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Anti-nicotinic Properties of Anticholinergic Antiparkinson Drugs

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Abstract

The nature of the antagonism by anticholinergic compounds of nicotine-induced convulsion in mice has not been defined clearly. Although, because they do not compete effectively for agonist binding to brain tissue in-vitro, these compounds are thought to be non-competitive antagonists in the brain, pharmacological evidence is lacking. This study describes the anti-nicotinic properties of the clinically used anticholinergic antiparkinson drugs, benztropine, biperiden, caramiphen, ethopropazine, procyclidine and trihexyphenidyl.

Nicotine-induced convulsion and arecoline-induced tremor in mice were effectively prevented by these drugs. The concentrations of benztropine, biperiden, caramiphen, ethopropazine, procyclidine and trihexyphenidyl affording 50% prevention of nicotine-induced convulsion (ED50 values) were 7·4, 4·6, 7·8, 4·9, 3·1 and 3·3 mg kg^{-1}, respectively. The classical muscarinic receptor antagonist atropine had potent anti-muscarinic effects but very weak anti-nicotinic activity. The classical nicotinic receptor antagonist mecamylamine had potent anti-nicotinic activity but no anti-muscarinic effects. The pattern of shift of the dose–response curve for nicotine-induced convulsion in mice was determined in the presence of increasing concentrations of the anticholinergic antiparkinson drugs. These drugs were found to increase the ED50 (0·49 mg kg^{-1}) of nicotine-induced convulsion in a dose-related manner. The maximum effect of nicotine and the slope of nicotine dose–response curve were not significantly influenced by either low or high doses of benztropine, procyclidine or trihexylphenidyl, which suggests competitive action. Biperiden, caramiphen and ethopropazine, at low doses which significantly increased the ED50 of nicotine, did not affect the maximum effect of nicotine or the slope of the nicotine dose–response curve; at higher doses, however, they reduced the maximum effect and the slope, which suggests that these drugs have both competitive and non-competitive properties in antagonizing nicotine-induced convulsion in mice.

The experiments demonstrate that the anticholinergic antiparkinson drugs and mecamylamine effectively antagonize nicotine-induced convulsion, but atropine does not; some of these drugs have competitive properties whereas others seem to have both competitive and non-competitive properties in antagonizing nicotine-induced convulsion in mice.

Nicotine has been shown to induce convulsions in mice, an effect antagonized by the nicotinic receptor antagonist mecamylamine but not by decamethonium, physostigmine, atropine, or by reserpine pretreatment. It has been demonstrated that nicotine-induced convulsion is accompanied by seizure as recorded by electroencephalograph, suggesting a central site of action (Zhang & Liu 1996). In the course of investigating various cholinergic antagonists to muscarinic and nicotinic receptors in our laboratory it was found that some anticholinergic drugs are antagonistic to the central action of both arecoline and nicotine (Niu et al 1990; Gao & Liu 1995; Gao et al 1995a, b, 1996a, b, 1997). Although it was found that most anticholinergic drugs could not displace the specific binding of [^{3}H]nicotine or [^{3}H]acetylcholine to nicotinic receptors in brain, some could antagonize nicotine-induced convulsions in mice (Schwartz et al 1982; Wonnacott 1990; Gao et al 1995a, 1996a, 1997), implying that these compounds might interact with nicotinic receptors in the brain in a different manner than does nicotine.

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The ability of anticholinergic antiparkinson drugs to alter the dose–response relationship of nicotine-induced convulsion in mice has been found to determine the nature of the antagonism of nicotine by its antagonists. It has been suggested that because nicotinic receptor antagonists do not displace [^{3}H]nicotine binding to brain tissue in-vitro they antagonize nicotine centrally in a purely non-competitive manner (Martin et al 1989). However, the correlation between the in-vivo pharmacology of these compounds and their ability to displace radiolabelled ligands from tissue determines the relevance of binding data. It is therefore necessary to evaluate the competitive or non-competitive nature of the effects of these compounds in the intact animal. The purpose of the study was to clarify the pattern of interaction of these drugs with the nicotinic receptors of the brain. Because these compounds also have anti-muscarinic activity, their effects on arecoline-induced tremor in mice and [^{3}H]quinuclidinyl benzylate binding were also studied.

Materials and Methods

Drugs

Atropine, arecoline, hexamethonium, nicotine free base, ethopropazine, procyclidine and trihexyphenidyl were from Sigma (St Louis, MO). Benztropine, biperiden, quinuclidinyl benzylate (QNB), phencynonate, tricyclopinate, 2-*R*-tropanyl benzylate (T9206), 3-(2′-phenyl-2′-cyclopentyl-2′-hydroxyethoxy)quinuclidine (P8018) and caramiphen were synthesized at our institute (the purity of these drugs was >99%). Mecamylamine was from the Sixth Pharmaceutical Factory of Shanghai (China). [^{3}H]QNB (43·3 Ci mmol^{-1}) was purchased from Amersham (Bucks, UK).

Antagonism of nicotine-induced convulsion in mice

The procedure used was similar to that described elsewhere (Gao et al 1995a). Five doses of each of the drugs were injected intravenously 10 min before administration of nicotine (1·0 mg kg^{-1}, i.v.) to determine the ED50 values of the antagonizing effect of the drugs. Ten Shanghai mice, 18–22 g, were used in each dose group. To determine the pattern of shift of the nicotine dose–response curve, different doses of each drug were administered intravenously 10 min before different doses of nicotine. The data were expressed as the percentage antagonism where:

$$\text{Antagonism (\%)} = \left[\frac{1 - (\text{\% effects of antagonist pretreatment})}{(\text{\% effect with nicotine alone})}\right] \times 100 \quad (1)$$

Antagonism of arecoline-induced tremor in mice

The method used was similar to that of Niu et al (1990). Five doses of each of the drugs were injected intraperitoneally 15 min before arecoline (10 mg kg^{-1}, s.c.) to estimate the ED50 values of the antagonistic effect of these compounds. Ten Shanghai mice, 18–22 g, were used in each dose-group.

Muscarinic-receptor binding

Rat brain membrane for [^{3}H]QNB binding studies was prepared by a procedure similar to that described elsewhere (Gao et al 1995a). Male or female Wistar rats, 180–220 g, were decapitated and the cerebral cortex was rapidly removed and homogenized in 10 vols (w/v) ice-cold 0·32 M sucrose in a glass homogenizer. The whole homogenate was centrifuged at 1000 *g* for 10 min at 4°C. The pellet was discarded and the supernatant was centrifuged at 20 000 *g* for 30 min at 4°C. The supernatant was poured out and the pellet (P_2) was suspended in 4 vols Na^+–K^+ phosphate buffer (pH 7·4, 4°C). P_2 was then rehomogenized in a glass homogenizer and stored at −20°C. Proteins were determined by the method of Lowry et al (1951) with bovine serum albumin as standard. To assay the specific binding of [^{3}H]QNB, protein (0·1 mg) was incubated with 0·01–1·0 nM [^{3}H]QNB at 35°C for 30 min in Na^+–K^+ phosphate buffer solution (50 mM; 1 mL) in the absence or presence of 1 μM unlabelled atropine to determine the total and non-specific binding, respectively. The incubation was stopped by addition of ice-cold buffer solution (3 mL). Bound [^{3}H]QNB was separated from the unbound ligand by vacuum filtration through Hongguang 49 glass filters (Shanghai, China). The filters were washed three times with ice-cold buffer solution (9 mL) and placed in vials containing scintillation liquid (3 mL, containing 0·3% 2,5-diphenyloxazol and 0·03% 1,4-*bis*-5-phenyloxazoyl-2-benzene) and maintained for >10 h at room temperature. The radioactivity was assayed by means of an LKB (Sweden) liquid scintillation counter at a counting efficiency of 36%. The binding of drugs to muscarinic receptors was determined by their ability to displace [^{3}H]QNB. The final concentration of [^{3}H]QNB in competition studies was 0·5 nM.

Every determination of binding was performed in duplicate.

Data analysis

ED50 values of these compounds against arecoline-induced tremor and nicotine-induced convulsion were calculated by use of the Quantal program (iteration-weighted probit analysis). Parallel tests

between different dose–response curves for nicotine in the presence or absence of different doses of drugs were also performed by use of the Quantal program. IC50 values (the concentrations resulting in 50% inhibition) were calculated by use of the Ligand program.

Results

Antagonism of nicotine-induced convulsion in mice

Figure 1 shows that nicotine-induced convulsions in mice were effectively prevented by the anti-cholinergic antiparkinson drugs, and that the anti-nicotinic potencies of these drugs were similar. The nicotinic receptor antagonist mecamylamine also potently antagonized the convulsion, but atropine had very weak anti-nicotinic activity. The ED50 values of the tested compounds are summarized in Table 1.

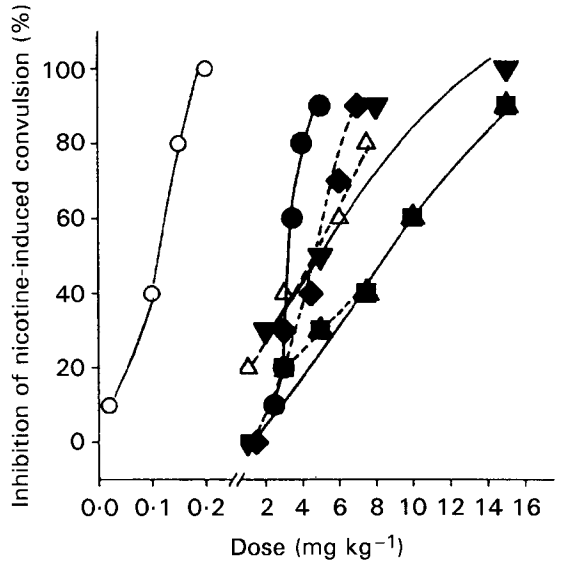

Figure 1. Dose–response curves for the effects of benztropine (■), biperiden (●), caramiphen (▲), ethopropazine (▼), procyclidine (◆), trihexyphenidyl (△) and mecamylamine (○) on nicotine-induced convulsion in mice. Different doses of the tested drugs were injected intravenously 10 min before nicotine (1·0 mg kg^{-1}, i.v.). Ten mice were used in each dose-group.

Shift of dose–response curve for nicotine-induced convulsion by anticholinergic antiparkinson drugs

The dose–response curves for nicotine-induced convulsion in mice are shown in Figure 2; the ED50 (confidence limits) was 0·49 (0·45–0·55) mg kg^{-1}. The anticholinergic antiparkinson drugs and the classical nicotinic receptor antagonist mecamylamine shifted the dose–response curve for nicotine to the right in a dose-related manner. The ED50 of nicotine was increased in the presence of the tested drugs. The maximum effect of nicotine and the slope of nicotine dose–response curve were not significantly affected by different doses of benztropine, procyclidine or trihexyphenidyl ($P > 0·05$). Although the slope of the nicotine dose–response curve and the maximum effect of the nicotine were not significantly influenced by low doses of biperiden, caramiphen, ethopropazine or mecamylamine, which is consistent with competitive antagonism, the maximum effect and the slope were significantly influenced by high doses of the drugs, which is consistent with non-competitive antagonism. In contrast, the classical muscarinic receptor antagonist atropine did not significantly influence the ED50 value, maximum effect or slope of the nicotine dose–response curve. The effect of tricyclopinate, a compound newly synthesized in our institute, was also studied; the results suggest that tricyclopinate shifted the dose–response

Table 1. Anticholinergic activity and muscarinic-receptor binding of anticholinergic antiparkinson drugs.

	ED50 against nicotine (mg kg^{-1})	ED50 against arecoline (mg kg^{-1})	IC50 against [^{3}H]QNB (nM)
Benztropine	7·4 ± 2·3	1·60 ± 0·30	19·6 ± 3·3
Biperiden	4·6 ± 1·4	3·40 ± 0·36	21·2 ± 2·2
Caramiphen	7·8 ± 2·1	6·1 ± 1·7	29·3 ± 11·1
Ethopropazine	4·9 ± 1·5	3·4 ± 1·1	12·9 ± 5·6
Procyclidine	3·1 ± 2·1	4·40 ± 0·93	43·2 ± 8·3
Trihexyphenidyl	3·3 ± 1·8	2·90 ± 0·52	19·1 ± 4·5
Atropine	>20	1·40 ± 0·31	20·1 ± 4·2
Mecamylamine	0·10 ± 0·02	Inactive	>10 000

For evaluation of anti-nicotinic effect five doses of each drug were injected into the caudal vein 10 min before nicotine (10 mg kg^{-1}, i.v.). For evaluation of anti-muscarinic effect the drugs were injected intraperitoneally 15 min before arecoline (10 mg kg^{-1}, s.c.). ED50 values are concentrations affording 50% prevention of nicotine-induced convulsion. Ten mice, 18–22 g, were used in each dose-group. The binding of various agents to muscarinic receptors was determined by their ability to displace [^{3}H]QNB. Protein (0·1 mg mL^{-1}) was incubated with [^{3}H]QNB (0·5 nM) in sodium-potassium phosphate buffer (50 mM, 1 mL) in the presence of the drugs. IC50 values (concentrations resulting in 50% displacement) were calculated by use of the Ligand program. Results are expressed as mean ± s.d., n = 4, for each compound.

Table 2. Effect of different doses of anticholinergic drugs on nicotine-induced convulsion in mice and on the slopes of nicotine dose–response curves.

Treatment	Dose ($mg\,kg^{-1}$)	ED50 (confidence limits) of nicotine ($mg\,kg^{-1}$)	Slope of nicotine dose–response curve
Control		0·49 (0·45–0·55)	5·80± 0·56
Benztropine	10	1·6 (1·4–1·9)*	6·1± 1·1
	15	1·9 (1·7–2·1)*	7·1± 1·6
Biperiden	3	0·82 (0·65–1·00)*	5·0± 1·7
	10	1·3 (0·9–1·9)*	2·90± 0·74*
Caramiphen	5	1·2 (1·1–1·4)*	6·90± 0·34
	10	3·1 (2·3–4·1)*	2·80± 0·35*
	13	12·5 (0·0–1000)*	1·5± 2·6*
Ethopropazine	4	0·96 (0·84–1·10)*	6·6± 0·63
	8	3·1 (0·54–19·80)*	0·78± 0·92*
	10	189·0 (0·00–1000)*	0·28± 0·23*
Procyclidine	3	0·80 (0·67–0·95)*	5·5± 1·0
	10	1·3 (1·1–1·6)*	4·7± 0·5
Trihexyphenidyl	3	0·93 (0·80–1·10)*	5·40± 0·79
	7	1·2 (1·1–1·4)*	5·9± 1·4
Mecamylamine	0·08	1·0 (0·88–1·2)*	6·7± 1·4
	0·12	2·4 (1·6–3·6)*	2·60± 0·56*
Atropine	10	0·52 (0·42–0·64)	5·30± 0·48
Tricyclopinate	0·5	0·99 (0·89–1·10)*	6·7± 1·1
	2	1·4 (1·2–1·6)*	7·3± 1·1

Different doses of each of the drugs were injected intravenously 10 min before intravenous injection of different amounts of nicotine. Ten mice, 18–22 g, were used in each dose-group. The ED50 values are the concentrations affording 50% prevention of nicotine-induced convulsion. The slopes are expressed as means± s.d. *Significantly different from result obtained in the absence of the tested drug, $P < 0·05$.

curve in a manner consistent with competitive antagonism. The ED50 of nicotine in the presence of different doses of the drugs and the slopes of the nicotine dose–response curves are summarized in Table 2.

Antagonism of arecoline-induced tremor in mice

As shown in Table 1, arecoline-induced tremor in mice was antagonized by the anticholinergic antiparkinson drugs. These drugs were similar to atropine in their anti-muscarinic potencies. Mecamylamine did not have anti-muscarinic activity.

Muscarinic receptor-binding studies

The anticholinergic antiparkinson drugs had similar potencies in displacing the specific binding of [^{3}H]QNB. Atropine, a classic muscarinic antagonist, also potently displaced specific binding of [^{3}H]QNB; other non-muscarinic agents, such as hexamethonium, mecamylamine and nicotine, at concentrations as high as 10 μM, displace <15% of the specific binding of [^{3}H]QNB (data not shown). The IC50 values of the drugs in inhibiting [^{3}H]QNB binding are summarized in Table 1.

Antagonism of nicotine-induced convulsion in mice by QNB and several newly synthesized anticholinergics

For further characterization of the antagonism of the central effects of nicotine, QNB and several anticholinergics newly synthesized in our institute were used to prevent nicotine-induced convulsion in mice. It was found that these drugs were more potent than the clinically used anticholinergic antiparkinson drugs in their anti-muscarinic effects, but were either less or more potent in their anti-nicotinic activities (Table 3), suggesting that the anti-nicotinic activity of the compounds was independent of their anti-muscarinic activity.

Discussion

This study has demonstrated that nicotine-induced convulsion in mice was prevented by anticholinergic antiparkinson drugs. The ratio of anti-nicotinic to anti-muscarinic activity was approximately unity for each drug, except for benztropine for which the ratio was approximately 4–5. The results showed that atropine was similar to or more potent than the anticholinergic antiparkinson drugs in its anti-muscarinic effects, but it had little anti-nicotinic activity. Mecamylamine had potent anti-nicotinic activity but no anti-muscarinic effect. Some anticholinergics, e.g. QNB and T9206, had anti-muscarinic activity approximately 1–2 orders of magnitude more potent than that of the clinically used anticholinergic antiparkinson drugs, but their anti-nicotinic activity was similar, suggesting that

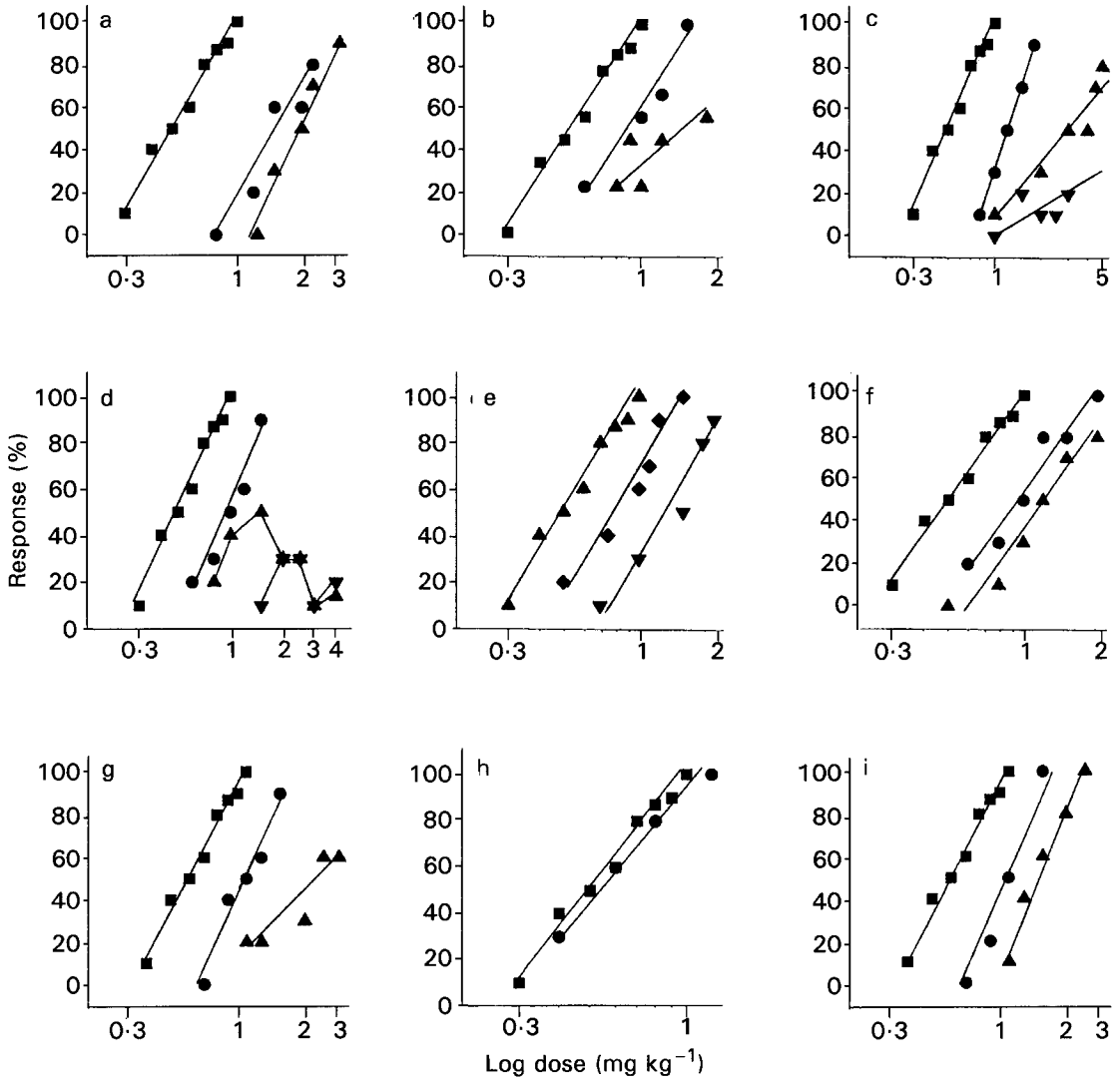

Figure 2. Dose–response curves for nicotine-induced convulsion in mice in the absence or presence of different doses of benztropine (a: ■ 0, ● 10, ▲ 15 mg kg^{-1}), biperiden (b: ■ 0, ● 3, ▲ 10 mg kg^{-1}), caramiphen (c: ■ 0, ● 5, ▲ 10, ▼ 13 mg kg^{-1}), ethopropazine (d: ■ 0, ● 4, ▲ 8, ▼ 10 mg kg^{-1}), procyclidine (e: ▲ 0, ◆ 3, ▼ 10 mg kg^{-1}), trihexyphenidyl (f: ■ 0, ● 3, ▲ 5 mg kg^{-1}), mecamylamine (g: ■ 0, ● 0·08, ▲ 0·12 mg kg^{-1}), atropine (h: ■ 0, ● 10 mg kg^{-1}) and tricyclopinate (i: ■ 0, ● 0·5, ▲ 2·0 mg kg^{-1}). Different doses of the tested drugs were injected intravenously 10 min before intravenous injection of different doses of nicotine. Ten mice were used in each dose-group.

Table 3. Anticholinergic activity of quinuclidinyl benzylate and several newly synthesized anticholinergics.

Drug	ED50 against nicotine (mg kg^{-1})	ED50 against arecoline (mg kg^{-1})
Quinuclidinyl benzylate	5·1 ± 1·4	0·03 ± 0·01
Phencynonate	7·9 ± 1·0	0·54 ± 0·11
Tricyclopinate	0·67 ± 0·14	0·030 ± 0·003
T9206	2·7 ± 1·2	0·013 ± 0·003
P8018	17·2 ± 5·7	0·97 ± 0·51

For evaluation of anti-nicotinic effect five doses of each drug were injected into the caudal vein 10 min before nicotine (1·0 mg kg^{-1}, i.v.). For evaluation of anti-muscarinic effect the drugs were injected intraperitoneally 15 min before arecoline (10 mg kg^{-1}, s.c.). Ten mice, 18–22 g, were used in each dose-group.

the anti-nicotinic activity of the anticholinergic drugs is independent of their anti-muscarinic effect.

It is well known that Parkinson's disease occurs as a result of degeneration of dopamine neurons in the substantia nigra. Because of the depression of the dopaminergic function, the cholinergic function is enhanced. As it is possible that both muscarinic and nicotinic cholinergic function are enhanced in parkinsonian patients, drugs with both anti-muscarinic and anti-nicotinic activity should be used for treatment. It has been shown clinically that drugs such as atropine and scopolamine with potent anti-muscarinic activity but no or very weak anti-nicotinic activity cannot be used to treat parkinsonism; the same is true of drugs such as mecamylamine and hexamethonium with potent anti-nicotinic activity but no antimuscarinic activity. In a clinical study we have found that phencynonate, a drug with both anti-muscarinic and anti-nicotinic activity (Table 3) newly developed in our institute, is more effective in the treatment of Parkinson's disease than clinically used anticholinergics. It is possible that drugs with appropriate anti-muscarinic to anti-nicotinic ratios would be most effective in the treatment of parkinsonism.

The shift of the dose–response curve of nicotine-induced convulsion in the presence of low doses of mecamylamine was consistent with competitive antagonism, inasmuch as the ED50 values of nicotine are increased by doses of drugs that do not alter its maximum effect. These data are consistent with the findings of Stolerman et al (1983) that the decreasing rate of antagonism of nicotine by mecamylamine in a drug-discrimination paradigm was completely overcome by increasing the dose of nicotine. At both high and low doses, benztropine, procyclidine and trihexyphenidyl also did not significantly influence the maximum effect of nicotine or the slope of the dose–response curve, implying competitive action of these drugs. However, the maximum effect and the slope of nicotine dose–response curve were reduced by high doses of biperiden, caramiphen, ethopropazine and mecamylamine. This reduction suggests that these drugs act non-competitively to antagonize nicotine-induced convulsion in mice. This type of antagonism was termed dualistic antagonism by van Rossum (1962), because the characteristics of both competitive and non-competitive antagonism are displayed.

Antagonists have been instrumental in the characterization of the pharmacology of nicotine. Because nicotine-induced convulsion is selectively antagonized by central nicotinic antagonists, it is thought that these effects are receptor-mediated. Although this antagonism is thought to involve direct interaction of the antagonists with nicotinic receptors, evidence is lacking. One of the pharmacological criteria for a receptor-mediated mechanism of action is that the behavioural effects mediated by the receptor can be antagonized by its antagonists. Initial evidence for the existence of central nicotinic receptors was based on the observation that the behavioural effects of nicotine were antagonized by ganglionic blockers, such as mecamylamine and pempidine, that penetrate the CNS. However, these compounds have not been shown to compete effectively for [^{3}H]nicotine or [^{3}H]acetylcholine binding sites in brain tissue (Marks et al 1986; Scimeca & Martin 1988). One possible explanation is that these compounds are non-competitive antagonists of nicotine in the CNS, although pharmacological studies to corroborate these findings have not been conducted for most of the behavioural effects of nicotine. Stolerman et al (1983) demonstrated that mecamylamine non-competitively antagonized the nicotine cue in rats, in that the antagonism could not be overcome by increasing the dose of nicotine.

Although it has been demonstrated that most of the anticholinergics could not directly influence nicotinic-receptor binding, they could antagonize the central effects of nicotine, suggesting that the nicotinic-receptor reaction sites with which anticholinergic antiparkinson drugs interact are not associated with nicotinic-receptor recognition site. One possibility might be that these drugs compete with nicotine for its receptor in-vivo, but the conditions under which in-vitro binding studies have been conducted are inappropriate for antagonist binding. Another possibility is that these anticholinergic antiparkinson drugs and nicotine are binding to distinctly different sites in brain. There is some evidence that the actions of nicotine in-vivo are mediated by two distinct receptor systems, which are differentially sensitive to antagonism by nicotinic receptor antagonists (Collins et al 1986).

It has been suggested that the reason for the low affinity to nicotinic receptors shown by some antagonists might be related to their binding at an allosteric site on the receptor ionophore complex, possibly at a site within the channel rather than at the binding site for nicotine (Lipiello & Fernandes 1988; Wonnacott 1990; Loiacono et al 1993). In this respect there is conflicting evidence from functional studies of some antagonists; the interaction of mecamylamine at neuronal nicotinic receptors has been described as competitive (Ascher et al 1979) or non-competitive (Trendelenburg 1961); in the central nervous system it has been suggested that it has both competitive and

non-competitive activity (Martin et al 1989, 1990). Binding studies have suggested that mecamylamine might act allosterically within the central nervous system (Takayama et al 1989; Banerjee et al 1990).

In this study it could not be demonstrated whether the anticholinergic antiparkinson drugs act on the allosteric site or the ionic channel of nicotinic receptors. To solve this problem, the Patch-Clamp technique should be used, and we have demonstrated that some of the anticholinergics interact with the allosteric site in the nicotinic receptors, but the detailed mechanism of action of these drugs needs to be studied further.

In conclusion, this study has demonstrated that nicotine-induced convulsion in mice was effectively prevented by the anticholinergic antiparkinson drugs. Although the potencies of these drugs in antagonizing nicotine-induced convulsion in mice are similar, their patterns of shift of nicotine dose–response curve are different; some of the anticholinergic antiparkinson drugs have competitive properties but others seem to have both competitive and non-competitive properties in antagonizing nicotine-induced convulsion in mice.

Acknowledgement

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