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Locomotor Activity After Nicotine Infusions Into the Fourth Ventricle of Rats

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SHOAIB, M. AND I. P. STOLERMAN. *Locomotor activity after nicotine infusions into the fourth ventricle of rats.* PHARMACOL BIOCHEM BEHAV 48(3) 749–754, 1994.—Microinjections of nicotine into the fourth ventricle of rats were reported previously to produce a characteristic prostration syndrome; similar microinjections have been investigated for effects on locomotor activity. It was confirmed that nicotine (4 μg) administered into the fourth ventricle of rats produced prostration which was also manifested on a second challenge with the drug. Increasing doses of nicotine produced increasing magnitudes of prostration and dose-related decreases in locomotor activity. In rats pretreated with nicotine (0.4 mg/kg SC) for 10 days, no tolerance was seen to either the prostration response or the locomotor depression. Mecamylamine (1.0 mg/kg SC) completely prevented the prostration response produced by 4 μg of nicotine, but the locomotor depression was still evident. The locomotor changes following intraventricular administration of nicotine appeared to be different from the locomotor depression seen following systemic administration because the posture of the animals was different and the latter effects showed tolerance with repeated exposures to nicotine and were fully blocked by mecamylamine. These findings suggested that the prostration response and the locomotor depression were mediated by different mechanisms.

Nicotine Fourth ventricle Prostration Locomotor activity Mecamylamine

ACCUMULATING evidence suggests that different behavioural effects of nicotine may be attributed to effects of the drug in different regions of the brain (26). Furthermore, biochemical (6,30), electrophysiological (10), and molecular biological (14) evidence suggests the existence of multiple subtypes of central nicotinic receptors. Certain behavioural and physiological effects of nicotine differ in their sensitivity to blockade by mecamylamine, which is consistent with the existence of multiple subtypes of central receptor (9).

A number of studies have utilised locomotor activity as a behavioural measure to determine the central actions of nicotine. Much of the previous work in rats has been devoted to characterising the nature of tolerance seen with repeated nicotine administration (27,28). Moderate doses of nicotine have an inhibitory effect on locomotor activity during the first 20 min after a systemic injection, followed by a longer period of facilitation. Tolerance develops rapidly to the initial depressant effect, whereas the activating effects become more pronounced after repeated administration of nicotine (8). Several more recent studies have been aimed at investigating the neuropharmacological mechanisms underlying the activation effects of nicotine on locomotion (16–18,21).

Less attention has been paid to the neural substrates underlying the locomotor depressant effects of nicotine. Abood and colleagues (2) observed a characteristic prostration syndrome (spreading of all limbs) when small doses of nicotine were administered directly into the fourth ventricle. Tremors, seizures, and total body rolling accompanied this event. To determine the neurochemical mechanisms underlying this syndrome, the ability of various psychotropic agents to prevent the prostration response was assessed. Intraventricular administration of hexamethonium completely blocked the syndrome, but mecamylamine produced only a partial blockade. Other nicotinic compounds such as *d*-tubocurarine and α-bungarotoxin were found to be ineffective (3). Following these preliminary studies, reports emerged of a strong correlation between the prostration response and radioligand binding with nicotinic analogues. The locus of nicotine action was investigated further in later studies using kainic acid lesions to abolish nicotine-induced prostrations (4). Hindbrain structures, in particular the vestibular nuclei and lobule X of the cerebellum, were reported to mediate the prostration response (4).

The experiments presented in this communication explore the behavioural consequence of administering nicotine into

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the fourth ventricle with quantified, automated measurements of locomotor activity as well as with ratings of prostration responses based on the scales used by Schwab and Kritzer (22). Further experiments determine whether tolerance can develop to these responses and whether mecamylamine blocks them. A preliminary account of the studies has been published (23).

METHOD

Animals

Male hooded Lister rats (200–300 g; Harlan Olac, Bicester) were used. Rats were housed individually with free access to food and water, in rooms maintained at 20–22°C with a regular light : dark cycle (lights from 0800–2000 h).

Apparatus

Locomotor tests were performed in photocell activity cages (30 × 30 × 30 cm), which were constructed from clear Perspex with wire mesh floors (21). Two parallel beams of infrared light were located 3 cm from the walls and 4 cm above the floor. Interruptions of the beams were recorded by a computer (CUBE system, Control Universal, Cambridge) in an adjoining room. During each session, interruptions of one beam that followed interruptions of the other beam were recorded as cage crosses (ambulation).

Surgery

One week prior to testing locomotor activity, guide cannulae (22 gauge, Plastic Products, Roanoke, VA) aimed at the fourth ventricle were implanted into rats under surgical anaesthesia (Halothane). Stereotaxic coordinates were A = −3.0 mm from the interaural line, L = 0.0 mm, and V = 2.0 mm below the horizontal zero plane according to the atlas of Pellegrino et al. (19). The coordinates had been verified previously by injection of dye in several subjects which were not included in the experiment.

General Procedures

All systemic injections were given in a volume of 1 ml/kg. All intracerebroventricular microinjections of nicotine were made with an injection cannula that extended beyond the guide to enter the fourth ventricle. Once the cannula was inserted, nicotine or vehicle was infused in a volume of 1 μl over 30 s. A further 30 s was allowed for complete delivery and diffusion. Immediately after termination of the infusion, the rat was observed and ratings of the magnitude of prostration were made. In most experiments, a rating system similar to that described by Schwab and Kritzer (22) was used to assess prostration. Ratings, therefore, started from 0 (no prostration) to 4 (complete immobilization). Intermediate scores were determined from the extent of immobilization seen with the four limbs, and scored with increments of 1 for each limb showing ataxia. Immediately following observation and rating, the rats were placed into locomotor boxes for 50 min (Experiments 1 and 2) or 60 min (Experiment 3) and the numbers of cage crosses were recorded.

Histology

At the end of each experiment, the locations of the injection cannulae were verified histologically. Of the 30 rats implanted, three were excluded from the experiments due to incorrect placement of cannulae. Correctly located injection cannulae produced tracks showing penetration through the cerebellum (cI Vermian lobule; Lingula) and into the fourth ventricle.

Drugs

Nicotine bitartrate (BDH, Dorset) was dissolved in distilled water. The pH of the solution was adjusted to 7.0 with dilute sodium hydroxide and the solution was then balanced isotonically with sodium chloride. Mecamylamine HCl was donated by Merck, Sharp & Dohme, Harlow, Essex, UK. All doses were calculated as those of the bases.

Statistical Analyses

Dose-related effects of nicotine on locomotor activity were analyzed using one- and two-factor analyses of variance and *t*-tests.

EXPERIMENT 1: EFFECTS OF NICOTINE ADMINISTERED INTO THE FOURTH VENTRICLE

The ability of nicotine to produce a prostration response after intraventricular administration was explored. A dose of 4 μg nicotine, previously reported as sufficient to produce the prostration response (2) was used. A second nicotine challenge (4 μg) was given to the same group of rats 48 h later, to assess the stability of responses.

Procedure

Each rat was tested after microinjection of nicotine (4 μg) and saline, with 1 day of rest between the tests (n = 7). The order of testing nicotine or saline was determined for each rat by a random method. Each rat, therefore, served as its own control. Descriptions of behavioural changes as a result of treatment were made, but were not rated on any scale in this initial experiment. Following the injections, the rats were placed immediately into photocell cages and locomotor activity was assessed in consecutive intervals of 5 min, for a total of 50 min.

Results

Administration of nicotine (4 μg) into the fourth ventricle produced a prostration syndrome similar to that reported by Abood et al. (5). All seven rats showed this response. None of the rats showed any behavioural changes when saline was administered into the ventricle. During the microinjection procedure, physiological effects of nicotine were apparent. These consisted of increased breathing (hyperpnoea), defecation, urination, and flaccidity of the limbs. The prostration effect persisted for approximately 2 min, after which three of the seven rats showed circling behaviour. The locomotor activity measurements that followed showed a depressant action of nicotine. Although the overall amounts of activity for the whole test did not differ following administration of nicotine or saline, $F(1, 6) = 1.6$, there was a significant interaction between drug effect and time after injection, $F(9, 54) = 3.38$, $p < 0.02$. Separate analyses of the results for different periods of time showed a significant effect of nicotine during the initial 5 min of the test, $t(6) = 3.1$, $p < 0.05$. Figure 1 illustrates these results.

The same dose of nicotine was administered into the fourth ventricle of six of the rats; prostration was seen again, but the effect was much weaker than observed following the first injection. Only two rats showed the marked prostration ob-

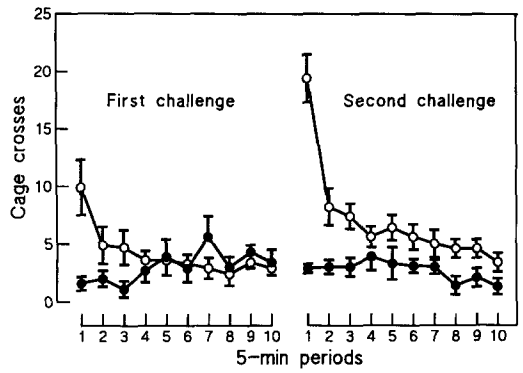

FIG. 1. Time-course of locomotor depression following administration of nicotine (4 μg) into the fourth ventricle (n = 6-7). The data represent mean numbers of cage crosses in 5-min periods following administration of nicotine (4 μg, ●) and saline (○). The left section shows activity determined on the first nicotine challenge and the right section shows results from the second challenge.

served after the first challenge with nicotine; the rest of the rats showed submaximal effects and were able to move their limbs despite maintaining a prostrated position. Physiological signs of nicotine administration (hyperpnoea and defecation) were still apparent after the second challenge. However, Fig. 1 shows that measurements of locomotor activity following these injections revealed marked depression as compared with activity following administration of saline, $F(1, 4) = 10.8$, $p < 0.05$; the means were 27 ± 3 cage crosses following nicotine and 70 ± 5 cage crosses after saline. The scores obtained after the second challenge with nicotine were similar to those obtained after the first challenge, $t(10) = 0.4$, whereas Fig. 1 shows that activity following saline injection was much greater during the second test than during the first test, $t(10) = 2.4$, $p < 0.05$. Time-course measurements revealed a persistently low level of activity throughout the 50-min test. The depressant phase was most prominent during the initial 5 min of the test (Fig. 1).

Discussion

The prostration syndrome produced by nicotine in Experiment 1 of the present study appeared to be very similar to that reported by Abood et al. (3), although the dose used was relatively large (4 μg, as compared with 0.81 μg or 5 nmol). The syndrome persisted for approximately 2 min; after this period, rats recovered the use of limbs but still showed an inability to move freely, and the time course of activity depression was similar to that seen following administration of a large systemic dose of nicotine (28). The main new finding was the locomotor depression. This effect was most prominent during the initial 5 min, suggesting a linkage between the prostration response and the locomotor depression.

The reproducibility of this syndrome following a second challenge was also examined. The results suggested that a degree of tolerance may have occurred to the prostration response. However, because prostration was not rated on a scale, firm conclusions cannot be made from this study. Rapid tolerance to the locomotor depression was not evident, because the effects were no less strong following the second nicotine challenge than following the first challenge; the increased activity after the second administration of vehicle makes precise comparisons difficult. Tolerance to the prostration syndrome has been reported with repeated intraventricular injections (4). Tolerance to locomotor depressant effects has been demonstrated after both single and multiple systemic (SC and IP) administrations of nicotine (7,11,28).

EXPERIMENT 2: DOSE RESPONSE AND TOLERANCE STUDY

Systemic administration of a single dose of nicotine has been shown to produce a parallel shift to the right of the dose-response curve for the locomotor depressant effect of nicotine in rats not previously exposed to the test apparatus (27), whereas chronic treatment produces an upward shift of the dose-response curve for the locomotor stimulant effect in rats previously exposed to the test apparatus (12). Abood et al. (1) demonstrated prostration using a single dose of nicotine (5 nmol, 0.81 μg) and found that administering nicotine into the fourth ventricle twice daily for 4 days was sufficient to produce tolerance in 70% of rats tested. However, chronic infusions of nicotine delivered via Alzet osmotic minipumps (50 nmol/h) into the lateral ventricle for 10 days failed to produce tolerance. In addition, a systemic chronic dosing regime (acute IP injection of 12 μmol/kg twice daily) for 14 days failed to produce tolerance (1). A limitation of these studies was the use of only a quantal score indicating the proportion of rats showing prostration. Later studies examining the prostration syndrome utilised a rating scale for grading prostration from 0 (no response) to 4 (complete prostration) in half point increments (22). This latter approach may provide a more sensitive and accurate index than the quantal score.

The aim of the present experiment was to investigate tolerance to the behavioural effects induced by injecting nicotine into the fourth ventricle using a graded scale for assessing the prostration response and the changes in locomotor activity.

Procedure

Guide cannulae aimed at the fourth ventricle were implanted in two groups of six rats. Following 7 days for recovery from surgery, the rats were pretreated with single daily systemic (SC) injections of nicotine (0.4 mg/kg) or saline for 10 days. Each rat was then tested with vehicle or doses of nicotine (1-8 μg) injected into the fourth ventricle in a randomised order. To confirm the effectiveness of the chronic nicotine treatment, the rats were also tested with a single systemic dose of nicotine (0.4 mg/kg SC). The rats were tested on alternate days, with chronic dosing taking place on the intervening days. The consequences of nicotine administrations were assessed by means of a rating scale for prostration and with photocell cages for locomotor activity (60-min sessions).

Results

Graded doses of nicotine administration into the fourth ventricle of drug-naive (saline pretreated) rats elicited dose-related prostration (Fig. 2). Locomotor activity determined following the prostration syndrome showed dose-dependent decreases as compared with controls (Fig. 2). The largest dose of nicotine (8 μg) reduced the number of cage crosses by 57%.

In rats pretreated with nicotine, a similar pattern emerged for both the prostration scores and locomotor activity. Maximal prostration also occurred at the 8 μg nicotine dose (Fig. 2), but no differences in the prostration scores of the two groups were apparent. Analysis of the locomotor activity data in a two-factor ANOVA confirmed the dose-related effects of

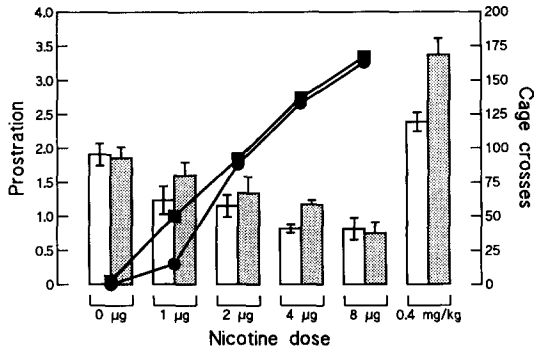

FIG. 2. Nicotine-induced prostration responses and locomotor depression observed in two groups of rats (n = 6). The line graph shows mean rating scores of prostration (0 = no detectable effects, 4 = complete immobilization) in rats chronically treated with either saline (●) or nicotine (■) for 10 days. The bar graph illustrates mean locomotor activity ± SEM measured during 60 min immediately after administration of nicotine (open bars, saline pretreatment; shaded bars, nicotine pretreatment). The bars on the extreme right of the figure represent activity following SC injection of nicotine (0.4 mg/kg).

nicotine administered intraventricularly, $F(4, 40) = 16.1$, $p < 0.001$, but there was no significant effect of chronic treatment with nicotine, $F(1, 40) = 1.7$, and there was no significant interaction between effects of pretreatment and test doses. Confirmation of the effectiveness of the chronic nicotine treatment came from the effects of the systemic challenge with 0.4 mg/kg of nicotine. Figure 2 shows that rats pretreated with nicotine produced a significantly greater, $t(10) = 3.6$, $p < 0.01$, mean number of cage crosses (169 ± 12) than the rats pretreated with saline (119 ± 7).

Discussion

The magnitude of the prostration response seen in Experiment 2 was not as large as that observed in Experiment 1. A possible explanation for this may lie in the much larger amount of handling received by the rats during the chronic pretreatment phase of the study. The main finding to emerge from Experiment 2 was the lack of tolerance to the depressant effects of intraventricular nicotine, under conditions that showed adaptation to systemic nicotine. A lack of tolerance to the prostration syndrome was also found by Abood et al. (1), who observed that systemic treatment (twice daily injections of 12 µM/kg nicotine for 10 days IP) failed to modify the symptoms induced by intraventricular infusions of 5 nmol of nicotine.

One plausible explanation for the apparent lack of tolerance to locomotor depressant effects may be that induction of locomotor depression via different routes of administration may be mediated by different mechanisms. Support for this hypothesis comes from recent demonstrations of locomotor depression produced by injecting nicotine directly into the nucleus accumbens (29). Once nicotine is infused into the fourth ventricle, the areas most likely to be exposed to nicotine are the cerebellar structures, and in particular lobule X; of the cerebellum (1). Hence, these sites may mediate prostrations, but whether they mediate locomotor depression produced by systemic injections of nicotine remains uncertain. Further, the prostration response (splaying of the four limbs) after injections of nicotine is distinct from the hindlimb ataxia seen after systemic administration of the drug (7). However, it is also possible that doses of nicotine larger than 0.4 mg/kg (SC) would produce tolerance to the effects of nicotine injected by the intracerebroventricular route.

Another explanation of this phenomenon may come from a consideration of the autonomic signs produced by intraventricular administration of nicotine. Observations of hyperpnoea and defecation suggest this action to resemble signs of direct autonomic stimulation. This form of stimulation has been widely observed in the cat (24). The autonomic effects fail to show tolerance and if the locomotor depression is in some way linked to the autonomic disturbances, it too would not be expected to show tolerance.

EXPERIMENT 3: EFFECT OF MECAMYLAMINE ON NICOTINE-INDUCED PROSTRATION SYNDROME

Mecamylamine is a noncompetitive antagonist of the nicotine receptor that can block almost all behavioural effects induced by nicotine (7,13). Together with hexamethonium that does not penetrate as well into the CNS, it has been used as a pharmacological tool to determine if effects of nicotine are mediated centrally or peripherally (8). Initial investigations by Abood et al. (2) found mecamylamine to be ineffective in blocking nicotine-induced prostration responses. In a subsequent study, Schwab and Kritzer (22) observed mecamylamine to completely block this parameter. In light of this variable outcome, the ability of mecamylamine to block the prostration and locomotor depression produced by nicotine administration into the fourth ventricle was examined again. The experiment utilised a single dose of mecamylamine (1.0 mg/kg SC) that was demonstrated previously to block both the depressant and stimulant effects of systemically administered nicotine on locomotor activity (7).

Procedure

Following recovery from surgery to implant guide cannulae, eight rats were screened for their ability to show the prostration response with 4 µg nicotine as compared with vehicle in two tests with an interval of 2 days between them. Each rat was then tested with a randomised sequence of treatments: a) two injections of saline (S-S); b) injections of saline and nicotine (S-N), and c) injections of mecamylamine and nicotine (M-N). There were 2 days without injections between successive tests. Saline (1 ml/kg SC) or mecamylamine (1.0 mg/kg SC) was given 30 min before injection of vehicle or nicotine (4 µg) into the fourth ventricle. Each rat, therefore, served as its own control. Ratings of the extent of prostration were made and locomotor activity was measured in 10-min intervals over 60 min.

Results

The screening test with nicotine (4 µg) injected into the fourth ventricle elicited a distinct prostration response with a mean rating score of 3.3 ± 0.3, as compared with a mean score of 0.0 following saline (Fig. 3). Following the second challenge with nicotine, ratings of prostration produced a mean score of 3.4 ± 0.3 with nicotine (S-N) in contrast to 0.0 following saline (S-S). Pretreatment with mecamylamine (1.0 mg/kg SC) completely abolished the prostration response in all eight rats (M-N). However, mecamylamine produced only marginal signs of blockade of the decreases in locomotor activity. Nicotine reduced the mean number of cage crosses in the 60 min tests from 48.9 ± 5.8 to 16.3 ± 2.0, $t(14) = 6.4$, $p < 0.01$; although there were 26.1 ± 4.1 cage crosses when

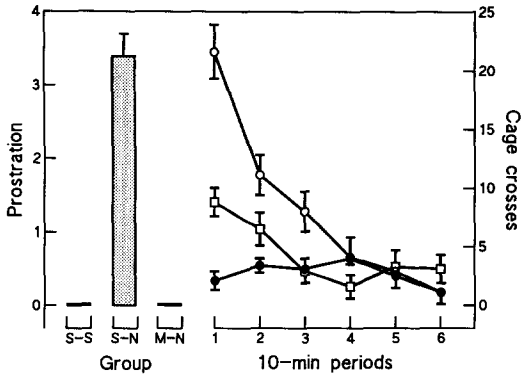

FIG. 3. The effect of mecamylamine (1.0 mg/kg SC) pretreatment on prostration and locomotor depression induced by nicotine (4 μg) administered into the fourth ventricle of rats (n = 8). The bar graph in the left part of the figure shows the mean rating of prostration following injections of saline (S-S), nicotine (S-N), and mecamylamine plus nicotine (M-N). Locomotor activity of the same rats is represented by the line graphs in the right section of the figure as mean cage crosses ± SEM in six successive 10-min periods (○, saline; ●, nicotine; □, mecamylamine plus nicotine).

mecamylamine was administered prior to the nicotine, there was no significant difference from the results with nicotine alone, $t(14) = 1.9$.

Figure 3 shows the time courses of the effects on locomotor activity. A two-factor ANOVA for repeated measures disclosed a significant effect of drug treatment, $F(2,14) = 48.4$, $p < 0.001$, a significant effect of time, $F(5,35) = 19.7$, $p < 0.001$, and a significant interaction, $F(10,70) = 9.8$, $p < 0.001$, indicating that the locomotor depressant action of nicotine was most prominent during the initial 10 min of the test. A one-factor ANOVA for the first 10-min period showed a significant effect of drug treatments, $F(2,14) = 42.2$, $p < 0.001$. Mecamylamine attenuated the nicotine-induced locomotor depression, $t(14) = 3.1$, $p < 0.05$, but the activity of rats treated with mecamylamine and nicotine was much less than that of saline controls, $t(14) = 9.0$, $p < 0.01$.

Discussion

Mecamylamine (1.0 mg/kg SC) completely blocked nicotine-induced prostration responses. This finding supports the previous report that mecamylamine can block nicotine-induced prostration when administered directly into the fourth ventricle (22). Surprisingly, the subsequent locomotor depression showed some resistance to 1 mg/kg of mecamylamine. Larger doses of mecamylamine were not evaluated, but with many nicotine-induced behavioural effects (in particular, changes in locomotor activity) a 1.0 mg/kg dose of mecamylamine has been found to be sufficient to produce complete antagonism of the response (8,25). From the present results it may be inferred that the prostration response is mediated by mecamylamine-sensitive nicotinic receptors and that the locomotor depressant response may be mediated via receptors that are relatively insensitive to mecamylamine. The results suggest that differences exist between the characteristics of locomotor depression after systemic and fourth ventricle administrations of nicotine.

GENERAL DISCUSSION

The present series of experiments confirm that nicotine can produce a prostration response when injected directly into the fourth ventricle of rats (1). The response is dose related and it fails to show tolerance after repeated systemic administration of nicotine. The doses of nicotine that produced prostration when injected intraventricularly also depressed locomotor activity in a dose-related manner, and the latter effect also failed to show tolerance. The systemic administration of mecamylamine blocked the prostration response, but did not completely block the locomotor depressant effect. Thus, the change in locomotor activity after intraventricular administration of nicotine may be distinguished from the locomotor depression seen following systemic administration of nicotine because the latter develops tolerance readily and can be fully blocked by mecamylamine (7,27). The gross behavioural effects are also different because, as noted above, nicotine administered systemically produces mainly ataxia of the hindlimbs, whereas administration into the fourth ventricle produces a different and characteristic splaying of all four limbs.

The differences between the prostration response and the locomotor depressant effects may be interpreted in two ways, the most probable explanation being that different brain regions mediate the different behavioural effects. Microinjection studies have implicated the nucleus accumbens as a possible site involved in mediating the depressant action of nicotine on locomotor activity (29); this effect showed tolerance in rats pretreated with nicotine (29) and may, therefore, be more closely related to the effects of systemically administered nicotine than the locomotor depression seen after fourth ventricle injections. The results can be explained if the behavioural changes are mediated by two populations of CNS nicotinic receptors, one of which is sensitive to blockade by mecamylamine and one that is relatively insensitive to the antagonist.

Previous studies have yielded some evidence for a dissociation in sensitivity to mecamylamine with other behavioural and physiological measures in mice (9). Studies have also yielded some evidence for dissociations between the effects of nicotinic compounds on locomotor activity and in drug discrimination experiments. The nicotine discriminative stimulus generalised to several nicotinic compounds and the ED_{50} values in the discrimination procedure correlated highly with their IC_{50} values for inhibition of binding of tritiated nicotine in vitro (20). However, some of these nicotinic compounds had effects on locomotor activity that were different from those of nicotine itself and these differences were presumed to reflect actions at different type of nicotine receptor (20). The different approach taken in the present experiments also suggests that nicotine may produce behavioural effects through actions at more than one type of receptor. It is intriguing that electrophysiological studies have reported on different subtypes of nicotinic receptor in the cerebellum, with differential sensitivities to the antagonists mecamylamine and α-bungarotoxin (10). More extensive work with a range of mecamylamine doses and with other nicotinic antagonists such as α-bungarotoxin may help to clarify the nature of these responses.

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