Br. J. Pharmac. (1984), **83**, 527–535

Chronic central nicotinic blockade after a single administration of the bisquaternary ganglion-blocking drug chlorisondamine

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1 Drug-naive rats were tested for horizontal and vertical activity in photocell cages, for up to 80 min starting immediately after a subcutaneous injection of (−)-nicotine bitartrate or 0.9% w/v NaCl solution (saline). Nicotine (0.1 to 0.4 mg kg^{-1} base) depressed vertical activity and induced ataxia in the first 20 min, but increased both horizontal and vertical activity later in the session; these actions were dose-dependent. A single intraventricular (i.v.t.) injection of chlorisondamine Cl (2 μg base), a quaternary ganglion-blocking drug, given one to two weeks before testing, blocked the ataxic and stimulant actions of nicotine.

2 The antagonistic actions of chlorisondamine (0.2, 1.0, 5.0 μg i.v.t., single administration) were shown to be dose-dependent. The stimulant actions of nicotine were blocked in a dose-dependent way for the duration of the experiment (5 weeks); nicotine's depressant actions were completely blocked at two weeks but not at five weeks.

3 A ganglion-blocking dose of chlorisondamine (0.1 mg kg^{-1}), given subcutaneously (s.c.), failed to reduce the behavioural actions of nicotine, whereas a much higher systemic dose (10 mg kg^{-1} s.c.) was effective for at least five weeks.

4 Chlorisondamine failed to alter the behavioural effects of (+)-amphetamine or apomorphine, while blocking those of nicotine.

5 It is concluded that chlorisondamine antagonizes some of nicotine's central actions in a potent, long-lasting and pharmacologically selective way.

Introduction

Acute studies with nicotinic antagonists indicate that it is nicotine's central actions which may be important in tobacco smoking (Stolerman *et al.*, 1973b). In rats chronically treated with nicotine, locomotor activity is stimulated (Morrison & Stephenson, 1969) through a direct central action (Clarke & Kumar, 1983a,b); in non-tolerant rats, a transient depressant phase may also occur (Stolerman *et al.*, 1973a; Clarke & Kumar 1983a,b). We recently described an experiment in rats, in which a single intraventricular injection of chlorisondamine ('Ecolid'), a bisquaternary ganglion-blocking drug (Plummer *et al.*, 1955; Stone *et al.*, 1958), blocked the central locomotor stimulant action of nicotine for several weeks (Clarke & Kumar, 1983b). Data are presented here which confirm and extend this unexpected finding.

The effects of nicotine on locomotor activity depend critically on the behavioural measure used (Bryson *et al.*, 1981). In our previous studies (Clarke & Kumar, 1983a,b), we investigated only gross horizontal locomotion. In the present series of experiments, both horizontal and vertical activity were measured. Drug-naive rats were used, since in non-tolerant rats, depressant as well as stimulant effects can be studied. The first experiment described here measured the effects of nicotine on both types of activity, over time and at different doses, and confirmed the existence of chronic blockade using the same dose of chlorisondamine (2 μg) as used previously. For subsequent experiments, a session duration of 60 min and a single challenge dose of nicotine (0.4 mg kg^{-1}) were selected; in diverse behavioural studies, this dose has been found to produce a near-maximal response (Clarke & Kumar, 1983a; 1984; Stolerman *et al.*, 1983). The second experiment investigated the ability of a range of doses of chlorisondamine, given in a single intraventricular injection, to counter nicotine's behavioural effects for up to five

weeks. In the third experiment, chlorisondamine was given systemically in a dose calculated to produce ganglionic blockade (Stone *et al.*, 1958; Morrison *et al.*, 1969), in order to check that nicotine's depressant and stimulant effects were not mediated via the autonomic nervous system. The fourth experiment addressed pharmacological specificity: would intraventricular chlorisondamine block locomotor stimulation produced by (+)-amphetamine as well as by nicotine? This question was also investigated in the final experiment, in which a high systemic dose of chlorisondamine was found to block nicotine's central actions for up to five weeks.

Methods

Male Sprague-Dawley rats (Taconic Farms, New York) were maintained on food and water *ad libitum*. They were housed in groups of four to eight subjects per cage on a random basis with respect to drug treatment, in a room illuminated from 07 h 00 min to 19 h 00 min. All rats were drug- and apparatus-naive at the start of testing.

Apparatus

Locomotor activity was tested in four Opto-Varimex-Minor activity meters (Columbus Instruments, OH, U.S.A.) interfaced with an ADL-80 microprocessor (Columbus Instruments). Each activity meter was housed in a ventilated sound-attenuating enclosure, illuminated overhead by fluorescent lighting, and masking noise was provided (BRS/LVE). The testing cage (46 by 46 by 20 cm) was made of clear perspex with a perforated lid to permit ventilation. The floor was coated with a thin layer of sawdust. Horizontal activity was measured by beam breaks among two orthogonal arrays each consisting of 12 parallel infra-red photobeams spaced 3.2 cm apart, running 3.8 cm above the cage floor. Only movements from one beam to an adjacent one were counted (the 'ambulatory movements only' output). Vertical activity was counted by a single horizontal array of 12 parallel infra-red photobeams running 14.5 cm above the floor designed to register rearing behaviour.

Data were printed every 20 min, unless otherwise indicated. The order of drug testing was counterbalanced as far as possible across test cages. Rats were placed in a holding cage in the testing room for 50 min before injection, and were tested immediately after injection, between 09 h 00 min and 17 h 00 min. Between one and two minutes after injection, each nicotine-injected rat was observed for 30 s, in order to rate the presence or absence of ataxia and prostration, transient effects which are particularly marked in drug-naive subjects (Clarke & Kumar 1983a,b). Prostration was scored if the rat lay with its abdomen on the floor without moving its hindlimbs for 10 s or more.

Drugs

(−)-Nicotine (+)-tartrate (BDH, Poole) was dissolved in 0.9% w/v NaCl solution (saline) and neutralized to pH 7.2 ± 0.2 with NaOH. Chlorisondamine Cl (CIBA-Geigy), (+)-amphetamine sulphate (Sigma) and apormorphine HCl (Merck) were dissolved in saline. All systemic injections were subcutaneous (s.c.), given in the flank in a volume of 1 ml kg^{-1}. Doses of nicotine and chlorisondamine refer to the base, those of (+)-amphetamine and apomorphine to the salt.

Analysis of data

The rat's activity scores were assessed by univariate and multivariate analysis of variance, each rat serving as its own control, where appropriate. A dose-dependent effect refers to a significant linear trend across absolute values of dose. Where a single dose of drug is used, the drug effect refers to the absolute difference in activity scores between the drug test and the saline test. Specific comparisons were made by paired and unpaired *t* tests, and probability values are two-tailed.

Intraventricular injections

Rats were anaesthetized with chloral hydrate 400 mg kg^{-1} intraperitoneally (Fisher Scientific Co.) and were placed in a stereotaxic frame (David Kopf Co., CA, U.S.A.) with the tooth bar positioned 3.5 mm below the interaural line. A 10 μl Hamilton syringe mounted on the frame was lowered vertically through a hole drilled in the skull, with the middle of the bevelled tip aimed at the right lateral ventricle (A 8.1, L 1.3, 4.2 mm below the dura). Suitable coordinates were determined by injecting six weight-matched rats with cresyl violet dye, followed by visual inspection of brain sections on a freezing microtome. Five microlitres of solution were injected over 30 s and the cannula was slowly withdrawn after a further minute. Chlorisondamine was dissolved in saline to a concentration of 0.04 to 1.0 μg base μl^{-1}, depending on the dose required. Control injections were of saline. The wound was dressed with Bacitracin ointment and was closed with stainless steel clips (Autoclips: Becton, Dickinson and Co., U.S.A.). Rats given intraventricular chlorisondamine did not differ from saline-injected controls either in terms of the amount of weight lost post-operatively, or in their subsequent rates of recovery.

Procedures

(1) *Dose- and time-related effects of nicotine and the effects of a single intraventricular (i.v.t.) dose of chlorisondamine* Sixteen rats were used, weighing 215–400 g at surgery. Eight randomly-selected subjects received 2 μg chlorisondamine i.v.t. (for details see above), and the remaining subjects received saline i.v.t. Starting 7 days after surgery, each rat was tested once with each dose of nicotine (0, 0.1, 0.2, 0.4 mg kg^{-1}) in a Williams square design (Cox, 1958), counterbalanced with respect to intraventricular pretreatment. Tests were spaced two days apart. Test sessions lasted 80 min.

(2) *Dose-related antagonism by intraventricular chlorisondamine of nicotine across five weeks* Thirty-two rats were used, weighing 260–370 g at surgery. Subjects were randomly allocated to four pretreatment groups ($n=8$), receiving i.v.t. saline or chlorisondamine 0.2, 1.0, and 5.0 μg, respectively. Following surgery, each rat was given six tests, grouped as three pairs. Each pair comprised one test with saline and one test with nicotine (0.4 mg kg^{-1}), given on successive days, and the order of drug testing was counterbalanced within each pretreatment group. A pair of tests occurred at one week, two weeks and at five weeks after surgery, i.e., 7 and 8, 14 and 15, and 35 and 36 days from surgery. Test sessions lasted 60 min.

(3) *Systemic pretreatment with a ganglion-blocking dose of chlorisondamine* Twelve rats (340–370 g) were each tested four times at intervals of two days. Each rat was tested with each combination of pretreatment (saline or chlorisondamine 0.1 mg kg^{-1} s.c.) and treatment (saline or nicotine 0.4 mg kg^{-1}), in a Williams square design. Pretreatment preceded the treatment injection by 20 min, and rats were returned to the holding cage for the interim. Test sessions of 60 min duration were begun immediately after the treatment injection.

(4) *Intraventricular pretreatment with chlorisondamine: effects on nicotine- and (+)-amphetamine-induced activity changes* Twenty rats (260–310 g at surgery) were randomly allocated to two pretreatment groups ($n=10$), receiving intraventricular saline or chlorisondamine 5.0 μg. Starting 6 days after surgery, each rat was tested on four occasions spaced two or three days apart, once with saline and once with each dose of amphetamine (0.375, 0.75 and 1.5 mg kg^{-1}). Drug testing followed a Williams square design, counterbalanced across pretreatment groups as far as possible. Test sessions lasted 30 min, beginning immediately after injection, and data were printed out at 15 and 30 min. The length of the session and doses were selected on the basis of pilot data. Subsequently (16 and 17 days from surgery), each rat received 60 min tests with saline and with nicotine 0.4 mg kg^{-1} (counterbalanced within the group), in order to check for nicotinic blockade.

(5) *Systemic pretreatment with a high dose of chlorisondamine and effects on nicotine-, (+)-amphetamine- and apomorphine-induced activity changes* Twenty-six rats (265–320 g) were randomly allocated to two pretreatment groups ($n=13$), each receiving a subcutaneous injection of saline or of chlorisondamine 10 mg kg^{-1}. This dose was selected on the basis of pilot data. Within 24 h, rats injected with chlorisondamine lost approximately 15 g of body weight compared to the controls. Seven days after pretreatment, body weights had recovered, and four tests were given, spaced two days apart. Each rat was tested once with saline, apomorphine 0.25 mg kg^{-1} (dose based on pilot data), (+)-amphetamine 0.375 mg kg^{-1} and nicotine 0.4 mg kg^{-1}, in a Williams square, counterbalanced as far as possible across pretreatment groups. Five weeks after the pretreatment injection, each rat was tested once with saline and once with nicotine 0.4 mg kg^{-1} (counterbalanced within group).

Results

(1) *Dose- and time-related effects of nicotine: intraventricular chlorisondamine pretreatment*

Saline activity scores in controls decreased markedly across successive 20 min periods, and by the end of the session, the rats were largely inactive; group means ± s.e.mean were, respectively: 336.2 ± 68.0, 32.5 ± 17.8, 11.9 ± 9.4 and 14.6 ± 5.6 for horizontal activity, and 136.1 ± 24.2, 6.2 ± 2.4, 0.6 ± 0.4 and 1.25 ± 0.9 for vertical activity. The effects of nicotine in control animals were largely as previously described (Clarke & Kumar, 1983a,b). In the first 20 min (Figure 1), nicotine produced a non-significant increase in horizontal activity (linear trend on dose $F=5.47$, d.f. 1, 7), and depressed vertical activity (rears) in a dose-dependent way $F=18.95$, d.f. 1, 7, $P<0.005$). Within a minute or so of injection, ataxia was commonly observed, especially at the highest dose, where all eight subjects became prostrated; these effects wore off within a few minutes. Ataxia was mainly associated with hindlimb immobility, and when not prostrated, an animal would typically circle its hindlimbs. In the three subsequent periods of 20 min (Figure 1), nicotine stimulated horizontal activity (respectively: $F=10.34$, 26.12, 7.78, d.f. 1, 7, $P<0.02$, 0.001, 0.05) and increased

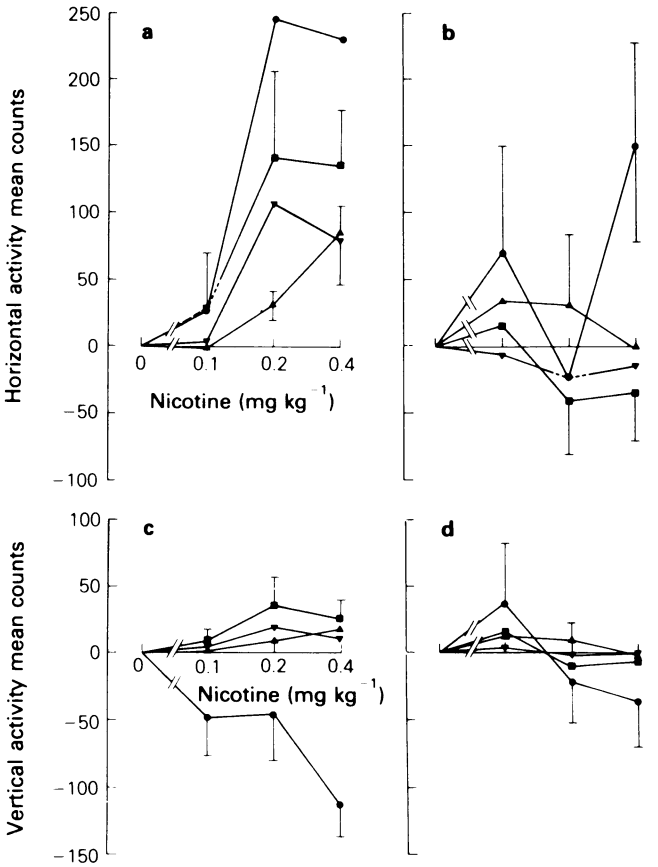

Figure 1 The effects of nicotine on horizontal (a and b) and vertical activity (c and d) in rats pretreated with saline (a and c) ($n = 8$), or with chlorisondamine 2 μg i.v.t. (b and d) ($n = 8$). Chlorisondamine did not alter saline test scores (see text). The vertical axes represent the mean differences of activity counts per 20 min (●, 0–20 min; ■, 20–40 min; ▲, 40–60 min; ▼, 60–80 min) between nicotine tests and the saline test and vertical lines show s.e.mean. In controls (a and c), nicotine reduced vertical activity (●, 0–20 min) (c) and then increased horizontal (a) and vertical (c) activity in a dose-dependent way. These latter stimulant effects were countered by chlorisondamine (b and d).

vertical activity weakly (respectively: $F = 4.77$, 10.96, 5.49, d.f. 1, 7, $P < 0.07$, 0.02, 0.06).

Chlorisondamine (2 μg, i.v.t.) affected neither horizontal nor vertical activity in saline tests (group main effect $F < 0.22$, d.f. 1, 14; group × time $F < 0.34$, d.f. 3, 12). The drug failed to counteract significantly the initial nicotine-induced depression of vertical activity (0–20 min), but countered the stimulant effects of nicotine in all three subsequent time periods (horizontal: $F > 7.90$, d.f. 1, 14, $P < 0.02$; vertical: $F > 4.62$, d.f. 1, 14, $P < 0.05$).

In subsequent experiments, nicotine either failed to alter or marginally increased horizontal activity early in the session (0–20 min). Since any increase probably reflected ataxic circling movements as well as actual place to place movement, the data are not presented here.

(2) *Dose-related antagonism by intraventricular chlorisondamine*

Saline scores differed across the three tests ($F > 7.44$, d.f. 2, 27, $P < 0.005$ for either variable). In control animals, nicotine appeared to exert a greater depressant action and smaller stimulant action on the first test than on subsequent weeks (Figure 2). Consequently, the three pairs of tests were analysed separately. Chlorisondamine appeared to reduce vertical activity in saline test sessions (see Table 1). However, there were no significant differences between groups for any variable (vertical 0–20, 20–60 min; horizontal 20–60 min) on any of the three occasions of testing (nine separate ANOVAs: group main effect $F < 2.26$, d.f. 3, 28).

Nicotine reduced vertical activity (0–20 min) on

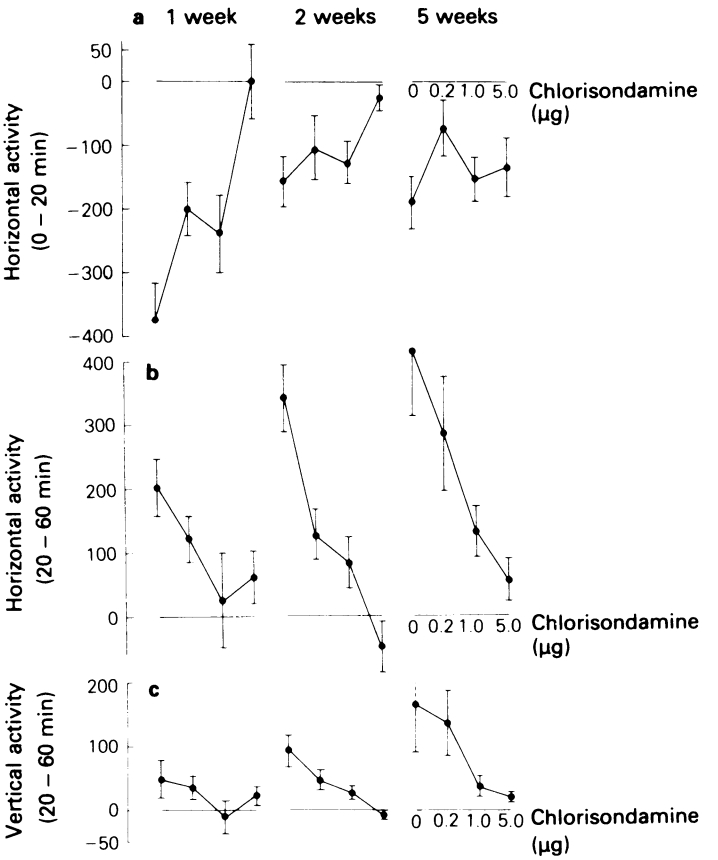

Figure 2 Dose-dependent effects of chlorisondamine, after a single administration, on the subsequent actions of nicotine. Rats were given an intraventricular (i.v.t.) injection of saline or chlorisondamine (0.2, 1.0, 5.0 μg) depending on group ($n = 8$ per group). At 1, 2 and 5 weeks after surgery, each subject was tested once with saline and once with nicotine (0.4 mg kg^{-1} s.c.). Test sessions lasted 60 min. Chlorisondamine did not significantly alter scores in saline tests (see Table 1). The vertical axes show the mean difference of activity scores (counts per 20 min) between each nicotine and saline test; s.e.mean represented by vertical lines. The depressant action of nicotine on vertical activity (0–20 min) was antagonized by chlorisondamine in a dose-dependent way at 1 and 2 weeks. Chlorisondamine blocked the stimulant actions of nicotine (20–60 min) for the duration of the experiment in a graded manner.

Table 1 Saline activity scores in rats pretreated with intraventricular saline or chlorisondamine

Week	*Pretreatment dose (μg)*	*Vertical 0–20 min*	*Horizontal 20–60 min*	*Vertical 20–60 min*
1	Saline	443.1 ± 61.2	172.4 ± 36.5	72.0 ± 19.2
	0.2	233.0 ± 49.1	82.6 ± 32.0	31.2 ± 15.3
	1.0	306.1 ± 61.7	189.4 ± 38.0	62.6 ± 21.4
	5.0	309.1 ± 60.4	105.9 ± 31.5	35.9 ± 11.2
2	Saline	238.5 ± 38.2	78.7 ± 22.5	21.2 ± 8.8
	0.2	185.4 ± 38.5	60.7 ± 12.8	11.6 ± 2.1
	1.0	188.0 ± 40.3	100.4 ± 22.4	25.3 ± 8.0
	5.0	165.1 ± 30.1	111.9 ± 28.8	19.0 ± 9.3
5	Saline	277.1 ± 45.9	91.7 ± 38.1	20.3 ± 8.8
	0.2	248.4 ± 62.6	67.4 ± 15.1	20.9 ± 14.1
	1.0	211.6 ± 48.8	66.4 ± 21.7	15.0 ± 6.5
	5.0	208.1 ± 37.8	47.9 ± 16.5	11.4 ± 6.2

Values shown are mean (± s.e.mean) activity counts per 20 min ($n = 8$).

Table 2 Ganglionic blockade and nicotine-induced activity changes

Pretreatment (s.c.)	*Treatment (s.c.)*	*Vertical 0–20 min*	*Horizontal 20–60 min*	*Vertical 20–60 min*
Saline	Saline	170.1 ± 24.9	91.1 ± 50.3	17.4 ± 10.5
Saline	Nicotine (0.4 mg kg^{-1})	52.1 ± 13.4	247.3 ± 30.8	43.2 ± 7.3
Chlorisondamine (0.1 mg kg^{-1})	Saline	159.2 ± 30.4	42.7 ± 16.0	8.9 ± 4.0
Chlorisondamine (0.1 mg kg^{-1})	Nicotine (0.4 mg kg^{-1})	32.8 ± 9.9	178.4 ± 26.3	24.6 ± 4.7

Values shown are mean (± s.e.mean) activity counts per 20 min ($n = 12$).

all three occasions in a highly significant manner (Figure 2). This depressant effect was reduced by chlorisondamine in a dose-related way at one week ($F = 19.54$, d.f. 1, 28, $P < 0.0001$) and at two weeks ($F = 5.77$, d.f. 1, 28, $P < 0.05$); complete blockade occurred at the highest dose. At five weeks, chlorisondamine did not significantly alter nicotine-induced depression.

Only the highest dose of the antagonist seemed to affect the incidence of prostration and ataxia following injection of nicotine. In all groups of subjects, except that receiving the high dose, seven or eight out of eight rats showed signs of ataxia or prostration at each nicotine test. In the high dose group, the corresponding incidence at 1, 2 and 5 weeks was 1/8, 1/8 and 6/8. Thus the protection afforded by the high dose was not permanent.

From 20 to 60 min after injection, nicotine increased horizontal activity in the control group. As Figure 2 shows, chlorisondamine countered this stimulant effect, and at 2 and 5 weeks, this antagonism was dose-related ($F = 26.74$, d.f. 1, 28, $P < 0.0001$; $F = 9.45$, d.f. 1, 28, $P < 0.005$, respectively). At 5 weeks, the high dose still provided a complete blockade. In control animals, nicotine weakly stimulated vertical activity from 20 to 60 min. This effect was significant only at week 2 ($t = 3.50$, d.f. 7, $P < 0.01$), and was reduced or blocked by chlorisondamine in a graded manner (Figure 2).

(3) *Systemic ganglion-blocking dose of chlorisondamine*

Analysis of variance showed that chlorisondamine had no effect on vertical activity (0–20 min), but reduced both horizontal and vertical activity from 20 to 60 min (main effect, respectively: $F = 5.21$, d.f. 1, 11, $P < 0.05$; $F = 5.62$, d.f. 1, 11, $P < 0.05$; Table 2). Chlorisondamine-treated rats became ptotic within a few minutes of injection. Nicotine depressed vertical activity (0–20 min: $F = 22.06$, d.f. 1, 11, $P < 0.001$; Table 2) and later increased horizontal and vertical activity (20–60 min: $F = 33.96$, d.f. 1, 11, $P < 0.001$ and $F = 8.00$, d.f. 1, 11, $P < 0.05$, respectively).

Table 3 Effects of pretreatment with intraventricular chlorisondamine (5 μg) on (+)-amphetamine and nicotine-induced activity changes

a *Tests at 1–2 weeks*

	Horizontal 15–30 min		*Vertical 15–30 min*	
Treatment	*Saline pretreatment*	*Chlorisondamine pretreatment*	*Saline pretreatment*	*Chlorisondamine pretreatment*
Saline	103.3 ± 47.3	246.0 ± 56.9	34.4 ± 17.1	65.1 ± 17.8
Amphetamine (0.375 mg kg^{-1})	547.6 ± 109.7	916.5 ± 95.0	136.4 ± 34.9	183.0 ± 34.1
Amphetamine (0.75 mg kg^{-1})	878.2 ± 75.3	1359.4 ± 180.9	211.4 ± 72.6	289.0 ± 102.9
Amphetamine (1.5 mg kg^{-1})	1064.2 ± 182.4	1583.2 ± 225.5	140.8 ± 48.7	273.2 ± 113.7

b *Tests on days 16 & 17*

	Vertical 0–20 min		*Horizontal 20–60 min*		*Vertical 20–60 min*	
Treatment	*Saline pretreatment*	*Chlorisondamine pretreatment*	*Saline pretreatment*	*Chlorisondamine pretreatment*	*Saline pretreatment*	*Chlorisondamine pretreatment*
Saline	147.4 ± 38.5	99.2 ± 17.2	43.4 ± 11.9	63.3 ± 27.2	9.2 ± 3.7	13.5 ± 6.8
Nicotine (0.4 mg kg^{-1})	15.5 ± 6.9	126.4 ± 39.1	159.2 ± 33.7	76.7 ± 22.4	53.2 ± 19.4	18.7 ± 6.1

Values shown are mean (± s.e.mean) activity counts per 15 min (a) or 20 min (b).

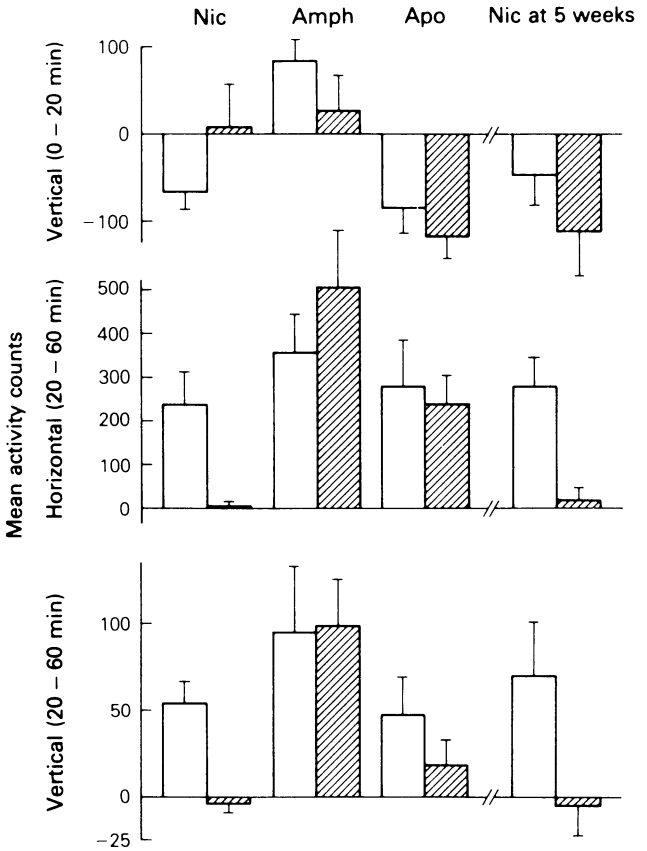

Figure 3 Selective effects of chlorisondamine on nicotine-induced changes in activity. Rats ($n = 13$ per group) were injected with a high systemic dose of chlorisondamine (10 mg kg^{-1} s.c.; hatched columns) or with saline (open columns). Between 1 and 2 weeks later, each rat was tested in four 60 min sessions, spaced two days apart: once with saline, nicotine (Nic; 0.4 mg kg^{-1}), (+)-amphetamine (Amph; 0.37 mg kg^{-1}) and apomorphine (Apo; 0.125 mg kg^{-1}). At 5 weeks, subjects were retested with saline and with nicotine (0.4 mg kg^{-1}). Activity scores in saline tests did not differ significantly between rats pretreated with chlorisondamine and controls (see text). The vertical axes show the mean difference of activity scores (counts per 20 min) between drug and saline tests; s.e.mean represented by vertical lines. Chlorisondamine blocked the depressant and stimulant effects of nicotine, but did not alter the actions of (+)-amphetamine and apomorphine.

None of these effects was altered by chlorisondamine (interaction terms NS). In addition, chlorisondamine did not alter the incidence of prostration or ataxia following nicotine injection (11/12 subjects in each condition).

(4) *Intraventricular chlorisondamine: nicotine- and (+)-amphetamine-induced changes*

The effects of (+)-amphetamine were examined from 15 to 30 min after injection, i.e., in the second half of the session. As shown in Table 3, (+)-amphetamine markedly increased horizontal activity in a dose-related way in rats pretreated with chlorisondamine as well as in controls. The chlorisondamine treated group tended to be more spontaneously active than controls, and so possible interactive effects between chlorisondamine and (+)-amphetamine were not analysed further. Clearly, however, chlorisondamine did not block the stimulant effects of (+)-amphetamine on horizontal or vertical activity (Table 3). As expected, nicotine initially depressed vertical activity (0–20 min: $t = 3.28$, d.f. 9, $P < 0.01$) and later enhanced activity (20–60 min: horizontal $t = 3.40$, d.f. 9, $P < 0.01$; vertical $t = 2.54$, d.f. 9, $P < 0.05$) in controls, but had no significant effects in rats which had received chlorisondamine more than two weeks previously (Table 3).

(5) *Systemic high dose of chlorisondamine: nicotine-, (+)-amphetamine- and apomorphine-induced activity changes*

Chlorisondamine pretreatment affected neither horizontal nor vertical activity within either saline test (group comparisons $P > 0.05$). Group means (±s.e.mean) were as follows (vertical 0–20 min, horizontal 20–60 min, vertical 20–60 min; first saline test followed by second): saline control group 127.0 ± 28.1, 41.0 ± 24.0, 10.5 ± 8.0, 140.0 ± 24.6, 84.2 ± 26.3, and 26.6 ± 14.6; chlorisondamine group 147.2 ± 27.2, 41.6 ± 19.1, 18.3 ± 10.0, 205.9 ± 57.1, 111.3 ± 37.4 and 47.5 ± 28.4. As Figure 3 shows, nicotine and apomorphine initially reduced vertical activity; and together with (+)-amphetamine, all three drugs increased activity later in the session. Group comparisons indicated that the effects of (+)-amphetamine and apomorphine were unaltered by chlorisondamine. In contrast, the actions of nicotine were blocked at 1–2 weeks. At 5 weeks, nicotine did not significantly depress vertical activity (0–20 min) in controls, but its stimulant actions were blocked by the antagonist.

Discussion

The behavioural experiments described in this paper clearly demonstrate that a single administration of chlorisondamine can result in an extremely long-lasting blockade of certain central actions of nicotine in rats. Blockade could be achieved by central administration of μg (i.e. nmol) quantities, and the degree of antagonism was related to the dose injected. When the drug was given systemically in a ganglion-blocking dose (some ten times higher than the centrally effective dose), it failed to alter the behavioural actions of nicotine. Chlorisondamine is a bisquaternary compound and hence does not pass freely through the blood-brain barrier (Mason, 1980). These observations, therefore, confirm that nicotine increases horizontal activity by acting centrally (Clarke & Kumar, 1983a,b), and show that the same is true for nicotine's effects on vertical activity, a new finding. Using a dose of chlorisondamine far in excess of that required for ganglionic blockade, it was possible to inject the drug systemically and arrive at a long-lasting central antagonism. Administered by either route, chlorisondamine reduced or blocked nicotine's actions for the duration of the experiment (five weeks), but failed to affect the depressant or stimulant actions of either (+)-amphetamine or apomorphine.

The actions of nicotine described here are largely in keeping with previous observations in non-tolerant rats using a different measure of horizontal activity (Clarke & Kumar, 1983a,b): prostration and motor disturbances were seen shortly after injection and a stimulant action emerged later in the session; with repeated administration of nicotine, its depressant actions appeared to wane, and locomotor stimulation became more pronounced. However, in contrast to our previous findings, horizontal activity was unaltered or actually increased in the first twenty minutes after injection. Because of the wide spacing of the photobeams employed in our previous work, the characteristic circling movements made by rats shortly after nicotine injection were not generally detected. For a few minutes after injection, muscle tone is reduced (Morrison & Stephenson, 1969; Clarke & Kumar, 1983a). As the animal circles its tail typically loops under its abdomen; this posture, which is not usually seen in undrugged rats, is conceivably relevant to reports of nicotinic 'antinociception' based on tests of tail-flick latency (Tripathi *et al.*, 1982). As noted by Stolerman *et al.* (1973a), vertical activity is a sensitive measure of the initial depressant actions of nicotine. The delayed stimulant effect of nicotine on rearing has not been described previously, but it is consistent with a rather general biphasic profile of action in non-tolerant rats (see Clarke & Kumar, 1983c).

How long does the antagonism last? The present experiments traced the actions of nicotine for up to five weeks after the administration of chlorisondamine. *Prima facie*, the results suggest that at this time, subjects were no longer protected from the depressant effects of nicotine occurring soon after injection. However, this interpretation overlooks the likelihood that the control (saline-pretreated) subjects altered their response to nicotine across successive tests. Specifically, the depressant action appeared to wane, and the stimulant action became more pronounced. Since tolerance of this sort depends on exposure to nicotine rather than on repeated testing (Clarke & Kumar, 1983b), chlorisondamine may have interfered with the development of tolerance, as well as blocking the acute effects of nicotine. Hence, at week 5, the chlorisondamine-pretreated animals were probably less tolerant than saline-pretreated control subjects. Although, at this time, chlorisondamine no longer offered complete protection from the depressant actions of nicotine, a residual antagonistic effect cannot be ruled out. In contrast, chlorisondamine clearly and completely blocked the stimulant effect of nicotine on horizontal activity until the end of the experiment. Among peripheral ganglion-blocking drugs, chlorisondamine is relatively long-acting (Schneider & Moore, 1955), but such an extended duration of action has not been described. Plummer *et al.* (1955) demonstrated a prolonged hypertension in dogs, with no recovery even eight hours after intravenous ad-

ministration, but in another study, chlorisondamine's actions were limited to a few hours' duration (Sethi & Gulati, 1973).

If chlorisondamine is similar to other ganglion blockers in the onium group, such as hexamethonium, it is excreted unmetabolized by the kidney (Mason, 1980). Onium compounds are completely ionized at physiological pH, and presumably only a small proportion of the high systemic dose required to produce central blockade actually penetrated the central nervous system. An attractive possibility is that chlorisondamine, having breached the blood-brain barrier, is trapped, unmetabolized, in pharmacological concentrations. However, quaternary nicotinic antagonists introduced into the cerebrospinal fluid appear to exit rapidly into the blood stream (Schanker *et al.*, 1962).

Iontophoresed onto single units in the brain, chlorisondamine antagonized the excitatory actions of acetylcholine (Bloom *et al.*, 1964). In common with ganglion-selective nicotinic antagonists, chlorisondamine fails to inhibit the high-affinity binding of nicotinic ligands to brain at pharmacological concentrations (Romano & Goldstein, 1980; Schwartz *et al.*, 1982; Clarke, Pert & Pert, unpublished). However, this lack of effect may merely reflect an artifact produced by *in vitro* incubation (see Romano & Goldstein, 1980). The turnover rate of central nicotinic receptors has not been described, and hence the possibility remains that chlorisondamine binds irreversibly to central nicotinic receptors. Whether or not this is the case, chlorisondamine may prove to be a useful pharmacological tool and may possibly be of clinical importance in combating the smoking habit.

I thank CIBA-Geigy for gifts of chlorisondamine. Drs A. Pert, I.P. Stolerman and R. Kumar made many helpful suggestions about the manuscript. P.B.S.C. is a Fogarty International Visiting Fellow.

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(*Received March 27, 1984. Revised May 1, 1984.*)