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Discriminative Stimulus Properties of Nicotine: Further Evidence for Mediation at a Cholinergic Receptor

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Abstract. Rats were trained to discriminate nicotine (0.4 mg/kg SC) from saline in a standard two-bar operant conditioning procedure with food reinforcement. The response to nicotine was dose-related and at the ED_{50} of 0.14 mg/kg, plasma nicotine concentrations were similar to those reported previously for cigarette smokers who inhale. The nicotine analogues anabasine and cytisine increased nicotine-appropriate responding in a dose-related manner. Animals predominantly responded on the saline-associated lever when administered drugs from a range of pharmacological classes, even at doses that were sufficiently large to reduce the overall numbers of responses. The results confirm that the nicotine discriminative stimulus is highly specific. Previous work has shown anabasine and cytisine to be active at nicotinic-cholinergic binding sites in rat brain. The finding that there is some correlation between the behavioural effects of these compounds and their actions at the nicotine binding site may indicate that the nicotine cue is mediated through a cholinergic receptor.

Key words: Nicotine – Drug discrimination – Cytisine – Anabasine – Nicotinic receptors

The ability of nicotine to serve as a discriminative stimulus (cue) was first reported over 10 years ago (Morrison and Stephenson 1969) and has subsequently been used as a behavioural assay for its central actions (Rosecrans and Chance 1977). The specificity of the nicotine cue and its central mediation was demonstrated by the findings that nicotinic but not muscarinic agents generalised with nicotine and that the cue was blocked only by those ganglion blocking drugs which penetrated into the CNS (Rosecrans and Chance 1977, Romano et al. 1981).

The current interest in the biochemical characterisation of central nicotine receptors has yielded conflicting data in terms of whether nicotine acts at a cholinergic receptor site. Romano and Goldstein (1980) showed that nicotine binds with high affinity in a stereospecific manner to rat brain membranes and that cholinergic agonists were extremely potent in displacing nicotine from this binding site. In contrast to these findings Sershen et al. (1981) showed that the binding of nicotine did not display stereospecificity in mouse brain and that nicotinic-cholinergic agents had little affinity for nicotine binding sites. They proposed that nicotine may bind to a non-cholinergic site in brain.

Results from studies of gross behavioural changes have not clarified whether nicotine acts through cholinergic or non-cholinergic mechanisms. Abood et al. (1978) reported that intraventricular injection of nicotine induced a prostration-immobilisation syndrome in rats which could not be mimicked or blocked by nicotinic compounds. They proposed that nicotine may act through non-cholinergic mechanisms. Schwab and Kritzer (1982), however, found mecamylamine and hexamethonium to antagonize the syndrome, and suggested an involvement of cholinergic mechanisms in this behavioural effect of nicotine.

The present behavioural investigation was designed to determine whether in a two-bar operant conditioning procedure, the properties of the nicotine discriminative stimulus could be correlated with the reported characteristics of nicotine's binding sites. A number of compounds from a range of pharmacological classes have been tested for nicotine-like effects. These include nicotinic and muscarinic agonists, the 5-hydroxytryptamine agonist quipazine, the benzodiazepine midazolam and the dopaminergic compounds apomorphine and amphetamine. In most cases the doses used had previously been shown to produce discriminative stimulus effects in other procedures. A preliminary account of some of this work has been given (Garcha et al. 1982, 1983; Stolerman et al. 1982). Plasma concentrations of nicotine have been measured in order to see if these correspond with those in cigarette smokers.

Materials and Methods

Animals. Male hooded rats (Olac, Bicester) which initially weighed 220–320 g were given restricted amounts of food so as to maintain their weights at about 80% of those under free-feeding conditions. They were housed individually in a room at a controlled temperature (20–22°C) and a regular light dark cycle was employed (light from 8 a.m. – 8 p.m.). Water was available at all times except during training or test sessions.

Apparatus. Standard experimental chambers (Campden Instruments) contained within sound-insulated, ventilated enclosures were used throughout. The chambers contained two retractable response bars separated by a recess in which 45 mg pellets of food could be presented by a dispenser. White noise at 78 dB above a reference level of 0.0002 dynes/cm^2 was present at all times to mask extraneous sounds. Solid-state

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recording and programming equipment was located in an adjoining room.

Drug Discrimination Training Procedure. The procedure was modified from that used by D'Mello and Stolerman (1977) and by Stolerman and D'Mello (1981). The rats were first trained to press the bars for food reinforcers delivered on a fixed ratio 10 schedule (FR10). In this schedule, every tenth bar-press was followed by delivery of one 45 mg food pellet. On any given day, only one of the two response bars was present in the chamber.

After this preliminary training to establish a baseline of responding, both bars were made available simultaneously and discrimination training began. All rats were trained to discriminate nicotine (0.4 mg/kg SC, 15 min prior to 15-min sessions), from saline administered at the same presession interval. In order to avoid possible position preferences, half the rats were reinforced with food for responding on the left bar following nicotine injections and the remaining rats reinforced for responding on the right bar following nicotine injections. Responses on the opposite bar were reinforced with food pellets after saline injections. Drug and saline training sessions took place in randomized sequences which were different for each rat, except that the same injections were not given to any rat for more than three consecutive sessions. The rats were trained 5 days per week. The retractable response bars were programmed to move into the chambers when the sessions began, with the rats already present.

After five drug discrimination training sessions the rats were required to make ten consecutive responses on the correct bar before receiving a reinforcement. This was to minimise any tendency for animals to alternate between bars. After five further discrimination sessions a variable interval (VI) component was introduced progressively into the schedule of reinforcement until after about 40 sessions, the final schedule of tandem VI 1 min FR10 was in effect. In this schedule, the tenth consecutive bar-press was reinforced after a randomly determined, variable interval of time (mean = 1 min; range 14 – 106 s). Responses during the intervening periods were recorded but not reinforced. This schedule was used because the FR10 component ensured high response rates, while the VI 1 component made it difficult for the rats to discriminate extinction test sessions from training sessions.

Generalisation Tests. After about 40 discrimination training sessions, tests for generalization to nicotine and other drugs began. Groups of 6 – 8 rats were used for this work. On days of the generalisation tests, rats were normally tested for 5 min, with no reinforcers presented regardless of which bar the rats pressed (extinction tests). All test days were preceded by a saline training day to minimize residual drug effects. In order to maintain drug discrimination a day of training with nicotine was given once a week. Thus during each week test doses of drugs were given on Tuesdays and Fridays, with saline training on Mondays and Thursdays, and nicotine training on Wednesdays.

For the nicotine dose-response study, nicotine was tested in doses ranging from 0.025 – 0.4 mg/kg SC 15 min prior to testing. Saline injections at the same presession interval provided the control data. All rats received each treatment once and the sequence of treatments was determined separately for each rat by a randomization procedure. In other experiments the time course of the nicotine cue was determined. To study the onset of the cue, animals were injected with nicotine (0.15 mg/kg SC) or saline and immediately placed in the testing chamber. The scores for drug-appropriate responding were then calculated 0 – 2.5 min, 2.5 – 5.0 min, 5.0 – 7.5 min and 7.5 – 10 min after the animals were placed in the chamber. The offset of the discriminative effects of nicotine at doses of 0.4 and 0.15 mg/kg SC was determined using a slightly different procedure. Animals were injected with either nicotine at the appropriate dose or saline, and placed in the test chamber for 2-min extinction tests at 10, 20, 40, 80 and 160 min after injections. The rats were returned to their home cages between tests.

For generalisation tests with other drugs, doses of drugs were administered in a random sequence and each series of tests included saline and nicotine (0.4 mg/kg SC) controls. Those animals which did not show a difference of at least 60% between the scores for drug-appropriate responding in saline and nicotine (0.4 mg/kg) test sessions were excluded. The following drugs were employed; (+)amphetamine, cocaine, apomorphine, cytisine, anabasine, oxotremorine, atropine, physostigmine, midazolam, quipazine and fenfluramine. All drugs were administered subcutaneously 15 min prior to the 5-min extinction tests with the exception of fenfluramine which was administered 30 min previously. The doses used were selected from previous work and all compounds were tested up to doses which decreased the overall number of responses on both bars. Occasional departures from random sequences were made when it was thought that testing a wider range of doses of drug would clarify the results.

Data Analysis. 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, taken over the whole 5 min of the extinction test. This index was calculated separately for each rat and means were then taken. ED_{50} values were calculated from the data according to Snedecor and Cochran (1967), yielding the modified ED_{50} defined by Barry (1974) as the dose of the test substance expected to produce 50% responding on the bar appropriate for the training drug. The percentage of rats selecting the bar appropriate for the training drug was also determined. This quantal index was calculated as the bar on which the rat first totalled ten responses during each test (Colpaert 1977).

Comparisons between different values of the quantitative index were made, after arc-sine transformations, by means of analyses of variance and Dunnett's t-test for multiple comparisons with a control group (Winer 1971). When a dose of a drug suppressed responding to the extent that a rat made a total of less than ten responses on both bars the index was not calculated. The total number of responses made on both bars throughout a 5-min extinction test was also used as an index of overall response rate. These scores were analysed by means of repeated measure analyses of variance and by Dunnett's t test (Winer 1971).

Drugs. (–)-Nicotine hydrogen-(+)-tartrate (BDH, Poole, Dorset, UK), was dissolved in isotonic saline. The pH was adjusted to 7 with 0.5 N NaOH. Cytisine (Koch-Light Laboratories, Haverhill, Suffolk, UK), was dissolved in isotonic saline and the pH adjusted to 7 with IN HCl. Cocaine HCl (B.P.), (+)-amphetamine sulphate (Smith, Kline and French, Welwyn Garden City, UK), *l*-anabasine sulphate (Pfaltz and Bauer, Stamford, CT, USA), oxotremorine sesquifumarate

(Sigma, Poole, Dorset, UK), atropine sulphate (Sigma, Poole, Dorset, UK), physostigmine sulphate (Sigma, Poole, Dorset, UK), midazolam maleate (Hoffmann-La Roche, Basel, Switzerland), quipazine maleate (Miles Laboratories, Slough, UK), and fenfluramine hydrochloride (Servier, Greenford, UK) were all dissolved in saline. Apomorphine hydrochloride (Macfarlan Smith, Edinburgh, UK) was dissolved in distilled water containing ascorbic acid (0.2 mg/ml). All injections were given in a volume of 1 ml/kg subcutaneously and all doses were calculated as those of the base.

Determination of Plasma Nicotine Concentrations. Groups of 6–8 experimentally naive rats were injected with saline or with nicotine (0.025–0.4 mg/kg SC) and decapitated 15 min later. Further groups of animals received nicotine (0.4 mg/kg SC) and were decapitated 2.5, 10, 40 and 160 min after injection. Two control animals received saline injections at each of the pretreatment times. Trunk blood was collected into 10 ml plastic tubes containing 15.0 I.U. lithium heparin. Plasma was separated by centrifugation and stored at −20° C until plasma nicotine concentrations were determined by gas chromatography using a nitrogen-selective detector (Feyerabend and Russell 1980). In order to simulate feeding conditions of animals in the discrimination experiments, a further group of seven rats was deprived of food so as to be maintained at 80% of their normal body weight. Another group of seven rats were allowed access to water for only 1 h per day between 10.00 h and 11.00 h for 1 week preceding the experiment. Animals were injected with nicotine (0.4 mg/kg SC) and blood samples were collected 15 min later for plasma nicotine determinations as described above. At the end of generalisation testing one group of nicotine-trained rats were similarly treated with nicotine (0.4 mg/kg SC) and nicotine plasma concentrations determined.

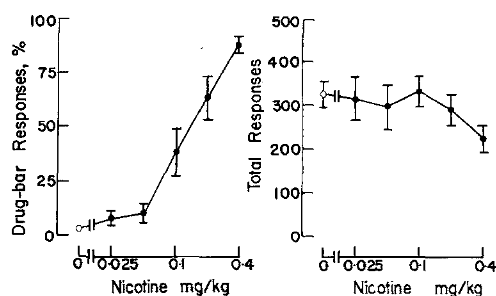

Fig. 1. Percentage responding (means ± SEM) on drug-appropriate bar and total number of responses (means ± SEM) as a function of nicotine dose in eight rats trained to discriminate nicotine (0.4 mg/kg SC) from saline. Results for control tests after saline injections are also shown. Data were collected in 5-min extinction tests

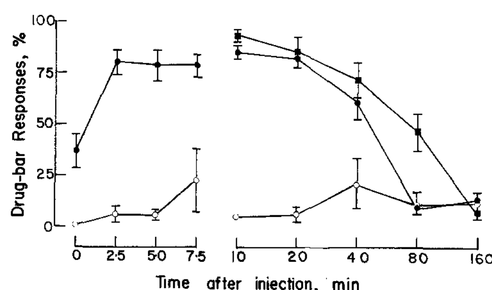

Fig. 2. Time course of the nicotine discriminative stimulus in seven rats trained to discriminate nicotine (0.4 mg/kg) from saline. Rats received either nicotine 0.15 mg/kg (●) or 0.4 mg/kg (■). In the left portion of the figure, data were collected at 2.5-min intervals in extinction sessions for a period of 10 min following drug administration. Data are shown for 5–7 rats which responded sufficiently for discriminative effects to be assessed. In the right portion of the figure, data were collected in 2-min sessions at the appropriate times after nicotine injection. Saline control data are also shown (○)

Results

Nicotine Cue: Dose-Response Relationship and Time Course. Following administration of the training dose of nicotine (0.4 mg/kg) the number of responses on the bar appropriate for nicotine expressed as a percentage of the total number of responses on both bars was 88.1 ± 4.1%, as compared with 2.8 ± 0.7% after saline (means ± SEM). The response to nicotine was strongly related to dose (Fig. 1); smaller doses than that used for training produced intermediate mean amounts of responding on the drug-appropriate bar. The ED_{50} was 0.14 mg/kg. Figure 1 also shows the effect of increasing doses of nicotine on the total number of responses. At doses between 0.025 and 0.2 mg/kg nicotine did not influence the overall number of responses when compared to control animals receiving saline. At the training dose of nicotine (0.4 mg/kg) the mean total number of responses was 223 ± 29 as compared with 326 ± 31 in control animals but this difference was not statistically significant.

Figure 2 shows the time course of the nicotine cue. Following administration of nicotine at a dose in the region of the cue ED_{50} value (0.15 mg/kg), nicotine-appropriate responding was detected as soon as 2.5 min after injection. At this time drug-appropriate responding was 36.7 ± 8.1% as compared with 1.3 ± 0.9% after saline. Drug-appropriate responding was maximal between 2.5–5.0 min after injection (79.8 ± 6.0%) and remained around this level for a further 15 min before returning to control values at 80 min. Nicotine at the training dose (0.4 mg/kg) showed a similar but slightly prolonged time-course. For example, 10 min after nicotine (0.4 mg/kg) administration drug-appropriate responding was 93.3 ± 2.8% as compared with 85.1 ± 3.0% after nicotine (0.15 mg/kg). The time of onset of the discriminative effects of the training dose of nicotine could not be determined, since at time intervals prior to 10 min the animals did nor produce a sufficient number of responses to allow the percentage of drug-appropriate responding to be determined.

Plasma Nicotine Concentrations: Dose-response Relationship and Time Course. The mean plasma concentration of nicotine rose almost linearly with increasing doses of nicotine in groups of 6–7 rats receiving nicotine 15 min previously (Fig. 3). At a dose corresponding to the nicotine training dose the mean nicotine plasma level was 146 ± 6 ng/ml and at the cue ED_{50} value (0.14 mg/kg) it was estimated as 48 ng/ml by interpolation from the data shown in Fig. 3.

In the time-course experiments, nicotine plasma concentrations were maximal 2.5 min following nicotine administration (0.4 mg/kg) and remained so for at least 10 min before beginning to decline 40 min after drug administration (Table 1). In those animals which were deprived of food so as to be maintained at 80% of their normal body weight the mean plasma nicotine concentration following nicotine ad-

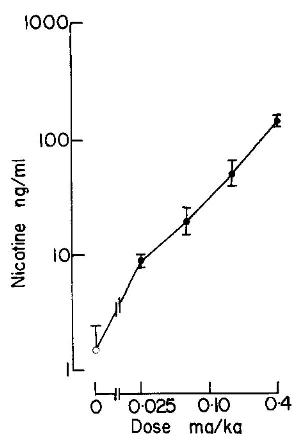

Fig. 3. Plasma nicotine concentrations (mean ± SEM) in groups of 6–7 rats receiving increasing doses of nicotine (0.025–0.4 mg/kg SC; ●) or saline (○) 15 min previously

Table 1. Plasma concentrations of nicotine (mean ± SEM) in rats receiving 0.4 mg/kg nicotine SC

Group	N	Plasma nicotine (ng/ml ± SEM)
Time-course experiment		
Saline, 2.5–160 min	8	0.3 ± 0.1
Nicotine, 2.5 min	5	130.8 ± 20.5
Nicotine, 10 min	6	130.2 ± 6.2
Nicotine, 40 min	6	101.4 ± 1.4
Nicotine, 160 min	6	24.0 ± 1.0
Deprivation experiment		
Saline, 15 min, not deprived	7	1.5 ± 0.4
Nicotine, 15 min, not deprived	7	146.1 ± 5.6
Nicotine, 15 min, food-deprived	7	123.5 ± 3.8[a]
Nicotine, 15 min, water-deprived	7	130.9 ± 7.1
Trained rats		
Nicotine, 15 min, food-deprived	6	147.2 ± 7.6

[a] $P < 0.05$ compared with control animals receiving nicotine (0.4 mg/kg SC) and not deprived of food

ministration (0.4 mg/kg) was slightly less than that in rats of the same age who were not food deprived ($P < 0.05$). A group of nicotine-trained rats displayed plasma nicotine concentrations almost identical to those in control animals, as did those rats which were water deprived (Table 1).

Generalization Tests with Nicotinic Agonists. Cytisine (0.4–3.2 mg/kg) increased nicotine-appropriate responding in a dose-related manner (Fig. 4). The maximal response occurred at a dose of 1.6 mg/kg; mean score was 59.0 ± 17.2% ($t = 4.96$, df 30, $P < 0.01$). Increasing the dose to 3.2 mg/kg did not further increase nicotine-appropriate responding, mean score being 50.0 ± 15.1%. Cytisine was then tested in eight different rats at a dose of 1.6 mg/kg; on this occasion that they received the drug, animals displayed a mean score of 73.7 ± 10.0% as compared with 2.2 ± 0.8% after saline.

Anabasine (1–4 mg/kg), like cytisine, produced a dose-dependent increase in nicotine-appropriate responding. In this case the maximal response occurred at the largest dose tested (4.0 mg/kg) mean score being 59.8 ± 12.7% ($t = 5.50$, df 48, $P < 0.01$). A repeat of the dose-response study with nicotine (0.05–0.4 mg/kg) yielded the expected dose-related increase in nicotine-like responding. Following the training dose of nicotine (0.4 mg/kg) drug-appropriate responding was 96.6 ± 1.0% as compared with 1.7 ± 0.7% after saline. Neither anabasine nor cytisine in any of the doses tested produced nicotine-appropriate responding to the extent observed following the training dose of nicotine. Larger doses of these drugs could not be tested because of dose-related suppression of responding (see Fig. 4).

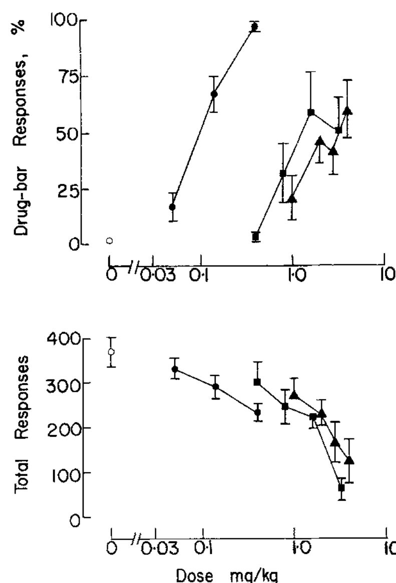

Fig. 4. Effect of anabasine (▲) and cytisine (■) on percent responding on nicotine-appropriate bar and on the total number of responses made by groups of 6–8 rats trained to discriminate nicotine (0.4 mg/kg) from saline. The pooled data for animals receiving nicotine (●; $N = 14$) are shown for comparative purposes. Results for control tests after saline injections are also shown. At the largest dose tested of anabasine and cytisine, one rat did not respond. Each point represents the mean ± SEM of observations made in 5-min extinction tests

In general, similar results were obtained when data were expressed in terms of the bar-selection index. For example, the percentages of rats selecting the drug appropriate bar, following administration of anabasine, cytisine and nicotine in doses which produced maximal drug-bar responding, were 50%, 66.7% and 100% respectively. The solutions of anabasine were assayed for nicotine, but none could be detected.

Generalization Tests with Non-nicotinic Compounds. Figure 5 A and B shows the results of generalization tests with a number of compounds from a range of pharmacological classes. The central stimulants amphetamine (0.075–1.2 mg/kg) and cocaine (1–8 mg/kg) did not produce any statistically significant increase in drug-appropriate responding when compared with saline controls. Amphetamine at doses of 0.3 and 0.6 mg/kg produced mean scores of 31.4 ± 17.3 and 43.0 ± 24.0% respectively. At the latter dose only five out of eight rats responded sufficiently for scores to be calculated, and in neither case were the scores statistically different from control values. Similarly the dopamine receptor agonist apomorphine did not increase nicotine-

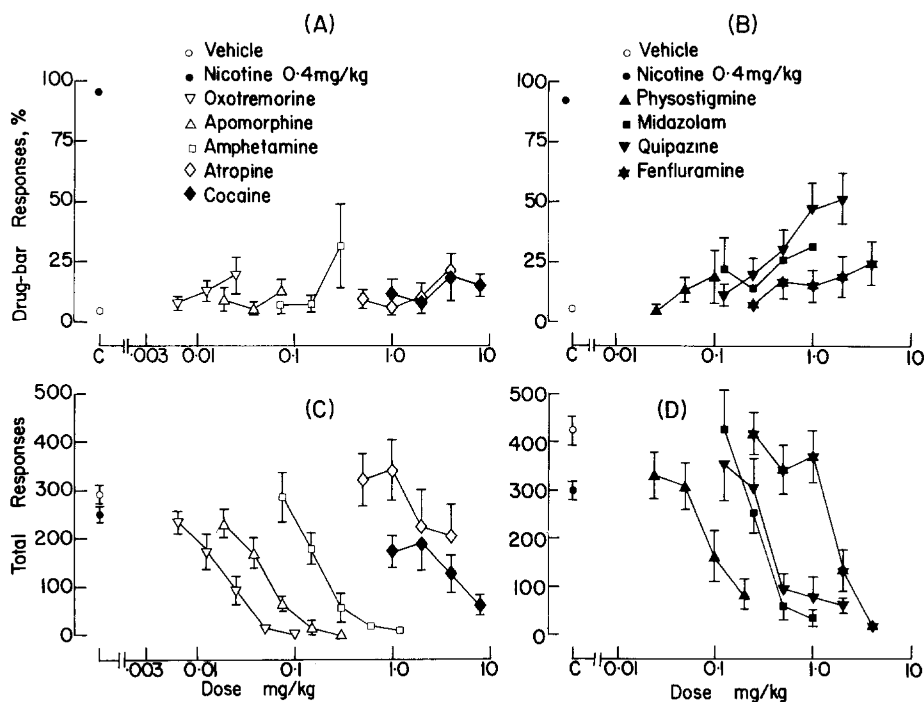

Fig. 5
Influence of drugs from a range of pharmacological classes on percent responding on nicotine-appropriate bar and on the total number of responses by groups of 6–8 rats trained to discriminate nicotine (0.4 mg/kg) from saline. Each point represents the mean ± SEM of observations made in 5-min extinction tests

appropriate responding over the dose range 0.019–0.3 mg/kg.

Compounds which either increase or reduce muscarinic-cholinergic function did not generalise with nicotine. Thus oxotremorine (0.0063–0.1 mg/kg), atropine (0.5–4.0 mg/kg) and physostigmine (0.025–0.2 mg/kg) all failed to significantly increase nicotine-appropriate responding when compared with values from vehicle controls. The 5-hydroxytryptamine (5-HT) agonist quipazine (0.125–2.0 mg/kg) increased drug-appropriate responding in a dose-related manner. At doses of 1.0 and 2.0 mg/kg drug-appropriate responding was 46.4 ± 10.5% ($t = 4.00$, df 42, $P < 0.01$) and 50.5 ± 10.6% ($t = 4.43$, df 42, $P < 0.01$) respectively. Fenfluramine (0.25–4.0 mg/kg) did not influence nicotine-appropriate responding. Midazolam, a short-acting benzodiazepine, did not generalise with nicotine over the dose range studied (0.125–1.0 mg/kg). Consideration of the percentages of rats selecting the drug-appropriate bar did not change any of these findings.

All drugs tested with the exception of atropine produced a dose-related decrease in the overall numbers of responses on both bars (Fig. 5C and D). This would indicate that despite being inactive in generalising with the nicotine cue, the drugs were active in the sense that they produced other behavioural effects in the dose ranges examined. For the majority of drugs examined the largest doses employed markedly suppressed responding and it was not, therefore, possible to assess discriminative effects in all rats at these doses.

Discussion

The present results add to the growing body of evidence that the main behavioural effects of nicotine are mediated through central cholinergic receptors. The investigations confirm and extend previous findings that the nicotine cue is a specific and powerful dose-related effect which is robust enough to serve as a behavioural marker in studies of the underlying central mechanisms (Chance et al. 1977).

Plasma concentrations of nicotine displayed dose-response and time-course functions paralleling the drug's stimulus effects (Fig. 3 and Table 1). Following the training dose, nicotine plasma concentrations compared well with those reported by Turner (1975) and Romano et al. (1981). Previous investigators have found maximal concentrations of nicotine in brain 10–20 min after peripheral administration (Yamamoto et al. 1968; Hirschhorn and Rosecrans 1974), lending further support to the early detection of the cue in our experiments. In cigarette smokers who inhaled, plasma nicotine concentrations ranged from 4–72 ng/ml with a mean concentration in the region of 30 ng/ml (Russell et al. 1980). These values are of similar magnitude to those reported in this study, where the plasma nicotine concentration was estimated as 48 ng/ml at the ED_{50} dose for the cueing effect. However, the concentration produced by the training dose itself of nicotine was considerably greater.

Earlier investigators have maintained that the nicotine discriminative stimulus is highly specific, and our observations support and extend these claims (Morrison and Stephenson 1969; Rosecrans and Chance 1977; Rosecrans et al. 1978). Although nicotine is considered a central stimulant, neither cocaine nor amphetamine generalised with nicotine. The possible weak effect with amphetamine is consistent with Morrison and Stephenson (1969) and Chance et al. (1977). However, rats trained with amphetamine display not more than 40% drug appropriate-responding in tests of generalisation to nicotine (Ho and Huang 1975; Pratt and Stolerman unpublished data). Thus, the stimulus effect of nicotine is not equivalent to that of the psychomotor stimulant drugs cocaine and amphetamine. Alternatively, the weak effect with amphetamine could have been attributed to its dopamine-releasing properties; in view also of the possible link between nicotine and dopamine systems (Giorguieff et al. 1977) we examined whether apomorphine could generalise with nic-

otine. The negative results obtained with this compound agree with those of Morrison and Stephenson (1969), and suggest that nicotine does not produce its stimulus effects through those dopamine systems on which apomorphine acts.

In addition to its central stimulant properties nicotine has been reported to produce feelings of tranquility (Gilbert 1979). We therefore carried out generalization tests with the short-acting benzodiazepine midazolam (Pieri et al. 1981). However, midazolam did not increase nicotine-appropriate responding. These findings agree with those reported for chlordiazepoxide (Morrison and Stephenson 1969) and indicate that the nicotine discriminative stimulus is probably not a result of a tranquillising action of the drug (Gilbert 1979), nor is it likely to be mediated by changes in GABA function, the neuronal system with which benzodiazepines are thought to interact primarily (Haefely et al. 1975; Guidotti et al. 1978).

The 5-HT agonist quipazine did partially generalise with nicotine in doses which themselves are discriminable (White et al. 1979). At present it is not known whether nicotine can generalise with quipazine in similar tests. One possible explanation of the partial transfer in our experiments would be that quipazine has some indirect affects on cholinergic function. Tests with fenfluramine revealed that the partial transfer obtained with quipazine appears not to be a characteristic common to all drugs which increase 5-HT function. It is therefore unlikely that the stimulus effect of nicotine is identical to that of drugs which enhance cerebral 5-HT function, which generalise with each other (White et al. 1979).

The lack of involvement of muscarinic receptors in the nicotine cue is demonstrated by the finding that atropine, physostigmine and oxotremorine did not influence the amount of nicotine-appropriate responding. The results for physostigmine and atropine are in agreement with those reported previously in drug-discrimination work using different experimental techniques (Morrison and Stephenson 1969; Hirschhorn and Rosecrans 1974). The muscarinic agonist arecoline also did not generalise with nicotine (Schechter and Rosecrans 1972). The ability of animals to distinguish between drugs acting on muscarinic or nicotinic receptors was further demonstrated by findings that oxotremorine but not nicotine generalised with arecoline, and by experiments with cholinergic antagonists (Meltzer and Rosecrans 1981).

The recent interest in characterization of central nicotinic receptors led us to examine two analogues of nicotine, anabasine and cytisine, which were active in displacing nicotine from its binding sites in rat brain (Romano and Goldstein 1980). Both compounds produced about 60% nicotine-appropriate responding in our procedure, compatible with the notion that the binding site studied by Romano and Goldstein (1980) was a functional receptor mediating the nicotine cue. Moreover, both anabasine, a tobacco alkaloid and cytisine, a laburnum alkaloid, have nicotine-like effects in a number of peripheral systems (Barlow and McLeod 1969; Haefely 1974; Romano 1981), indicating that the cue receptor may be related to the peripheral ganglionic nicotinic receptor.

Our findings do not wholly agree with those reported by Romano et al. (1981) who observed nicotine-like discriminative effects with anabasine but not cytisine in a T-maze shock-escape paradigm. The reasons for this difference is unclear; Romano et al. (1981) attributed their negative result with cytisine to the poor penetration of cytisine into the CNS. However, we employed smaller doses of cytisine than Romano et al. (1981) yet were able to obtain some generalisation with nicotine; the differences might therefore reflect differences in the sensitivity or specificity of the two procedures. Neither cytisine nor anabasine completely generalised with nicotine in our studies and larger doses of these drugs could not be tested because of the overall suppression of responses. Clearly the pharmacological profile of these agents is not completely identical to that of nicotine. Perhaps these compounds did not reach the brain in sufficient quantities to activate the nicotine receptor prior to the onset of other less specific behavioural effects. Recently, we have found that greater generalization can occur in rats trained with a lower dose of nicotine (Garcha et al. 1983).

Taken together our results constitute further evidence that nicotine produces a highly specific cue, probably mediated through cholinergic receptors related to those in peripheral ganglia.

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References

Abood LG, Lowy K, Tometsko A, Booth H (1978) Electrophysiological, behavioural, and chemical evidence for a non-cholinergic stereospecific site for nicotine in rat brain. J Neurosci Res 3:327–333

Barlow RB, McLeod LJ (1969) Some studies on cytisine and its methylated derivatives. Br J Pharmacol 35:161–174

Barry H III (1974) Classification of drugs according to their discriminable effects in rats. Fed Proc 33:1814–1824

Chance WT, Murfin D, Krynock GM, Rosecrans JA (1977) A description of the nicotine stimulus and tests of its generalization to amphetamine. Psychopharmacology 55:19–26

Colpaert FC (1977) Drug-produced cues and states: Some theoretical and methodological inferences. In: Lal H (ed) Discriminative stimulus properties of drugs. Plenum, New York, pp 5–21

D'Mello GD, Stolerman IP (1977) Comparison of the discriminative stimulus properties of cocaine and amphetamine in rats. Br J Pharmacol 61:415–422

Feyerabend C, Russell MA (1980) Assay of nicotine in biological materials: sources of contamination and their elimination. J Pharm Pharmacol 32:178–181

Garcha HS, Giardini V, Pratt JA, Stolerman IP (1983) Anabasine and cytisine: nicotine-like behavioural effects in a drug-discrimination procedure. Br J Pharmacol 79:272P

Garcha HS, Kumar R, Pratt JA, Stolerman IP (1982) Characteristics of the nicotine cue in rats. Br J Pharmacol 76:227P

Gilbert DG (1979) Paradoxical tranquilizing and emotion-reducing effects of nicotine. Psychol Bull 86:643–661

Giorguieff MF, Le Floc'h ML, Glowinski J, Besson MJ (1977) Involvement of cholinergic presynaptic receptors of nicotinic and muscarinic types in the control of the spontaneous release of dopamine from striatal dopaminergic terminals in the rat. J Pharmacol Exp Ther 200:535–544

Guidotti A, Toffano G, Costa E (1978) An endogenous protein modulates the affinity of GABA and benzodiazepine receptors in rat brain. Nature 275:553–555

Haefely W (1974) The effects of various "nicotine-like" agents in the cat superior cervical ganglion in situ. Naunyn-Schmiedeberg's Arch Pharmacol 281:93–117

Haefely W, Kulcsar A, Möhler H, Pieri L, Polc P, Schaffner R (1975) Possible involvement of GABA in the central actions of benzodiazepines. In: Costa E, Greengard P (eds) Advances in biochemical psychopharmacology, vol 14. Raven Press, New York, pp 131–151

Hirschhorn ID, Rosecrans JA (1974) Studies on the time course and the effect of cholinergic and adrenergic receptor blockers on the stimulus effects of nicotine. Psychopharmacology 40:109–120

Ho BT, Huang J-T (1975) Role of dopamine in *d*-amphetamine-induced discriminative responding. Pharmacol Biochem Behav 3:1085–1092

Meltzer LT, Rosecrans JA (1981) Discriminative stimulus properties of arecoline: A new approach for studying central muscarinic receptors. Psychopharmacology 75:383–387

Morrison CF, Stephenson JA (1969) Nicotine injections as the conditioned stimulus in discrimination learning. Psychopharmacologia 15:351–360

Pieri L, Schaffner R, Scherschlicht R, Polc P, Sepinwall J, Davidson A, Möhler H, Cumin R, Da Prada M, Burkard WP, Keller HH, Müller RK, Gerold M, Pieri M, Cook L, Haefely W (1981) Pharmacology of midazolam. Drug Res 31:2180–2201

Romano C (1981) Nicotine action on rat colon. J Pharmacol Exp Ther 217:828–833

Romano C, Goldstein A (1980) Stereospecific nicotine receptors on rat brain membranes. Science 210:647–650

Romano C, Goldstein A, Jewell NP (1981) Characterization of the receptor mediating the nicotine discriminative stimulus. Psychopharmacology 74:310–315

Rosecrans JA, Chance WT (1977) Cholinergic and non-cholinergic aspects of the discriminative stimulus properties of nicotine. In: Lal H (ed) Discriminative stimulus properties of drugs. Plenum, New York, pp 155–185

Rosecrans JA, Kallman MJ, Glennon R (1978) The nicotine cue: an overview. In: Colpaert FC, Rosecrans JA (eds) Stimulus properties of drugs: Ten years of progress. Elsevier-North Holland, Amsterdam, pp 69–81

Russell MAH, Jarvis M, Iyer R, Feyerabend C (1980). Relation of nicotine yield of cigarettes to blood nicotine concentrations in smokers. Br Med J 280:972–976

Schechter MD, Rosecrans JA (1972) Nicotine as a discriminative cue in rats: Inability of related drugs to produce a nicotine-like cueing effect. Psychopharmacologia 27:379–387

Schwab LS, Kritzer MF (1982) The effect of cholinergic antagonists on a central response to nicotine. Experientia 38:119–120

Sershen H, Reith MEA, Lajtha A, Gennaro J (1981) Non-cholinergic, saturable binding of (±)-[^{3}H]nicotine to mouse brain. Journal of Receptor Research 2:1–15

Snedecor GW, Cochran WG (1967) Statistical methods, 6th edn. Iowa University Press, Iowa

Stolerman IP, D'Mello GD (1981) Role of training conditions in discrimination of central nervous stimulants by rats. Psychopharmacology 73:295–303

Stolerman IP, Pratt JA, Garcha HS (1982) Further analysis of the nicotine cue in rats. In: Drug discrimination: applications in CNS pharmacology. Elsevier, Amsterdam, pp 203–210

Turner DM (1975) Influence of route of administration on metabolism of [^{14}C] nicotine in four species. Xenobiotica 5:553–561

White FJ, Appel JB, Kuhn DM (1979) Discriminative stimulus properties of quipazine: direct serotonergic mediation. Neuropharmacology 18:143–151

Winer BJ (1971) Statistical principles in experimental design, 2nd edn. McGraw-Hill, New York

Yamamoto I, Inoki R, Iwatsubo K (1968) Penetration of nicotine-^{14}C into several rat tissues in vivo and in vitro. Toxicol Appl Pharmacol 12:560–567

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