kg did not attenuate the CTA. Intraventricular injection of the quaternary ganglion-blocker chlorisondamine (5 μg) blocked the CTA produced by nicotine (0.4 mg/kg). This block appeared despite a period of nine days between the single injection of chlorisondamine and the commencement of the CTA procedure [22].

The specificity of action of mecamylamine was examined by means of the CTA produced by apomorphine. In control rats receiving saline before each conditioning trial, clear CTA developed (Fig. 5C). However, mecamylamine did not prevent the development of this CTA, as can be seen most clearly from the results for trial 3 (Fig. 5D). The effect of mecamylamine on CTA produced by nicotine cannot, therefore, be attributed to learning processes such as stimulus preexposure [5]. It can only be understood in terms of a specific pharmacological interaction. Chlorisondamine (5 μg intraventricularly) also failed to weaken the CTA produced by apomorphine [22].

The possible role of dopaminergic mechanisms in CTA produced by nicotine was tested by experiments with the neuroleptic pimozide [20]. In animals receiving pimozide (1.2 mg/kg) 3.25 hr before each conditioning trial, nicotine (0.4 mg/kg) produced clear CTA which did not differ appreciably from that in control animals receiving the vehicle for pimozide (Fig. 6C and D). The same dose of pimozide completely blocked the CTA produced by apomorphine (Fig. 6A and B). Pimozide slightly reduced the baseline consumptions of flavoured solutions, but it did not itself produce a CTA under the conditions of this experiment. In contrast, the dopamine antagonist domperidone, which penetrates poorly into the CNS, did not prevent apomorphine (0.4 mg/kg) from producing CTA [20].

In experiments where mecamylamine (2 mg/kg) was administered immediately *after* exposure to flavoured solutions, a moderate degree of CTA was detected with the very sensitive four-trial procedure (Fig. 7). Across trials 1–4 (one-stimulus tests) CTA was not significant, $t(7)=2.00$. On trial 5 (two-stimulus test), the mean intake of the drug-paired flavoured solution was 16.0±5.6% of total fluid intake, representing a significant CTA, $t(7)=4.66$, $p<0.01$.

This finding may be contrasted with the lack of any detectable CTA when the mecamylamine was given as a pretreatment prior to exposure to flavoured solutions (Fig. 5).

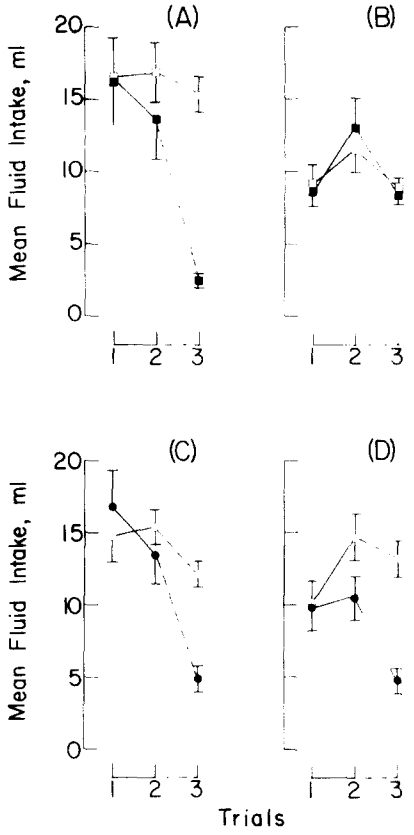

FIG. 6. Conditioned taste aversions in four groups of rats (n=6–8). In (A) apomorphine produced CTA which in (B) was blocked by pimozide (1.2 mg/kg SC). In (C) nicotine produced CTA which in (D) was *not* blocked by same dose of pimozide. Doses of nicotine (●) and apomorphine (■) were 0.4 mg/kg (SC). Intakes of control flavoured solutions for each group of rats are also shown (○ and □). In these experiments, pimozide or saline was administered 3.25 hr before presentation of flavoured solutions for trials 1 and 2 [20].

DISCUSSION

The discriminative stimulus effect of nicotine provides a pharmacologically specific behavioural procedure for quantifying certain aspects of the actions of nicotine in the CNS. The recent studies comparing the *in vitro* biochemical effects and the behavioural effects of nicotine support the view that binding sites for tritiated (−)-nicotine constitute a pharmacologically relevant, functional receptor. In this work, it was possible to establish correlations at both the qualitative and quantitative levels. Qualitatively, only compounds that inhibited the binding of tritiated nicotine produced nicotine-like discriminative effects. Compounds from a wide range of pharmacological classes were inactive (Table 2). Quantitatively, there is a reasonable correlation emerging between the relative potencies of compounds in inhibiting nicotine binding, and their relative potency in the behavioural procedure. The development of both the qualitative and quantitative aspects of the correlation has been hampered by the extremely small numbers of suitable nicotinic agonists. This situation is changing as more groups become interested in the possible importance of CNS nicotinic mechanisms in psychiatric and neurological states.

Present investigations of the CTA produced by nicotine suggest that the CNS receptors through which it is mediated may not be greatly different from those mediating the nicotine cue. Both effects are produced by the same range of doses of nicotine, which can include doses within the "smoking" range. The relative potencies of the stereoisomers of nicotine are not very different in the two procedures, although there is a slightly greater degree of stereoselectivity for the discriminative effect. Both effects can be blocked by systemic administration of mecamylamine, and the quaternary ganglion-blocker chlorisondamine is in both cases a potent, long-acting antagonist when injected intraventricularly. The 0.75 mg/kg dose of mecamylamine needed to block the discriminative effect of nicotine is smaller than

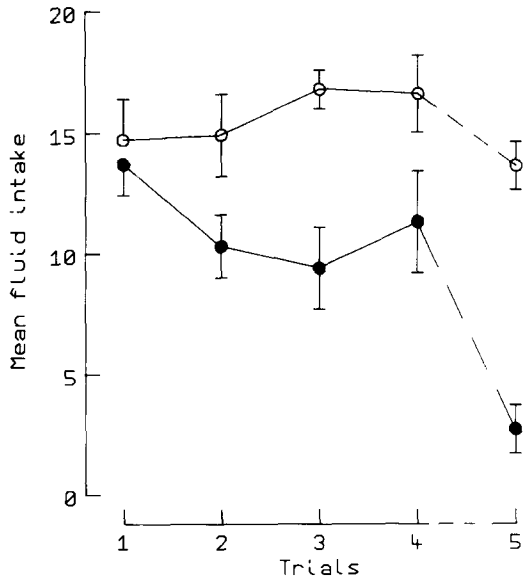

FIG. 7. Conditioned taste aversion produced by mecamylamine (2.0 mg/kg SC) in rats (n=8). In these experiments, mecamylamine (●) or saline (○) was injected in trials 1–4, immediately after removal of the flavoured solutions, and nicotine was not administered at all.

the 2 mg/kg dose needed to fully block the CTA effect, but this cannot be taken as evidence for different subpopulations of receptors; in the discrimination experiments, mecamylamine was given at the optimal time in relation to nicotine, whereas to block the CTA effect it is probably necessary to give enough mecamylamine to prevent the effects of nicotine throughout its time-course of action. There does not appear to be a discrepancy in the doses of chlorisondamine needed to block the two effects, presumably because this compound acts over a long period of time. The possibility remains that the two effects are mediated through different regions of the brain and this requires further investigation.

Some of the ligand-binding studies with tritiated nicotine indicate the presence of multiple binding sites and as many as five different sites have been reported [28,29]. These sites include one for the (+)-isomer of nicotine which is not present in tobacco, although there have been suggestions that it is present in tobacco smoke. Some investigators have only been able to identify one saturable binding site for tritiated (−)-nicotine [12]. The ligand alpha-bungarotoxin binds to a different subpopulation of sites, and it has been suggested that these constitute CNS nicotinic receptors resembling those at the neuromuscular junction [4]. However, there is no evidence to date that these binding sites are functional receptors mediating behavioural effects [1, 4, 32].

The behavioural studies to date provide only hints of support for the notion of multiple types of central nicotinic receptors. The nicotinic-cholinergic agonists anabasine and cytisine produce discriminative effects fully equivalent to those of a 0.1 mg/kg training dose of nicotine [33]. However, anabasine and cytisine are not fully equivalent to a 0.4 mg/kg training dose of nicotine [21,33]. One of several possible explanations of these data is that nicotine acts on more than one subtype of receptor, and that the analogues show some selectivity. This explanation assumes that the cue produced by a 0.4 mg/kg training dose of nicotine involves at least two receptor subtypes, whereas the cue produced by a 0.1 mg/kg training dose involves only the receptor subtypes upon which the analogues act. Another suggestion of the possible complexity of the CNS nicotinic receptor comes from the evidence that the quaternary ganglion-blocking drugs are not all able to block the nicotine cue, even when they are injected intraventricularly [34]. There is clearly scope for much more behavioural work to test ideas about multiple nicotinic receptors. Drug discrimination methods provided some of the best early evidence that CNS cholinoceptive sites consisted of separate muscarinic and nicotinic subpopulations [26], and the use of such methods in the future may help to reveal the functional significance of multiple nicotinic receptors.

The finding that mecamylamine (2 mg/kg) alone produced a moderate degree of CTA suggests a possible tonically-activated state of nicotinic receptors. However, a very wide range of different drugs can produce CTA, and it has not been established that mecamylamine produces CTA through central actions. The finding that this CTA occurred with post-trial but not with pre-trial administration of the drug is consistent with the usual temporal relationships for classical conditioning.

The significance of the discriminative and CTA effects of nicotine in relation to its other actions also needs further clarification. The negative results from generalisation tests with convulsant and tranquilizing drugs suggest that such effects of nicotine do not form the basis of the cue. The partial generalisation to (+)-amphetamine suggests a possible arousal-related component in the cue, although cocaine did not produce the same effects as amphetamine. There are also several poorly understood aspects of CTA, and the results of the studies on the nicotine (and apomorphine) CTA help to clarify certain points. The chemoreceptor trigger zone of the *area postrema* lacks a blood-brain barrier and it is accessible to drugs (such as hexamethonium and domperidone) which do not penetrate to many other regions of the brain. Thus, hexamethonium can block the emetic effect of nicotine [15,31]. The present experiments show that hexamethonium does not block the CTA effect of nicotine. Similarly, domperidone can block the emetic effects of apomorphine and yet domperidone does not attenuate the CTA produced by apomorphine [20]. The results suggest that the CTA's produced by both nicotine and apomorphine are mediated centrally, and that they have nothing to do with the *area postrema* or the emetic effects of the drugs. This may be of interest in relation to previous uses of nausea and vomiting produced by these drugs as unconditioned stimuli in aversion therapies for smoking and other addictions.

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NOTE ADDED IN PROOF

After preparation of this manuscript was complete, Collins *et al.* presented behavioural evidence that mecamylamine may be able to distinguish between sub-types of nicotinic receptors (*Pharmacol Biochem Behav* **24:** 1767–1773, 1986).

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