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PSYCHOTOMIMETICS AS ANTICHOLINERGIC AGENTS—I

1-CYCLOHEXYLPIPERIDINE DERIVATIVES: ANTICHOLINESTERASE ACTIVITY AND ANTAGONISTIC ACTIVITY TO ACETYLCHOLINE

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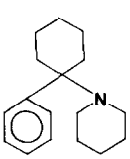
Abstract—Phencyclidine (sernyl®, 1-[(1-phenylcyclohexyl)piperidinel]) and ten of its derivatives, known for their psychotomimetic activity, are potent competitive inhibitors of butyrylcholinesterase and acetylcholinesterase. The drugs also protect the enzyme against inactivation by an organophosphate (sarin), presumably by their direct interaction with the active site. In addition to the acetylcholine-like structural factors identifiable in these molecules, the cyclohexyl moiety is considered necessary for the interaction. The drugs are also competitive antagonists of acetylcholine in perfused organs (guinea-pig ileum, frog rectus abdominis) and in the eye of three mammals. This peripheral activity is three orders of magnitudes less potent than that of atropine. Antiacetylcholine activity in the central nervous system was studied through the antidotal effect of THA (tacrine), before and after injection of the drugs. The correlation between the anticholinesterase activity and the CNS activity as well as the structural relation of the drugs to agonists and antagonists of the cholinergic system is discussed

ALTHOUGH PHENCYCLIDINE [1-(1-phenylcyclohexyl) piperidine] and its derivatives have been known for more than fifteen years as CNS stimulants or depressants,[1,2] interpretation of data concerning their mode of action in the nervous system is still ambiguous and sometimes contradictory.



Phencyclidine

Thus, Ilet and co-workers[3] ascribe direct and indirect sympathomimetic properties to phencyclidine, while Kotev *et al.*[4] suggest that phencyclidine is an anticholinergic

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Abbreviations: BuChE, butyrylcholinesterase (EC3.1.1.8); AcChE, acetylcholinesterase (EC3.1.1.7); 5-HT, 5-hydroxytryptamine; Sarin, isopropyl methylphosphonofluoridate.

agent. These conclusions were drawn from an investigation of phencyclidine in systems with a relatively high organization level, e.g. the third eyelid of the cat and the whole brain[4] and the blood pressure of adrenalectomized animals.[3] The mechanism of action of phencyclidine at the molecular level has remained essentially unexplored.[5,6] The reports by Leonard and Tonge on its effect in rat brain on the metabolism of 5-hydroxytryptamine,[7] noradrenaline,[8] acetylcholine and glutamic acid[9] are at most suggestive.

Recently phencyclidine has gained popularity as a hallucinogen among drug addicts under the name of 'Hog'.[10]

We have studied the effects of phencyclidine and its derivatives at three different levels of biological organization: (1) the intact animal; (2) three isolated organs representing cholinergic tissues, i.e.: (i) the smooth muscle of the guinea-pig ileum, (ii) the iris in three different mammals, (iii) and a striated muscle of the frog (rectus abdominis); (3) soluble enzymes, i.e. BuChE and AcChE. They were found to compete with acetylcholine on the cholinergic receptor in all the organs studied. The responses recorded both with these organs and on whole animals (guinea pigs and mice) indicate that phencyclidine and its derivatives act as mild anticholinergics. In addition these compounds interact with cholinesterases at their active site proving their ability to compete with acetylcholine on one of its specific "receptors".

It is inferred that the activity of these drugs is related to their geometry and electronic structure which are complementary to that of the cholinergic receptor. Thus, the positive head at the heterocyclic nitrogen on the one hand, and a region of high electron density (e.g. aromatic ring or multiple bonds) on the other, mimic, respectively the trimethylammonium group and the ester oxygen in the acetylcholine molecule. The cyclohexyl moiety is instrumental in keeping these two sites at a proper orientation and may also contribute to additional binding of the hydrophobic type.

EXPERIMENTAL PROCEDURE

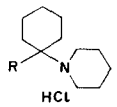
Chemicals. Twelve phencyclidine derivatives (Table 1) were prepared according to published methods.[11–13] In addition to these, two separated 'fragments' of the phencyclidine molecule were also prepared. These are: (i) *N*-cyclohexylpiperidine, obtained by a catalytic reduction of *N*-1(-cyclohexenyl) piperidine[14] and (ii) *N*-benzylpiperidine, prepared from piperidine (2 moles) and benzyl bromide (1 mole), in anhydrous ether.[15]

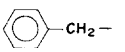
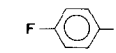
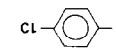
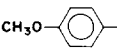
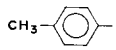
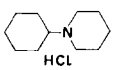
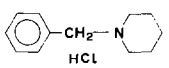
Where necessary, the free base of the phencyclidine derivative was purified by chromatography on an alumina (neutral, Merck) column, with benzene–chloroform (1:1) as eluent. The hydrochlorides were prepared by bubbling HCl gas in anhydrous ether solution and recrystallized from ethanol–ethyl acetate. The compounds were tested for purity in two different TLC systems (Table 1).

Acetylcholine perchlorate, propionylcholine iodide, butyrylcholine iodide and atropine sulfate hydrate were supplied by Sigma. Sarin (isopropyl methylphosphonofluoridate) was a gift from Dr. H. Leader to whom we are thankful.

Enzymes. A hemolysate of fresh human red blood cells, free of any butyrylcholine hydrolyzing activity, was used to test acetylcholinesterase activity. Butyrylcholinesterase from human or horse serum was purified 400-fold in two consecutive steps: (1) ammonium sulphate fractionation, according to Sterlitz,[16] and (2) DEAE column chromatography according to Das and Liddell.[17]

TABLE 1. PHENCYCLIDINE DERIVATIVES



No.	R—	m.p. (°C)*	R_f (TLC)†	Method of preparation (ref.)
I		229	0·4(A); 0·5(B)	Maddox *et al.*[12]
II		184	0·4(A); 0·8(B)	Kalir *et al.*[13]
III		233	0·7(A); 0·9(B)	ibid.
IV		221	0·4(A); 0·8(B)	ibid.
V		225	0·8(A); 0·7(B)	ibid.
VI		183	0·2(A); 0·5(B)	Maddox *et al.*[12]
VII		217	0·2(A); 0·7(B);	ibid.
VIII	$CH_2{=}CH-$	218	0·2(A); 0·4(B)	Mousseron *et al.*[11]
IX	$CH_2{=}CH-CH_2-$	110	0·4(A); 0·8(B)	ibid.
X	$HC{\equiv}C-$	247	0·3(A); 0·7(B)	§
XI	$NC-$	234	1·0(A); 0·9(B); 0·5(C)	Kalir *et al.*[13]
XII	C_2H_5-	221	0·3(A); 0·6(B)	Mousseron *et al.*[11]
		293	0·3(A); 0·6(B)	
		177	0·6(A); 0·6(B)	
		247	0·0(A); 0·0(B); 0·5(C)	

* m.p. are not corrected.

† The following systems were used: A—alumina (acidic, Woelm), chloroform. B—alumina (neutral, Woelm), chloroform-benzene (1:1). C—silica (Woelm), methanol-methylenchloride-acetic acid (1:1:1).

§ We thank Dr. E. Oppenheimer (Org. Chem. Dept., The Hebrew University, Jerusalem) for her generous gift.

METHODS

Acetylcholinesterase activity was measured with a Radiometer pH-stat, under nitrogen, at pH 7·4, 37°. The two titrants, NaOH (0·005 M) and the substrate (0·005 M) were added simultaneously to the reaction vessel, according to Roufogalis and Thomas.[18] The assay mixture (22·5–27·0 ml) contained acetylcholine perchlorate as substrate ($1–10 \times 10^{-4}$ M), 0·2 M NaCl and 0·5 units of the enzyme.

Butyrylcholinesterase activity was determined similarly. The assay mixture contained in 4·0 ml, the substrate (acetyl propionyl or butyrylcholine iodides 1·8–37·5 × 10^{-3} M), phosphate buffer (5 × 10^{-3} M) and 0·6–1·0 enzyme units, using 0·02 N NaOH as the titrant. The enzymic activity of BuChE on benzoylcholine chloride was determined according to Zimmerman and Goyan.[19]

The rate of phosphorylation of BuChE (horse serum) with sarin, in the presence of both the substrate and phencyclidine and its derivatives, was followed titrimetrically, according to Main and Dauterman[20] and Volkova,[21] at pH 7·4, and 37°. The reaction mixture (10·7 ml) contained phosphate buffer (5 × 10^{-3} M), enzyme (1·0 units) sarin (2 × 10^{-7} M) and various concentrations of substrate and phencyclidine derivatives.

The values of kinetic parameters and mode of inhibition of the phencyclidine derivatives were calculated from Lineweaver–Burk plots.

Mydriasis. The drugs studied were dissolved in 0·1 M phosphate buffer pH 7·4, to give a final concentration of 10^{-4} M to 10^{-2} M, and were locally applied on one eye of the test animals (mice and guinea-pigs), while the other eye was untreated and used as a control. This procedure gave no measurable response in the rabbit. Buffered solutions of the compounds were therefore injected under the cornea. The pupil diameter was measured as a function of time when the eye was under strong illumination.

Local anaesthesia. This activity was measured qualitatively in the eyes of mice, rabbits and guinea-pigs. After local application of the drug to one eye of the test animal, the sensitivity in both eyes to direct scratching of the cornea was tested and compared.

Antiacetylcholine activity. The antiacetylcholine activity of phencyclidine and its derivatives was measured in isolated perfused guinea-pig ileum, and in frog rectus abdominis muscle, according to Livingstone.[22]

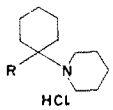
Pharmacology. Saline solutions of the compounds tested were administered either subcutaneously (s.c.) or intraperitoneally (i.p.) to mice, rabbits or guinea-pigs. The volumes used were 0·1 ml/kg for mice, 0·2 ml/kg for guinea-pigs and 0·25 ml/kg for rabbits.

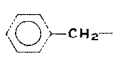
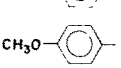
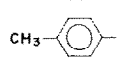
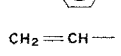
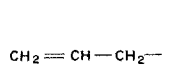
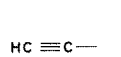
RESULTS

Phencyclidine and some of its derivatives were found to inhibit reversibly both AcChE and BuChE *in vitro*. The effect was far greater towards BuChE than towards AcChE (Table 2). In all cases the inhibition of both enzymes was competitive (Fig. 1a). Since the inhibition of BuChE catalyzing the hydrolysis of acetyl, propionyl and butyrylcholine is very similar, the data for one substrate only is sometimes shown. However when benzoylcholine is the substrate the inhibition of BuChE by phencyclidine is non-competitive (Fig. 1b). The degree of BuChE purification did not affect the inhibition constant e.g. for phencyclidine reasonably similar values were obtained for the enzyme in native serum and for 20- and 400-fold purified samples.

An indication of the type of interaction between inhibitor and enzyme may be derived by applying the Hill equation.[23] At ACh concentration of 3·7 × 10^{-2} M, n = 0·89. One may conclude that presumably one molecule of inhibitor is involved in the interaction with the catalytic site of the enzyme.[23] In order to validate further this assumption, it became necessary to show that phencyclidine could significantly

TABLE 2. INHIBITION CONSTANTS OF PHENCYCLIDINE DERIVATIVES ON CHOLINESTERASES ACTIVITY



No.	R =	K_i	Enzyme	Enzyme source*
I		1.0×10^{-6}	BuChE	A
		5.1×10^{-7}	BuChE	B
		2.4×10^{-6}	BuChE	C
		8.0×10^{-5}	AcChE	D
II		6.6×10^{-7}	BuChE	B
		1.7×10^{-7}	BuChE	C
III		4.1×10^{-7}	BuChE	A
		2.2×10^{-6}	BuChE	B
		1.0×10^{-5}	BuChE	C
		2.0×10^{-5}	AcChE	D
IV		6.3×10^{-7}	BuChE	B
V		7.5×10^{-7}	BuChE	A
VI		5.6×10^{-7}	BuChE	A
		1.0×10^{-7}	BuChE	B
VII		2.6×10^{-7}	BuChE	B
VIII		4.5×10^{-7}	BuChE	A
IX		6.0×10^{-7}	BuChE	A
		2.7×10^{-7}	BuChE	B
		2.4×10^{-6}	BuChE	C
X		1.2×10^{-6}	BuChE	A
		1.2×10^{-6}	BuChE	B
		1.2×10^{-4}	AcChE	D
XI		1.0×10^{-3}	BuChE	A
		2.0×10^{-4}	AcChE	D
XII		1.5×10^{-6}	BuChE	C

* A Human plasma, B—Horse plasma, C—Homogenate of guinea-pig ileum. D—Hemolysate of fresh human red blood cells.

The inhibition constants were determined at pH 7·4 and 37°, on pH-stat (Radiometer), from Lineweaver Burk plots. Experimental procedure as described in methods.

prevent a reaction at the active site of the cholinesterase, e.g. by sarin which is known to phosphorylate selectively the hydroxyl group of serine in the esteratic site of the enzyme.[24] If phencyclidine were to compete for the same site, then the time course of inhibition by sarin should slow down in the presence of increasing concentrations of phencyclidine. Thus, the half-life time ($\tau_{1/2}$) in presence of phencyclidine should be greater than $\tau_{1/2}$ in its absence, under a given set of conditions. Therefore, a prerequisite in this investigation is the knowledge of $\tau_{1/2}$ in the inhibition of BuChE by sarin in the presence of various substrates. The value of $\tau_{1/2}$ is given in Table 3,

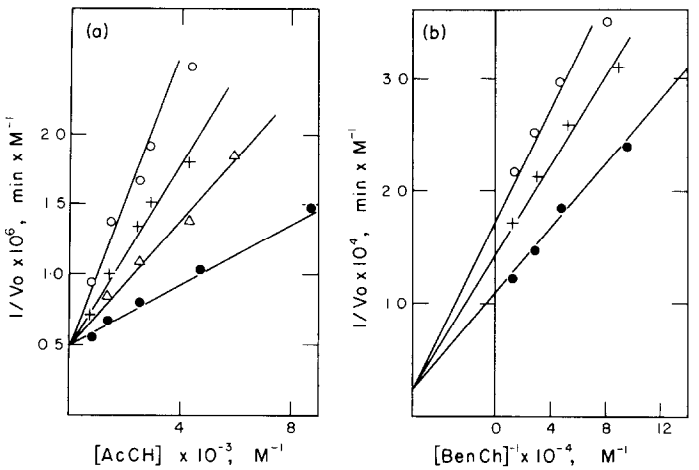


FIG. 1. Lineweaver–Burk plots of the BuChE (horse plasma) catalyzed hydrolysis of acetylcholine (a) and benzoylcholine (b) at pH 7·4, 37°, in the presence of phencyclidine (HCl); (●): no inhibitor; (△): 5×10^{-7} M; (×): 1×10^{-6} M; (○): 2×10^{-6} M. Reaction conditions: (a) 0·6–1·0 units of enzyme, 1·8 37·5 $\times 10^{-3}$ M substrate, 5×10^{-3} M phosphate in a volume of 9 ml; titrant 0·2 N NaOH. (b) 0·2 units of enzyme, 0·1 M NaCl, 2·5–6·5 $\times 10^{-4}$ M substrate, in a volume of 25 ml; titrant 0·005 M NaOH. Assays carried out under nitrogen.

together with the K_m values for four substrates. At comparative concentrations, only butyrylcholine displayed protection of the enzyme important enough to allow a measurable inhibition rate, while in the presence of acetyl- and propionylcholine, the inactivation was still too fast to be measured. In general however, a good correlation was found between $\tau_{1/2}$ and $1/K_m$, as would be expected for inhibitor and substrate that compete for the same site.

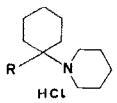
Addition of phencyclidine to the system enzyme–substrate–inhibitor confers additional protection on the enzyme and at a rate that is dependent on phencyclidine concentration (Fig. 2). However phencyclidine itself is a competitive inhibitor of BuChE which must be taken into consideration when interpreting the results. Also, the design of experiments to measure the protective effect of phencyclidine precludes the use of high concentrations of this agent. Yet, the results shown in Fig. 3 demonstrate,

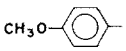
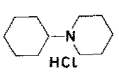
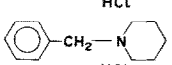
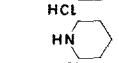
TABLE 3. EFFECT OF SUBSTRATES ON THE RATE OF INHIBITION OF BuChE BY SARIN

Substrate	K_m (M)	Concn (M)	$\tau_{1/2}$ (min)
Acetylcholine	$2{\cdot}0 \times 10^{-3}$	$2{\cdot}7 \times 10^{-2}$	2·3
Propionylcholine	$1{\cdot}5 \times 10^{-3}$	$2{\cdot}7 \times 10^{-2}$	6·1
Butyrylcholine	$1{\cdot}0 \times 10^{-3}$	$2{\cdot}7 \times 10^{-2}$ $1{\cdot}0 \times 10^{-3}$	10·4 2·8
Benzoylcholine	$1{\cdot}7 \times 10^{-5}$	$1{\cdot}0 \times 10^{-3}$	5·2

Rates of the phosphorylation reaction were measured at pH 7·4, 37°, in the presence of 2×10^{-7} M sarin.

TABLE 4. EFFECT OF PHENCYCLIDINE DERIVATIVES ON THE RATE OF BuChE INHIBITION BY SARIN: COMPARISON WITH THE CORRESPONDING K_i VALUES



R—	K_i (M)	Protective activity: Concn (M)	Protective activity: $\tau_{1/2}$ (min)
	$5{\cdot}1 \times 10^{-7}$	$2{\cdot}7 \times 10^{-6}$	4·0
		$5{\cdot}3 \times 10^{-6}$	5·2
		$1{\cdot}1 \times 10^{-5}$	7·6
		$2{\cdot}2 \times 10^{-5}$	11·5
	$1{\cdot}0 \times 10^{-7}$	$9{\cdot}2 \times 10^{-7}$	5·0
		$2{\cdot}7 \times 10^{-6}$	8·0
		$4{\cdot}9 \times 10^{-6}$	11·0
$HC \equiv C-$	$1{\cdot}2 \times 10^{-6}$	$2{\cdot}6 \times 10^{-6}$	4·3
		$5{\cdot}3 \times 10^{-6}$	6·6
		$1{\cdot}1 \times 10^{-5}$	8·0
$NC-$	$>10^{-3}$	1×10^{-3}	2·3
	$1{\cdot}3 \times 10^{-6}$	$4{\cdot}5 \times 10^{-6}$	3·5
		$2{\cdot}7 \times 10^{-5}$	6·0
		$9{\cdot}2 \times 10^{-5}$	12·0
	3×10^{-5}	1×10^{-4}	2·8
	$>10^{-3}$	1×10^{-3}	2·3
Without phencyclidine derivatives			2·3

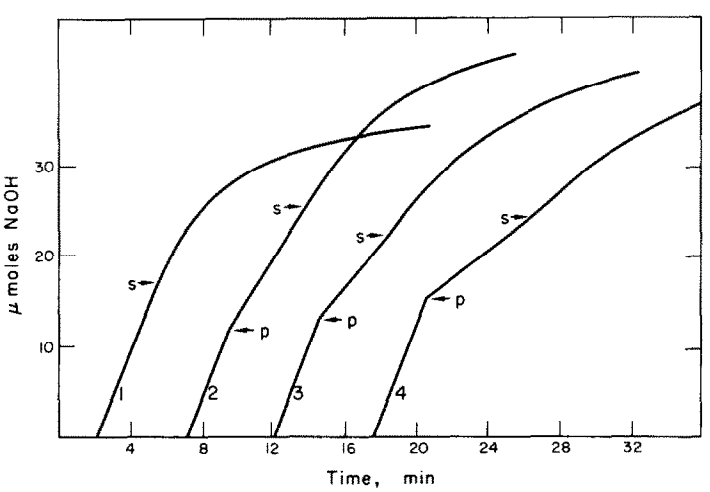


FIG. 2. Progress curves for BuChE inhibition at pH 7·4, 37° in the presence of: the substrate (acetylcholine $2{\cdot}7 \times 10^{-2}$ M), sarin (2×10^{-7} M) and various phencyclidine concentrations (curve 1—control, curve 2—$2{\cdot}7 \times 10^{-7}$ M, curve 3—$5{\cdot}3 \times 10^{-6}$ M, curve 4—$1{\cdot}1 \times 10^{-5}$ M). The arrows indicate time of additions of sarine (S) and phencyclidine (P).

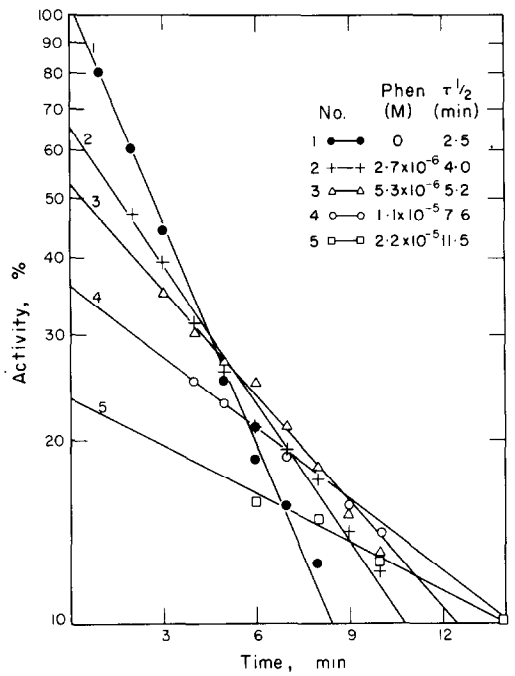


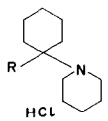
FIG. 3. Plot of per cent log activity of BuChE (horse plasma) hydrolyzing acetylcholine ($2{\cdot}7 \times 10^{-2}$ M) vs time at pH 7·4 and 37°, in the presence of 2×10^{-7} M sarin and various phencyclidine concentrations. Insert shows $\tau_{1/2}$ values calculated from the data in this figure.

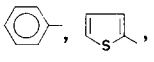
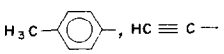
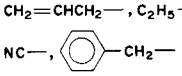
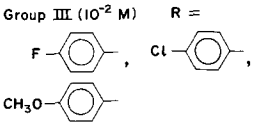
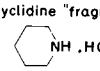
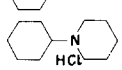
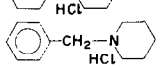
that $\tau_{1/2}$ increased from 2·5 minutes in the absence of phencyclidine to 11·5 minutes in presence of $2{\cdot}2 \times 10^{-5}$ M phencyclidine (Figs. 2 and 3). Measurements of $\tau_{1/2}$ values have been extended to the whole series of phencyclidine derivatives (Table 4). Remarkably, *N*-cyclohexylpiperidine, which represents a fragment of the phencyclidine molecule, possesses K_i and $\tau_{1/2}$ values similar to those of phencyclidine itself, but pipderidine and its *N*-benzyl derivative lacked activity as inhibitors or protectors of the active site (Table 4).

Mydriasis and local anaesthesia. Phencyclidine has a dual action when applied locally to the eye of experimental animals: it acts both as a short acting mydriatic and as a local anaesthetic.

The eye of the mouse was found to be the most sensitive to the mydriatic activity of phencyclidine, while that of the rabbit was the least responsive. In the latter it was necessary to inject the phencyclidine under the cornea in order to get the maximum possible effect. Atropine on the other hand, caused mydriasis after local application in all the test animals. The onset time for full mydriasis caused by phencyclidine ($\leqslant 10^{-2}$ M) was two minutes, compared to ten minutes for atropine (10^{-4} M). On the other hand, the duration of phencyclidine activity was relatively short (15–20 min) and could not be prolonged even when phencyclidine concentration was higher than 10^{-2} M. Atropine, at $\leqslant 10^{-3}$ M caused a long-lasting mydriasis (over 60 min).

TABLE 5. MYDRIATIC ACTIVITY OF PHENCYCLIDINE DERIVATIVES



Compounds	Onset time (min)	Degree of the effect	Duration (min)	Antagonism
Group I (10^{-3} M) R =  	2	maximum	10–15	5×10^{-2} M THA
Group II (10^{-2} M) R = $CH_2{=}CH-$, $CH_2{=}CHCH_2-$, C_2H_5-, 	2	70% of the maximum	3	
Group III (10^{-2} M) R = 		inactive as mydriatics		
Phencyclidine "fragments" (10^{-2} M) 		little myosis in mouse eye only		
		little myosis in mouse eye only		
		acute mydriasis in mouse eye, and little dilatation in the guinea-pig eye		

Under strong illumination buffered solutions of the drugs were dropped directly on one eye of the test animal (mice and guinea-pigs). The pupil diameter of the treated eye was measured as a function of time, during 15 min. The other eye or a saline solution was used as the control.

The local anaesthetic activity of phencyclidine was also of short duration, compared to benzylalcohol. The characteristics of this anaesthetic effect of phencyclidine, currently under investigation, will be described elsewhere.

The study of the mydriatic activity of phencyclidine derivatives was based on the following parameters: (1) the onset time; (2) the duration of the effect; (3) its magnitude and (4) reversal by tacrine. Accordingly, the twelve derivatives studied may be divided into three subgroups (Table 5). The first group includes the most active phencyclidine (I—according to Table 1) and its thienyl (II) ethynyl (X) and *p*-methyl (VII) analogs. The mydriatic activity of these three drugs is concentration dependent: an increase in concentration from 1×10^{-4} M to 1×10^{-2} M is associated with rapid

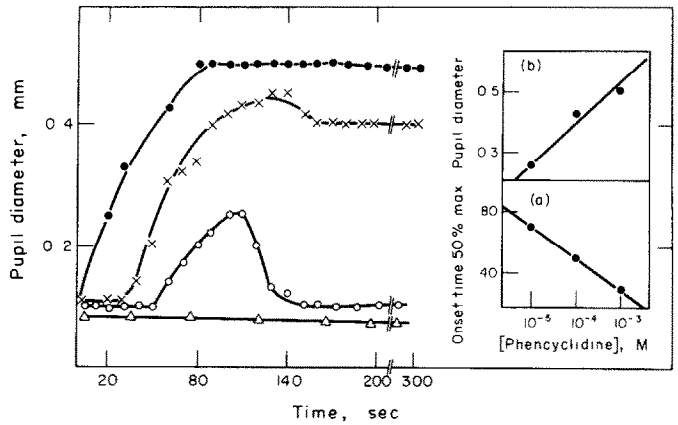


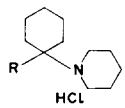
FIG. 4. The mydriatic activity of phencyclidine in the mouse eye. See Table 5 for experimental details. Phencyclidine concentrations: (●) 10^{-3} M, (×) 10^{-4} M, (○) 10^{-5} M, (△) saline. Insert shows plots of: (a) the onset time for 50 per cent of the maximum response, and (b) the maximum pupil diameter, as a function of phencyclidine concentration.

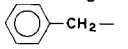
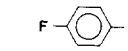
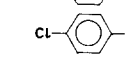
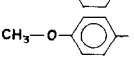
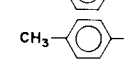
onset, wider pupil and larger duration. These effects are exemplified in Fig. 4 for phencyclidine. Tacrine (9-amino-1,2,3,4-tetrahydroacridine, $\leqslant 1 \times 10^{-1}$ M), a cholinesterase inhibitor,[39] completely blocked the mydriatic activity of 10^{-3} M phencyclidine, when applied simultaneously. At these concentrations tacrine alone caused an acute myosis which lasted for more than thirty minutes. Local application of tacrine three minutes after phencyclidine application, completely reversed the already developed mydriasis. The second group includes four less potent phencyclidine derivatives (Table 5). These compounds, caused only a transient mydriasis in the mouse (<3 min), and no effect at all in the guinea-pig and rabbit, even at concentrations as high as 10^{-2} M, when the maximum possible effect was scarcely attained. The vinyl derivative (VIII) is perhaps exceptional in this group, for its extremely short duration ($\leqslant 1{\cdot}5$ min). Difference between phencyclidine and atropine as mydriatics are further exemplified by parenteral administration of these drugs. A weak mydriatic effect developed in phencyclidine treated animals (4 mg/kg) and lasted for about thirty minutes. At this level phencyclidine caused noticeable behavioural changes in the mouse.[13] Atropine at the same concentration caused an acute mydriasis.

For a comparison, three compounds which structurally represent 'fragments' of the phencyclidine molecule were also applied locally to the eye of the mouse (Table 5). Only *N*-benzylpiperidine caused a pronounced mydriasis, while piperidine and cyclohexyl piperidine were slightly miotic.

Antispasmodic activity. Phencyclidine and some of its derivatives have a dual effect on the perfused guinea-pig ileum. Generally, low concentrations of phencyclidine derivatives cause a potentiation of the spasmodic activity induced by acetylcholine, while higher concentrations inhibit the same effect. The potentiation and the inhibition activities of phencyclidine were both determined at the ED_{50} of acetylcholine (usually $3\text{–}6 \times 10^{-8}$ M). These activities are expressed in Table 6 as the maximum potentiation obtainable as a function of the drug concentration, for one effect, and the concentrations needed for a 50 per cent and 100 per cent block of the spasmogenic activity for the other.

TABLE 6. THE EFFECTS OF PHENCYCLIDINE DERIVATIVES ON SMOOTH MUSCLE



No.	R—	Potentiation of the muscle contraction*		Inhibition of the muscle contraction*	
		Concn range (M)	Potentiation percentage	50% (M)	100% (M)
I		$5–50 \times 10^{-7}$	30	1×10^{-5}	5×10^{-5}
II		—	not detected	7×10^{-6}	2×10^{-5}
III		$5–50 \times 10^{-7}$	100	no effect†	
IV		1×10^{-6}	40	1×10^{-5}	5×10^{-5}
V		1×10^{-6}	50	1×10^{-5}	5×10^{-5}
VI		$1–50 \times 10^{-6}$	50	no effect†	
VII		—	not detected	1×10^{-5}	6×10^{-5}
VIII	$CH_2=CH-$	$2–20 \times 10^{-7}$	50	2×10^{-5}	6×10^{-5}
IX	$CH_2=CH-CH_2-$	$2–8 \times 10^{-6}$	70	5×10^{-5}	4×10^{-4}
X	$HC \equiv C-$	$1–5 \times 10^{-6}$	100	1×10^{-5}	1×10^{-4}
XI	$N \equiv C-$	$1–10 \times 10^{-6}$	70	no effect§	
XII	CH_3CH_2-	$2 \times 20 \times 10^{-6}$	40	5×10^{-5}	5×10^{-4}
Atropine		no effect		100% inhibition at 2×10^{-8} M	

* The standard response—100%, is at the ED_{50} of acetylcholine.
† This derivative caused spontaneous spasms at about 10^{-4} M.
§ Spontaneous spasms above 5×10^{-4} M.
The spasmodic activity of the smooth muscle from guinea-pig ileum was measured according to Livingstone,[22] in tyrode solution, at 37°. The drugs were added to the perfusion vessel 1 min before the addition of acetylcholine, at its ED_{50} concentration ($\sim 3 \times 10^{-8}$ M), at 37°.

The potentiation and the inhibition activities of phencyclidine and its derivatives may also be induced in the already twitched muscle at the ED_{50} of acetylcholine (Fig. 5). Distinction between the spontaneous relaxation of the contracted muscle and the effects of phencyclidine derivatives could be achieved by increasing the rotation rate of the kimograph recorder. In this way, the time axis was extended enough to make possible a separate recording of the twitched muscle responses.

As shown in Fig. 5, an already twitched smooth muscle further contracts or relaxes after addition of the phencyclidine derivatives to the perfusion vessel. The concentration needed to induce relaxation in the twitched muscle equals that needed to inhibit the contraction of the same relaxed muscle (Table 6). Thus, the order of phencyclidine and acetylcholine addition is, within a reasonable period of time, unimpor-

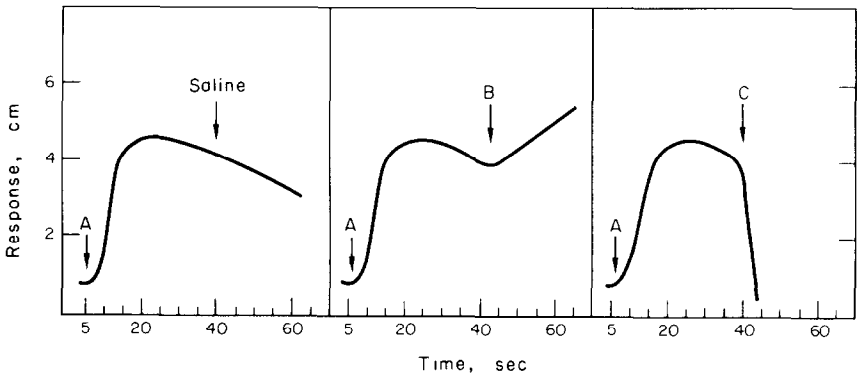


FIG. 5. The inhibition and the potentiation effects of the ethynyl (X) analog of phencyclidine on smooth muscle. See text for experimental details. (A) Acetylcholine (4×10^{-8} M), (B) the ethynyl analog (4×10^{-6} M), (C) the same analog (10^{-4} M).

tant as far as the combined effect is concerned, implying a fully competitive process for the same site. Phencyclidine derivatives were both potentiators and inhibitors of the spasmodic activity evoked by acetylcholine in the smooth muscle (Table 6). However, their potency to cause each effect differed widely and this was reflected in the concentrations needed either for contraction or relaxation. The most active inhibitor was the thienyl (II) derivative, while the ethynyl (X), the allyl (IX) and the benzyl (III) derivatives were the most active potentiators.

Only the *p*-methoxy (VI), the benzyl (III) and the nitrile (XI) derivatives were found to be potentiators (Table 6). The last two compounds caused a pronounced spontaneous spasm of the smooth muscle in concentrations exceeding 10^{-4} M, without addition of exogeneous acetylcholine.

The competitive nature of phencyclidine inhibition was further verified by the slope of the dose-response curve obtained in the presence of phencyclidine (10^{-5} M) which is the same as the slope determined in its absence. The affinity constant of phencyclidine, determined according to Gaddum[25] is 1×10^5.

Since atropine is a standard inhibitor of the smooth muscle contraction caused by acetylcholine, its action on relaxed and contracted smooth muscle was compared to that of phencyclidine. There are three points of divergence between the action of atropine and that of phencyclidine. (1) Phencyclidine and some of its derivatives are potentiators of the contraction of the smooth muscle, at concentrations which are lower by 1 log unit than these needed to inhibit contraction. Atropine is devoid of such a property. (2) Phencyclidine and its derivatives are easily removed from the perfused organ. Thus, simple washing of the muscle with a small volume of the perfusate, restores it to its original state before application of phencyclidine. In the case of atropine, about 30 min of intense washing are required to free the muscle from this agent. (3) Molar wise atropine is more active than phencyclidine by four log units as indicated by its affinity constant (10^9).[22]

The same concentrations of the drugs were also found to inhibit competitively the striated muscle contraction by acetylcholine. Phencyclidine and its thienyl (II) derivative were again found to be potent antispasmodics while the nitrile (XI) derivative remained the least active.[26]

Antagonism of phencyclidine activity in the whole animal. Phencyclidine induced two entirely different behavioural effects in mice and guinea-pigs: while 4–8 mg/kg (s.c.) caused hyperactivity in mice (rapid jumps from side to side of the cage), 5 mg/kg (s.c.) phencyclidine caused a general anaesthesia in guinea-pigs that lasted about 90 min. Tacrine (10 mg/kg, i.p.) administered 15 min after phencyclidine (4 mg/kg, s.c.) completely reversed the arousal effect in mice. Pre-injection of tacrine to mice evoked a characteristic sedation which changed gradually to normal behaviour fifteen minutes after phencyclidine injection. The deep anaesthesia caused in guinea-pigs by phencyclidine itself (5 mg/kg, s.c.), was prevented if tacrine (15 mg/kg, i.p.) and phencyclidine were injected simultaneously. Tacrine administered 15 min after the injection of phencyclidine, shortened the anaesthesia from 90 to about 40 min.

DISCUSSION

Our results suggest that molecules possessing the structural features of phencyclidine bind selectively and reversibly to both the active site of cholinesterases and the cholinergic receptor. The binding is of a competitive nature (Figs. 1, 5) with affinity constants of *ca.* 10^{-7} M and *ca.* 10^{-5} M towards cholinesterases and the cholinergic receptor, respectively. Binding to the cholinesterases entails masking the hydroxyl group of the active site serine, even though this group may not be involved directly in the interaction. Moreover, the rate of protection of the hydroxyl group is inversely proportional to the K_i values of the agents investigated. This is analogous to the relation between $\tau_{1/2}$ and $1/K_m$ for various substrates also found by Bogolyubova *et al.*[27] suggesting a similar mechanism in both cases. In his review on competitive inhibitors of cholinesterases, Long[28] presents a very large number of molecules with various degrees of activity. Three groups in this list should be considered of particular relevance to this study: the CNS—anticholinergic drugs, the central nervous system stimulants, and depressants. However, none of these drugs has an inhibition constant (K_i) value similar or lower than that of phencyclidine and its derivatives, and thus are less potent. An exception is lysergic acid diethylamide (LSD) which was found by Thompson *et al.*[29] to be a rather good competitive inhibitor of mammalian cholinesterases. Recently, Ferko and Gero[30] investigated the interactions of narcotics and their antagonists with human serum esterase. These drugs were also found to be potent competitive inhibitors of cholinesterases.

The usual meaning attached to competitive inhibition is that the inhibitor reacts reversibly with the active center on the enzyme. The accommodation of phencyclidine in a site normally reserved for acetylcholine or butyrylcholine is unexpected, in view of the apparent dissimilarity in structure of the two systems. However, quantum mechanical calculations reveal the structural similarity between the electron charge distribution of the active molecules in the phencyclidine series and the natural neurotransmitter.[31] It is, therefore, reasonable to assume that the binding of the piperidine ring at the "anionic" site, of the active center of the enzyme, while the cyclohexyl moiety is involved in "hydrophobic interactions" but is oriented near the esteratic site[32-33] which becomes blocked to the attack by sarin. This view finds support in the suggestion[32] that BuChE, unlike AcChE, has many 'hydrophobic areas' surrounding its active site. This is consistent with the weak inhibitory effect of phencyclidine upon AcChE, as compared to BuChE (Table 2).

Phencyclidine and its derivatives block acetylcholine activity in both the isolated perfused frog rectus abdominis and guinea-pig ileum, and in the eye of three mammals, but are far less active than atropine in the last two cholinergic organs. The mild nature of the peripheral anticholinergic symptoms obtained with these drugs may have escaped earlier detection by Domino,[1] Chen *et al.*[34] and Mousseron.[11]

Antiacetylcholine effects could have been evoked in two of the organs investigated here both by sympathomimetic and by anticholinergic agents. Consequently, Ilet *et al.*[3] ascribe phencyclidine activity to direct sympathetic stimulation, while Kotev *et al.*[4] related it to an anticholinergic effect. Three lines of evidence support the latter hypothesis: (i) the drugs block acetylcholine activity in the frog rectus abdominis[26] which has no adrenergic innervation;[35] (ii) phencyclidine has been found here to inhibit competitively the spasmogenic activity of acetylcholine in the guinea-pig ileum; and (iii) the molecular structure of these drugs defines interaction patterns, based on the electron charge distribution, which are compatible with geometry of the muscarinic pharmacophore. In phencyclidine analogs, we suggest that a region of high electron density (e.g. the phenyl, ethynyl moieties) may interact with the cholinergic receptor similarly to the ester oxygen in the usual muscarinic agents (Fig. 6). Thus the quantum mechanical calculations reveal that corresponding fragments which can be identified in the two types of molecules, generate surrounding electrostatic potential fields of a very similar pattern, (Fig. 6). These patterns provide the features which are necessary for the specific orientation and binding at sites which have a complementary electrostatic field pattern. This suggestion is supported by the strong muscarinic activity observed for oxotremorine and other acetylenic amines (Fig. 7).

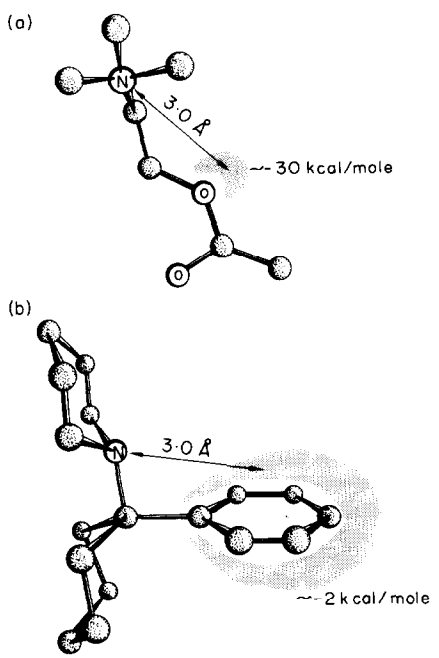


FIG. 6. Elements of the spatial interactions pattern of acetylcholine (a) and phencyclidine (b) with the cholinergic receptor. Distances between the center of the positive group (nitrogen atom) and the region of negative interaction potential[31] are calculated for an energetically preferred conformation of acetylcholine[47] and crystallographic data for phencyclidine.[48]

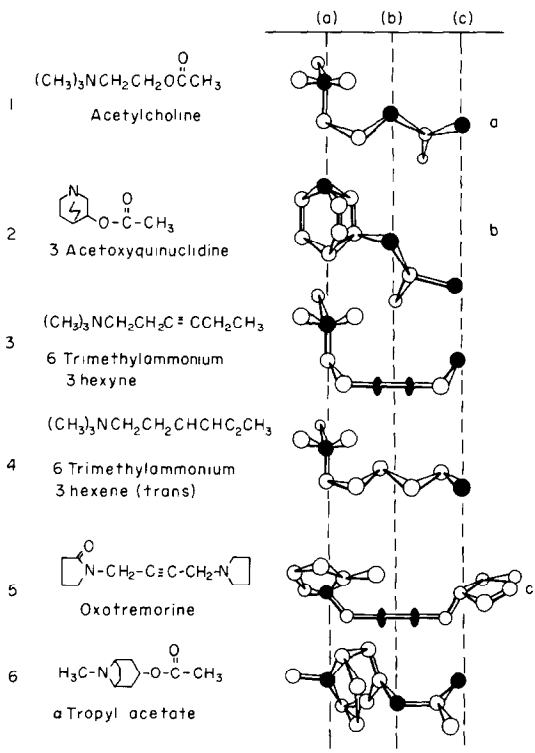


FIG. 7. Correlation of functional groups in molecular structural models of acetylcholine agonists. The molecular models were drawn according to: (a) Canepa *et al.*[49] and Beveridge and Radna;[47] (b) Weinstein *et al.*;[50] (c) Kier.[51]

Indeed, phencyclidine derivatives with polarizable π-electron groups, localized in the region corresponding to the ester oxygen in the pharmacophore (Fig. 6), have been shown to be consistently more active than derivatives lacking this property. Halogen substitution in the phenyl ring, replacement of the phenyl ring with an ethyl group, the incorporation of one methylene group between the phenyl and the cyclohexyl ring, or its complete removal, lead to loss of mydriatic activity (Table 5). The ethynyl (X) and the nitrile (XI) derivatives represent a case of special interest: the apparent similarity between these compounds (Fig. 8, compound 3) is in striking contrast to the difference in their respective effect on the ileum (Table 6), the eye (Table 5) and on cholinesterase activity (Table 2). This emphasizes the importance of this site for the specific interaction at the receptors since the two compounds are quite different in their charge distribution pattern and the corresponding electron polarizabilities. In the nitrile (XI) the triple bond region is not nucleophilic as required for association with the pharmacophore but highly repulsive to a positively charged region.[36] On the other hand, an increase in π-electron density and polarizability in the drug series is accompanied by a parallel increase in anticholinergic activity. Thus, the 2 or 3 thienyl analogs, which are known to be highly aromatic, are also active anticholinergics.

The requirements discussed here for the direct interaction with the receptor being the same for drugs activating the cholinergic receptor. It must be emphasized that

the transition from agonistic to antagonistic properties in acetylcholine-like molecules have been shown to be associated with increasing hydrophobicity, structural rigidity[37] and excess binding from groups responsible for hydrogen bonding near the receptors.[38,39] Most of these features are satisfied by the derivatives of phencyclidine investigated here (Table 2). Moreover, the cyclohexane group which is common to all drugs in the present series has been directly implicated in antagonistic activity[40] (Fig. 8 compound 2). The results of structure-activity relationship studies reported here are consistent with these findings.

The competitive nature of the antiacetylcholine activity of phencyclidine relates well with the antidotal effect of tacrine in the systems studied. This antagonistic effect can be interpreted as a result of direct competition on the cholinergic receptor by the increased endogeneous concentration in the presence of the anticholinesterase

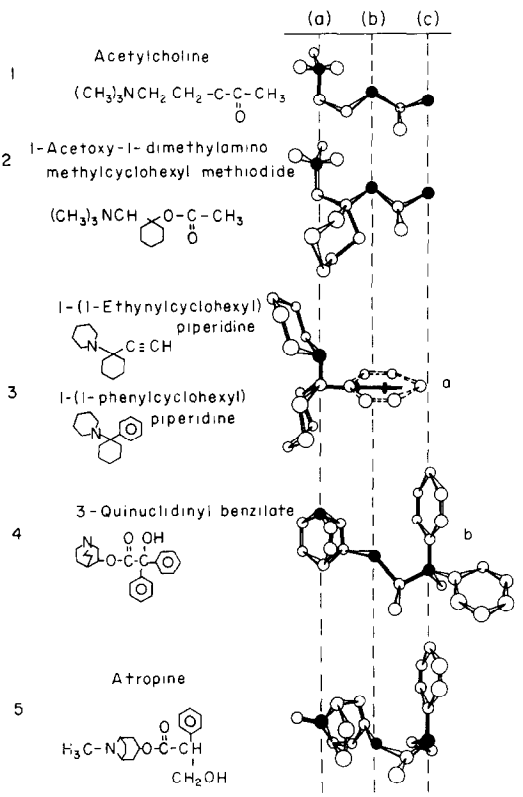


FIG. 8. Correlation of functional groups in molecular structural models of acetylcholine antagonists. The molecular models were drawn according to: (a) Argos *et al.*;[48] (b) Meyerhöffer and Carström.[52]

agent tacrin. In the absence of phencyclidine, tacrine caused myosis in mouse eye. Gershon and Olarin[2] however, could not prove that tacrine antagonizes the central effect of phencyclidine. Because phencyclidine itself has anticholinesterase properties it may increase the local concentration of acetylcholine. This may be the basis of 'potentiation', observed in the perfused guinea pig ileum (Table 6) (Fig. 5). This property has also been observed for other cholinesterase inhibitors, e.g. organophosphate compounds and eserine.[41] It has however been suggested that enzyme inhibi-

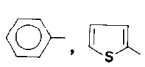
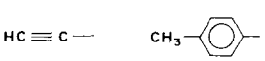
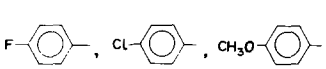
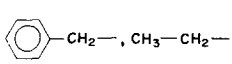
tion might not be the only explanation for the effect.[42] Remarkably lower concentrations of phencyclidine are required for potentiation than the inhibition yet the overall effect of the drug is anticholinergic.

The most pronounced effects of phencyclidine are central rather than peripheral. Since these effects are efficiently antagonized by tacrine, we may reasonably infer that the reversal of hyperactivity in mice or anaesthesia in guinea pigs is acetylcholine mediated. This assumption is supported by the activity of tacrine *in vitro*.

The role of BuChE in the nervous system is still not clear, although the enzyme is widely distributed both in the central and in the peripheral nervous system.[43] The distribution patterns of BuChE and AcChE in the CNS are different.[44–45] At this state, it is not known whether the inhibitory effects of phencyclidine are relevant to this CNS activity. However, there are two observations which deserve special attention: (i) the presence of a nitrile group (instead of the phenyl ring) hinders both the inhibitory capacity ($K_i > 10^{-3}$ M, Table 6) and the CNS activity,[13] and (ii) the cyclohexyl moiety or an equivalent structure is an absolute requirement for both of these activities.[13]

A comparison of the biological activity of the twelve derivatives of phencyclidine in the various organs (Table 7) reveals that the activity on the CNS is better correlated with the iris than with the response of the smooth muscle. In general, the smooth muscle seems to be less selective towards changes in the phenyl ring region. Yet, the compounds most active on the CNS and on the iris [the phenyl (I) and the thienyl (II) derivatives] are also the most active anticholinergics in the smooth muscle. One of the seven compounds which are totally inactive on the CNS and on the

TABLE 7. SOME BIOLOGICAL EFFECTS OF PHENCYCLIDINE DERIVATIVES

Group No.	R–N–.HCl	Biological activity*		
		Iris†	CNS§	Smooth muscle‡
	R–			
1		+++	+++ (1)	+++
		+++	++ (2)	+++
2	$H_2C{=}CH{-}$, $H_2C{=}CH{-}CH_2{-}$	+	+ (2)	+++
3		O	O(1)	+++
		+	O(1)	+++
	$N{\equiv}C{-}$	+	O(1)	O

* Key: (+++) very active; (+) moderately active; (O) not active.
† See legend to Table 5 for experimental details.
‡ See legend to Table 6 for experimental details.
§ The number in parenthesis refer to the following references: (1) Kalir *et al.*[13]; (2) Mousseron *et al.*[11].

iris [the nitrile derivative (XI)], is still a very weak anticholinergic in the smooth muscle. Such lack of specificity in this organ has been reported by others.[46] The reasons for the observed discrepancies between the activities at the different receptors are most probably structural differences in the vicinities of receptors of different origins rather than dissimilarity of the receptor pharmacophores. However, the peripheral activity of these compounds is accompanied by the general behavioural effects in the CNS, which strongly supports the suggestion that the psychotomimetic activity is due to their anticholinergic effect, and caused by a direct interaction with the biological receptor.

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