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Characterization of the locomotor stimulant action of nicotine in tolerant rats

P.B.S. Clarke[1] & R. Kumar

Institute of Psychiatry, De Crespigny Park, London SE5 8AF

1 Tests of locomotor activity (photocell cages) were used to investigate the development of tolerance to nicotine in rats. Repeated exposure to the apparatus did not influence the rate at which tolerance was acquired.

2 Comparisons of (+)-nicotine (0.4–1.6 mg kg^{-1}, s.c.) and (−)-nicotine (0.1–0.4 mg kg^{-1}, s.c.) in tolerant rats showed that the (−)-isomer was at least ten times more potent in stimulating motor activity.

3 Subcutaneous pretreatment with mecamylamine (1.0 mg kg^{-1}) completely prevented the locomotor stimulant action of nicotine in tolerant rats, whereas chlorisondamine (0.01 or 0.1 mg kg^{-1} s.c.) only partially reduced it. When mecamylamine was given after an injection of nicotine, the locomotor stimulant action of nicotine was blocked, and nicotine actually reduced activity.

4 A single intraventricular dose of chlorisondamine (2μg) blocked the stimulant actions of nicotine for the duration of the experiment (23–24 days).

Introduction

Habitual tobacco smokers report that, depending on the circumstances, nicotine can have either sedative or stimulant effects (see review by Kumar & Lader, 1981). In non-tolerant animals, initial depressant effects may be followed by stimulation, for example, of locomotor activity (Clarke & Kumar, 1983a) or of learned behaviour (Morrison, 1967; Pradhan, 1970; Stitzer, Morrison & Domino, 1970; Clarke & Kumar, 1983b); the pattern of change may, however, be critically influenced by the dose and by the 'baseline' (pre-drug) rate of responding. The depressant effects of nicotine wane upon repeated treatment with the drug (Domino & Lutz, 1973) and tests in tolerant rats have shown that nicotine markedly stimulates both locomotor activity (Kuschinsky & Hotovy, 1943; Morrison & Stephenson, 1972; Clarke & Kumar, 1983a) and also rewarded responding (Clarke & Kumar, 1983b). It is not clear whether the apparent emergence of an increasingly pronounced stimulant action merely reflects the development of tolerance to the depressant effects of nicotine, nor how far tolerance is some sort of acquired behavioural adaptation to the effects of the drug. It has, for example, been suggested that environmental cues, such as exposure to the locomotor test apparatus can influence the acquisition of nicotine tolerance (Schlatter & Bättig, 1978) although other workers (Mansner, 1972; Morrison & Stephenson, 1972; Stolerman, Fink & Jarvik, 1973) have shown that injections of nicotine given to rats or mice in their home cages can reduce or prevent locomotor depression and promote an enhanced stimulant effect (Clarke & Kumar, 1983a). In a recent study of operant behaviour, Hendry & Rosecrans (1982) demonstrated the importance of pharmacological rather than behavioural variables in the development of tolerance to a depressant action of nicotine. Mice were trained to lever press for sweetened milk, and tolerance subsequently occurred at the same rate in subjects given nicotine before the daily test as in other subjects receiving nicotine directly after each session instead.

The first experiment described here traces the development of tolerance to the depressant effect of nicotine and records the emergence of motor stimulation. Half the rats were not tested every day but

[1]Present address: Biological Psychiatry Branch, NIMH, Bldg. 10, Rm. 3N212, 9000 Rockville Pike, Bethesda, MD 20205, U.S.A.

were replaced in their home cages after their daily medication. It was therefore possible subsequently to examine the influence of exposure to the apparatus on the development of tolerance to nicotine.

Binding of nicotine in the rodent brain is stereospecific (Romano & Goldstein, 1980; Marks & Collins, 1982) and behavioural studies have shown that the (−)-isomer is the more potent one (Meltzer, Rosecrans, Aceto & Harris, 1980; Kumar, Pratt & Stolerman, 1983). In the second experiment described below, the potencies of a range of doses of (+)- and (−)-nicotine were compared on a measure of locomotor activity in rats that had already been made tolerant to (−)-nicotine.

Tolerance does not develop to the locomotor stimulant effects of nicotine (Kuschinsky & Hotovy, 1943; Clarke & Kumar, 1983a) and such stimulation can be prevented by systemic injection of the secondary amine, mecamylamine, but not by similar doses of the quaternary ganglion blocking agent, hexamethonium (Clarke & Kumar, 1983a). These two antagonists are nearly equipotent at peripheral sites in the cat (Stone, Torchiana, Navarro & Beyer, 1956) and although equivalent tests in rats are lacking, it seems reasonable to assume that nicotine acts centrally when increasing locomotor activity in rats. We report analogous tests of motor activity with chlorisondamine which is also a quaternary ganglion blocking drug, but its peripheral actions in rodents are somewhat better characterized than those of hexamethonium (Stone, Meckelnburg & Torchiana, 1958; Morrison, Goodyear & Sellers, 1969). In order to examine further the question of central versus peripheral actions of nicotine we have compared the effects of central versus systemic injections of chlorisondamine on measures of locomotor activity in nicotine tolerant rats.

Mecamylamine by itself does not alter the locomotor activity of rats, but pretreatment with this drug prevents the stimulant effect of nicotine in tolerant rats (Clarke & Kumar, 1983a). Electrophysiological evidence suggests that mecamylamine acts at nicotinic cholinoceptors on parasympathetic ganglion cells (Ascher, Large & Rang, 1979). If mecamylamine has a similar action in the central nervous system *in vivo* (see Schwartz, McGee & Kellar, 1982), then nicotine presumably increases locomotor activity by stimulating central receptors. This behavioural action lasts typically between 1 and 3 h, depending on dose (unpublished results), and brain levels of the drug follow a similar time course (Hirschhorn & Rosecrans, 1974). These observations are consistent with a tonic drug action, a suggestion which was tested more directly in the final experiment, by giving mecamylamine after a dose of nicotine, to see whether it would still block the stimulant effect of nicotine.

Methods

Male hooded rats (OLAC 76 Ltd, Bicester, UK) were maintained on food and water *ad libitum*. They were housed in pairs, on a random basis with respect to drug treatment, in a room illuminated from 08 h 00 m to 20 h 00 min.

Apparatus

Test cages (approximately 30 × 30 × 30 cm) were made of clear perspex with wire grid floors. Parallel, infra-red light beams 23 cm apart were projected 4 cm away from two opposite walls of the chamber; the beams ran 4.5 cm above the floor. Beam breaks were recorded by a solid state programming device and locomotor activity was measured as counts of the number of times a rat moved from one beam to the other. Counts occurring less than 0.5 s apart were not registered. Data were printed every 10 min by an electromagnetic counter (BRS/LVE) in an adjacent room. Rats were tested for 80 min immediately after injection. Tests were carried out between 10 h 00 min and 15 h 00 min.

Drugs

(−)-Nicotine hydrogen (+)-tartrate (BDH, Poole) and (+)-nicotine hydrogen (−)tartrate were dissolved in saline and neutralised to pH 7.2 ± 0.2 with NaOH. Mecamylamine HCl (Merck) and chlorisondamine Cl (Ciba-Geigy) were dissolved in saline. Unless otherwise indicated, all drugs were injected subcutaneously in the flank in a volume of 1 ml kg^{-1}. All injected doses refer to the base. Control injections were of saline.

Induction of tolerance to nicotine

Experiments 2, 3, 4, and 5 were done in rats which were already tolerant to nicotine. For two weeks the animals were injected daily with nicotine (0.4 mg kg^{-1} s.c.) and immediately replaced in their home cages. Each rat was then tested on two consecutive days, once with nicotine (0.4 mg kg^{-1}) and once with saline, in order to verify that locomotor activity was stimulated by the drug, and the experiment was begun between two and six days later. The level of tolerance to the locomotor depressant actions of nicotine does not fall off within a week of abstinence and it declines gradually over several weeks (Clarke & Kumar, 1983a).

Analysis of data

The rats' activity scores were tested over four consecutive periods of 20 min. Multivariate analysis of

variance was used, each rat serving as its own control, where appropriate. Specific comparisons were made by paired or unpaired *t*-tests and probability values are 2-tailed.

Intraventricular injections

Rats were anaesthetized with 1–2% halothane (May and Baker) in oxygen and were placed in a stereotaxic frame (Stoelting Co. USA). A 28-gauge cannula, external diameter 0.72 mm, (Plastic Products Co. C313) was lowered vertically through a hole drilled in the skull, with the tip aimed in the left lateral ventricle (De Groot, 1959; A 6.2, L 1.5, 4.5 mm below surface of skull). Correct placement was verified by injecting six weight-matched control rats with Luxol fast blue dye, followed by visual inspection of brain sections on a freezing microtome. The injection cannula was connected via polythene tubing to a 10 microlitre Hamilton syringe which was driven by a pump and delivered 5 μl of solution in 1 min. The cannula was left in place for a further minute and was then slowly withdrawn. Chlorisondamine was dissolved in 0.9% saline to a concentration of 0.4 μg base μl^{-1}. Control injections were of saline. The wound was dressed with Cicatrin power and was closed with stainless steel clips (Autoclips: Becton, Dickinson and Co. U.S.A). All rats recovered from anaesthesia in a few minutes and no abnormal motor effects were observed. The chlorisondamine-treated rats and the controls did not differ either in terms of the amounts of weight lost post-operatively or in their subsequent rates of recovery.

Procedure

(1) *Tolerance to nicotine and influence of exposure to test apparatus* Thirty two rats (223–300 g) were used, all were initially naive to the apparatus and to the drug; they were randomly allocated to 4 groups ($n = 8$). Two of the groups were tested daily for five consecutive days after injection of nicotine (0.4 mg kg^{-1} s.c.) or of saline. The other two groups were similarly injected but were immediately replaced in their home cages. All the subjects were tested on the 6th and 7th days; each rat was tested once with nicotine (0.4 mg kg^{-1}) and once with saline and the order of drug testing was counterbalanced.

(2) *Comparison of (+)-and (−)-nicotine in tolerant rats* Ten rats were first made tolerant to (−)-nicotine (for details see Methods) and they were then tested seven times at intervals of 48 h. The order of treatments were randomised and the following treatments were compared: saline, (−)-nicotine (0.1, 0.2, 0.4 mg kg^{-1}) and (+)-nicotine (0.4, 0.8, 1.6 mg kg^{-1}).

(3) *Pre-treatment with subcutaneous macamylamine or chlorisondamine in nicotine-tolerant rats: effects on locomotor stimulation* Each of 8 nicotine-tolerant rats (387–436 g) received 8 tests spaced 48 h apart. Each combination of treatment and pretreatment occurred once in a Williams Square design (Cox, 1958). Pretreatment consisted of saline, mecamylamine (1.0 mg kg^{-1}) and chlorisondamine (0.01, 0.1 mg kg^{-1}). The rats were returned to their home cages after pretreatment. Twenty minutes later, each rat was injected with saline or nicotine (0.4 mg kg^{-1}) and testing was immediately begun.

(4) *Pre treatment with intraventricular chlorisondamine in nicotine-tolerant rats* Twenty-four nicotine-tolerant rats (406–551 g) were randomly allocated to two equal groups and each subject was tested once with nicotine (0.4 mg kg^{-1}) and once with saline. The order of drug treatment was counterbalanced within each group. On the next day the rats in one group were injected intraventricularly with chlorisondamine (2 μg) and the control rats were injected with saline. The dose of chlorisondamine was suggested by pilot data from rats with chronic indwelling intraventricular cannulae. After two days of recovery (days 5 and 6) the rats were tested as before with nicotine and with saline. Pairs of such tests were repeated on days 9 and 10, 15 and 16 and finally on days 26 and 27 from the start of the experiment.

(5) *Antagonism of nicotine-induced locomotor stimulation by a post-treatment dose of mecamylamine* Eight nicotine-tolerant rats (348–472 g) were tested four times at intervals of 48 h. The two treatment conditions were nicotine (0.4 mg kg^{-1}) and saline, and after these injections the rats returned to their home cages. Twenty minutes later the post-treatment injection was given, either mecamylamine 1.0 mg kg^{-1} or saline, and the 80 min test session was begun immediately. Each rat was tested once with each combination of treatment and post-treatment medication, in a Williams Square design.

Results

(1) *Development of tolerance to nicotine and influence of environmental factors (exposure to apparatus)*

Activity was initially depressed after the first injection of nicotine (Figure 1, day 1) ($t = 4.70$, d.f. 14, $P < 0.0005$) and during the first few minutes the rats were ataxic and moved their hind legs with difficulty. Figure 1 also shows that, relative to the control

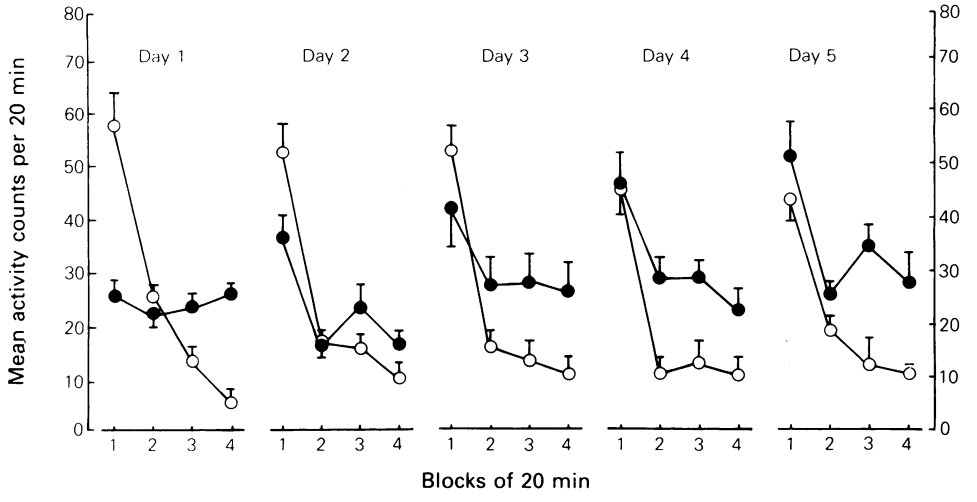

Figure 1 Locomotor activity of rats tested daily with nicotine or saline. Two groups of rats ($n = 8$/group), which were initially drug- and apparatus-naive, were tested daily, immediately after a subcutaneous injection of nicotine, 0.4 mg kg^{-1} (●), or of saline (○). The tests lasted for 80 min and on the first day nicotine initially depressed activity. By the fifth day the initial depressant effect had disappeared and nicotine increased activity throughout the test session. Bars represent 1 s.e. about the mean.

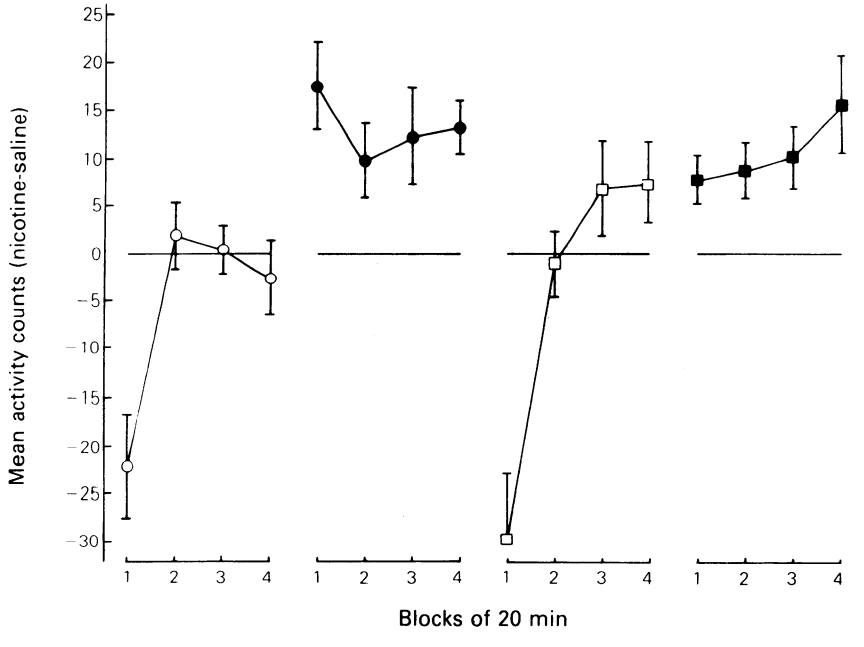

Figure 2 Effects of repeated exposure to apparatus and/or to nicotine upon the locomotor response to nicotine. Two groups of rats ($n = 8$/group) had already had 5 tests in the photocell cages either under the influence of nicotine, 0.4 mg kg^{-1} (●), or saline (○) (see Figure 1). Two other groups of rats ($n = 8$) were given comparable injections (■, nicotine; □, saline) but were replaced in their home cages each time. All the rats were then tested on the 6th and 7th days, once with nicotine 0.4 mg kg^{-1} and once with saline. The effect of nicotine is shown as the mean of the difference scores between the nicotine and the saline tests for each of the 4 groups. Bars represent 1 s.e. about the mean. The development of tolerance to nicotine was not influenced by exposure to the test apparatus.

group, the nicotine-treated rats were more active 60–80 min after the first injection ($t = 5.43$, d.f. 14, $P < 0.0001$). The ataxia and motor depression waned rapidly over successive days and the stimulant effect became increasingly pronounced, occurring sooner after injection (Figure 1, days 2–5).

All the animals were tested on days 6 and 7, and their activity scores were examined by a 2 × 2 analysis of variance; the two factors being previous experience of nicotine up to day 5 and previous experience of the apparatus up to day 5. Saline activity scores (0–80 min) were unaffected by either factor and no interaction was found. The drug effect was defined as the difference between the scores on the nicotine and the saline tests for each subject (Figure 2). Over the whole 80 min session, the drug effect was dependent on previous treatment with nicotine ($F = 32.2$, d.f. 1, 28, $P < 0.0001$), but it made no difference whether the rats had been tested after the injection each day or whether they had remained in their home cages ($F < 1$, see Figure 2). Previous injections of the drug on days 1 to 5 not only resulted in tolerance to the locomotor depressant action occurring in the first 20 minutes, but also increased the locomotor stimulant action of nicotine seen later in the test session (e.g. 60–80 min., main effect of drug pretreatment on drug effect $F = 8.75$, d.f. 1, 28, $P < 0.01$).

(2) *Relative potencies of (+) and (−)-nicotine in tolerant rats*

Dose-related stimulation of locomotor activity was seen with both isomers and the natural form (−)-nicotine was over 10 times more potent (Figure 3).

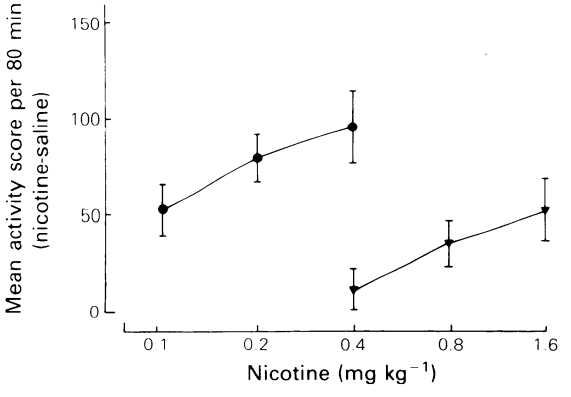

Figure 3 Stimulation of locomotor activity by (+)- and (−)- isomers of nicotine in rats already tolerant to (−)-nicotine: three doses each of the (+)-isomer (▼) and (−)-nicotine (●) were tested in 10 rats and the scores are expressed as mean differences of activity counts from saline-control levels (absolute counts: mean ± s.e. mean = 120.2 ± 31.2). The vertical bars show the s.e. about the mean.

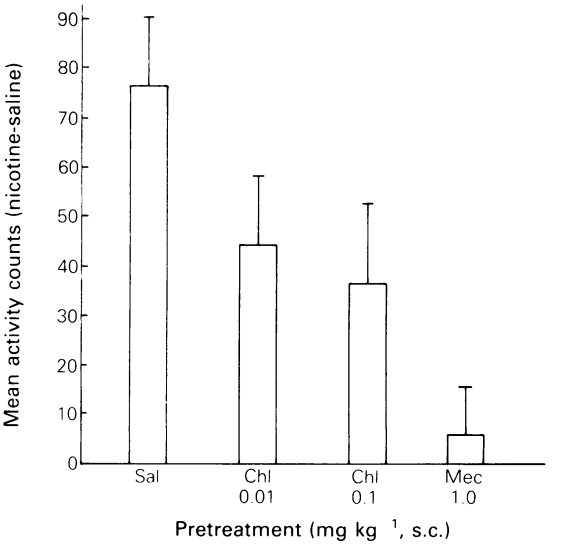

Figure 4 Comparisons of pretreatments with subcutaneous mecamylamine (Mec) or chlorisondamine (Chl) in tolerant rats tested with nicotine 0.4 mg kg^{-1}, s.c. The histograms represent the mean differences (± s.e. mean) between the nicotine and saline tests in the presence of each pretreatment condition; $n = 8$. Sal = control.

(3) *Pretreatment with subcutaneous mecamylamine or chlorisondamine in nicotine-tolerant rats*

Compared to saline pretreatment, neither chlorisondamine (0.01, 0.1 mg kg^{-1}) nor mecamylamine pretreatment altered activity during saline test sessions ($t < 1.89$, d.f. 7 for each paired comparison with saline: mean scores ± s.e. mean were respectively 97.2 ± 14.3, 102.2 ± 15.6, 84.3 ± 9.75, 108.7 ± 15.8). As expected, nicotine increased activity when it was preceded by saline pretreatment ($t = 5.15$, d.f. 7, $P < 0.001$) and also after either dose of chlorisondamine ($P < 0.05$). Mecamylamine completely blocked the stimulant action of nicotine (see Figure 4). There was nevertheless some attenuation of locomotor stimulation after chlorisondamine as well ($t = 2.54$, d.f. 7, $P < 0.05$ and $t = 2.30$, d.f. 7, $P < 0.06$ for the 0.01 and 0.1 mg kg^{-1} doses respectively, see Figure 4).

(4) *Pretreatment with intraventricular chlorisondamine in nicotine-tolerant rats*

Figure 5 shows that on the first pair of test days, nicotine produced a clear rise in activity scores in comparison with the saline tests in the same rats ($t = 6.77$, d.f. 23, $P < 0.0001$). In the group of rats that received intraventricular injections of chlorison-

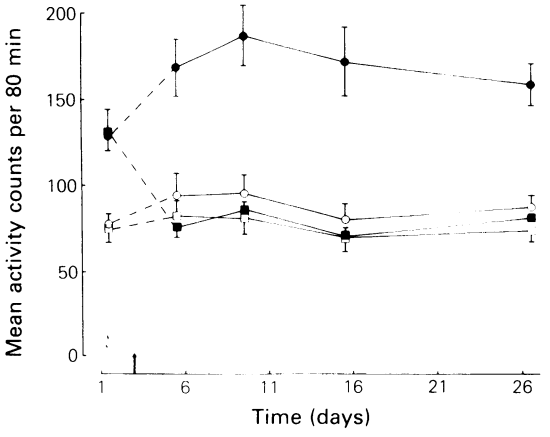

Figure 5 Effect of intraventricular chlorisondamine on nicotine-induced locomotor activity in tolerant rats. Chlorisondamine (2 μg) (■□) or saline (●○) was injected into the ventricles of 2 groups of rats (n = 12/group) indicated by arrow on abscissa scale. The rats were tested on pairs of days, either with nicotine (■●) or with saline (□○). Stimulation of activity by nicotine was completely blocked by the single treatment with chlorisondamine, but the same rats' activity scores under the saline condition were unaffected. The block by chlorisondamine persisted for the duration of the experiment. Bars show s.e. mean.

damine (2 μg) on day 3, the stimulant effect of nicotine was blocked for the rest of the experiment (24 days). The scores of these rats during tests with saline did not differ from the saline scores of the other group which had had the control intraventricular injections of saline. Chlorisondamine also blocked the residual signs of ataxia which could still be seen in the tolerant rats in the first 5 min after injection of nicotine 0.4 mg kg^{-1} s.c. Some recovery was, however, seen by the end of the experiment; in the four successive tests after the intraventricular injection, the numbers of rats found to show some signs of ataxia in the chlorisondamine group were 1, 2, 2 and 6 out of 12 and the corresponding numbers for the 12 controls were 11, 11, 12 and 12.

(5) *Antagonism of nicotine-induced locomotor stimulation by a post-treatment dose of mecamylamine*

In saline pretreated rats, mecamylamine did not significantly alter locomotor activity and, as Figure 6 shows, in the absence of mecamylamine, nicotine stimulated motor activity during the whole session. However, in the presence of mecamylamine, nicotine significantly depressed motor activity in the 80 min session as a whole ($t = 2.50$, d.f. 7, $P < 0.05$), and as Figure 6 shows, this depressant action was detected even in the first 20 min after administration of mecamylamine.

Discussion

Tolerance develops quickly to the depressant action of nicotine which is progressively replaced by stimulation and, as Figure 2 shows, this change is most evident in the first 20 min after injection. How is the altered response to nicotine mediated? The weight of evidence is against explanation in terms of changes in the metabolism of the alkaloid (see Clarke & Kumar, 1983a). Possibly some form of behavioural adaptation occurs to the depressant effects of the drug, but if so, our findings suggest that this is not specific to the particular environmental cues present in the testing situation. Such a conclusion is consistent with the results of the parametric study of Hendry & Rosecrans (1982), but is at variance with the data of Schlatter & Bättig (1978). The stimulant effect of nicotine in tolerant rats is stereospecific (Experiment 2), and the comparisons of mecamylamine and

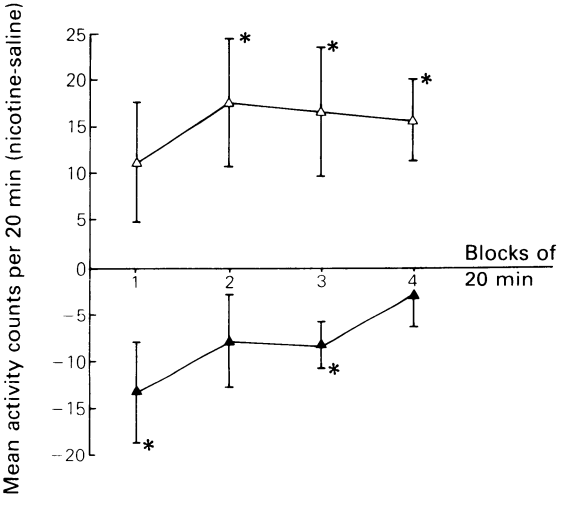

Figure 6 Effects of post-treatment with mecamylamine on locomotor stimulation induced by nicotine in tolerant rats. Animals were injected with saline or nicotine (0.4 mg kg^{-1} s.c.) in their home cages, and 20 min later were tested immediately after administration of saline or mecamylamine (1.0 mg kg^{-1} s.c.). Each subject (n = 8) received each of the four drug combinations. Mean differences (± s.e. mean) between the nicotine and saline treatment are shown over successive 20 min periods. In the absence of mecamylamine (△), nicotine increased locomotor activity as expected. After post-treatment with mecamylamine (▲), nicotine reduced activity instead. Significant deviations ($P < 0.05$) from baseline are indicated by*.

chlorisondamine point strongly to a central site of action. Pretreatment with subcutaneous mecamylamine, which is thought to enter the brain readily, completely prevented the increased activity. Chlorisondamine is about 100 times more potent than mecamylamine in peripheral tissues (Morrison *et al.*, 1969) and in the third experiment this drug only marginally counteracted motor stimulation by nicotine. There is probably little passage of chlorisondamine across the blood brain barrier by virtue of its bisquaternary structure and systemic doses that produce ganglion blockade fail to modify the central discriminative properties of nicotine (Morrison & Stephenson, 1969). In mice, nicotine acts centrally to cause convulsions and death, actions that are prevented by extremely large systemic doses of chlorisondamine or by much smaller amounts injected directly into the cerebral ventricles (Aceto, Bentley & Dembinski, 1969). We are therefore consistent in finding that intraventricular chlorisondamine (2 μg) completely blocked the stimulant actions of nicotine in tolerant rats.

The prolonged blockade of nicotine by a single dose of chlorisondamine seems to be a unique effect; it persisted for over three weeks and at the same time there was no discernible change in the rats' baseline (saline) activity levels nor in gross observations of behaviour. The central injection of chlorisondamine did not lead to weight loss; intraventricular administration of anti-acetylcholine receptor antibodies has been reported to produce aphagia, adipsia and eventually spastic paralysis in rabbits (Tarrab-Hazdai & Edery, 1980). Chlorisondamine may be of value in elucidating the central actions of nicotine, since it appears to have certain advantages over the more commonly used nicotinic antagonists, mecamylamine and hexamethonium. Mecamylamine is surprisingly lacking in potency when injected into the ventricles (Aceto *et al.*, 1969; Stolerman, Pratt, Garcha, Giardini, & Kumar, 1983), and it may be that this secondary amine passes rapidly out of the brain. Quaternary compounds may therefore provide a better means of localising nicotine's sites of action in the brain. Recent evidence (Romano, 1981) suggests that hexamethonium is a relatively ineffective peripheral antagonist in the rat; its status as a central antagonist e.g. of the nicotine, cue is also uncertain (Hazell, Peterson, and Laverty, 1978; Stolerman *et al.*, 1983). Whilst chlorisondamine, hexamethonium, and mecamylamine all have low potency in displacing nicotine or acetycholine from receptors in brain (Romano and Goldstein, 1980; Schwartz *et al.*, 1982; Marks & Collins, 1982), this may be a result of *in vitro* incubation (see Schwartz *et al.*, 1982).

Mecamylamine by itself does not affect locomotor activity in nicotine-tolerant rats, but pretreatment with this drug reduces motor stimulation by nicotine in a dose-related way (Clarke & Kumar, 1983a and see also Experiment 3). In the last experiment, giving the same dose of mecamylamine (1.0 mg kg^{-1}) 20 min *after* the nicotine injection produced an unexpected fall in motor activity. Thus, in the presence of mecamylamine, nicotine reduced motor activity in tolerant rats and such a depressant action, unlike that previously identified (Clarke & Kumar, 1983a b; Stitzer, Morrison & Domino, 1970; Barthelemey, Tremblay & Jacob, 1970), may not be mecamylamine-sensitive.

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