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STEREOCHEMICAL REQUIREMENTS FOR CENTRAL AND PERIPHERAL MUSCARINIC AND ANTIMUSCARINIC ACTIVITY OF SOME ACETYLENIC COMPOUNDS RELATED TO OXOTREMORINE

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1 The enantiomers of some analogues of the central muscarinic agent, oxotremorine, were prepared and investigated for tremorogenic and tremorolytic activity in intact mice and for muscarinic and antimuscarinic activity on the isolated ileum of the guinea-pig.

2 The R-isomers were more potent than the S-isomers both *in vivo* and *in vitro* regardless of whether the compounds are agonists, partial agonists or competitive antagonists.

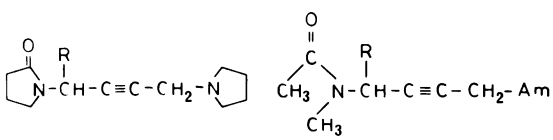
3 It is suggested that in the oxotremorine series, agonists and antagonists interact with a common receptor site, in contrast to classical muscarinic antagonists which are believed to bind also to accessory receptor areas, located close to the agonist binding site.

Introduction

Considerable effort has been devoted to elucidating the stereoselectivity of action of muscarinic agonists and antagonists (Inch & Brimblecombe, 1974; Casy, 1975; Triggle & Triggle, 1976). Such stereoselectivity may yield information not only on the geometry of the relevant binding sites but also on the relationship between agonist and antagonist sites. The results from studies of optical isomers of classical muscarinic agonists and antagonists suggest that the stereochemical requirements for muscarinic and antimuscarinic activity are quite different (Inch & Brimblecombe, 1974; Casy, 1975; Triggle & Triggle, 1976). For example, whereas the potency of the agonist acetyl-β-methylcholine depends greatly on the configuration at the chiral centre, enantiomers of benziloyl-β-methylcholine with antagonist properties show practically no difference in potency (Ellenbroek, Nivard, Van Rossum & Ariens, 1965). Recent results, obtained with enantiomers of tertiary 3-quinuclidinol derivatives, clearly illustrate the different stereochemical demands of muscarinic agonists and antagonists. Thus the potencies of the agonist 3-acetoxyquinuclidine (Barlow & Casy, 1975; Lambrecht, 1976; Ringdahl, Ehlert & Jenden, 1982) and the antagonist 3-quinuclidinyl benzilate (Rehavi, Maayani & Sokolovsky, 1977; Lambrecht, 1979) are governed critically by the configuration of the chiral centre. However, the more potent enantiomers of these compounds have opposite absolute configuration (Rehavi *et al.*, 1977; Lambrecht, 1979).

Classical muscarinic antagonists, obtained from agonists by the introduction of large hydrophobic groups, may give misleading information regarding the stereoselectivity of muscarinic interactions. Binding of the hydrophobic groups to accessory receptor areas (Ariens & Simonis, 1967; Ariens, Beld, Rodrigues de Miranda & Simonis, 1979) may over-ride the three-dimensional requirements of the agonist binding site itself (Beckett, 1967). Studies with chiral antagonists, produced by small changes in agonist molecules and for which binding to accessory receptor areas is not likely to be involved, should be more informative.

It was previously shown (Dahlbom, Lindquist, Lindgren, Svensson, Ringdahl & Blair, 1974) that the compund (2) (in Figure 1), obtained by the introduction of a methyl group at the 1-position of



1 R = H

2 R = CH_3

	R	Am
3	H	N
4	CH_3	N
5	CH_3	$N(CH_3)_2$
6	CH_3	$\overset{+}{N}(CH_3)_3$

Figure 1 Compounds tested for muscarinic and antimuscarinic activity.

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the butynyl chain of the tremorogenic muscarinic agent oxotremorine (1), is a potent antagonist to oxotremorine, and that its enantiomers differ widely in potency. The optical isomers of a number of analogues of 2 also showed varying degrees of stereoselectivity in their antimuscarinic actions (Ringdahl & Dahlbom, 1978; 1979; Ringdahl, Resul & Dahlbom, 1979). Although the stereochemical demands for central and peripheral antimuscarinic potency in the oxotremorine series thus are well established no chiral analogues of oxotremorine with muscarinic properties have yet been described. The reason for this might be sought in the fact that the introduction of substituents in the oxotremorine molecule to make it chiral almost invariably leads to loss of muscarinic activity and to the appearance of antagonist properties (Dahlbom, 1980).

We recently showed that the addition of a methyl group in the 1-position of the butynyl chain of the potent oxotremorine-like agent 3 affords a potent antagonist (4) to oxotremorine *in vivo* (Resul, Dahlbom, Ringdahl & Jenden, 1982). On the guinea-pig isolated ileum, 4 behaved in a manner typical of a partial agonist. However 5, the dimethylamino analogue of 4, showed oxotremorine-like activity both *in vivo* and *in vitro*, which offered us an excellent opportunity to investigate a chiral agonist closely related to oxotremorine. We have now prepared the enantiomers of compounds 4 and 5 as well as of compound 6, the *N*-methyl quaternary derivative of 5. Thus a series of chemically closely related enantiomeric pairs (2, 4–6), with chiral centres at corresponding positions in the molecules, became available for a comparison of the stereochemical requirements for muscarinic and antimuscarinic activity.

Methods

Determination of the tremorogenic dose

Tremor intensity was estimated using an electronic device to achieve an objective measurement. The method used has been described in detail (Ringdahl, Muhi-Eldeen, Ljunggren, Karlen, Resul, Dahlbom & Jenden, 1979). In short, the test drug was given by intravenous injection to groups of six male NMRI mice weighing 20–24 g, and the median dose required to evoke a predetermined tremor intensity was calculated.

Determination of the tremorolytic dose

Fifteen minutes before oxotremorine administration, three linearly spaced doses of the antagonist were given intraperitoneally to groups of six mice, while six control animals remained untreated. The median effective dose of oxotremorine, which for the untreated animals was approximately 0.5 μmol/kg, was plotted against the dose of the test compound. That dose of antagonist which doubled the median effective dose of oxotremorine was estimated by linear regression analysis (Ringdahl *et al.*, 1979).

Muscarinic and antimuscarinic activity in the guinea-pig isolated ileum

Guinea-pigs (male, English short-hair, 350–400 g) were killed by a blow to the head and bled. Segments of the ileum, 2–3 cm long, were removed and suspended in a 10 ml organ bath containing Tyrode solution at 37°C and aerated with O_2 containing 5% CO_2. Contractions were recorded isotonically at 1 g tension, by means of an electromechanical displacement transducer and a potentiometric recorder.

Agonists were compared on the same preparation with carbachol using the cumulative dose-response technique. Potencies were expressed as pD_2 values (negative logarithm of the ED_{50} values). The preparation was allowed to equilibrate with each concentration of antagonist for 20 min before dose-response curves to carbachol were obtained. pA_2 values were calculated according to the method of Arunlakshana & Schild (1959).

Drugs

The following drugs were used: oxotremorine sesquioxalate (Bebbington & Shakeshaft, 1965), carbamylcholine chloride (Aldrich Chemical Co.), hexamethonium chloride (K & K Laboratories), atropine sulphate (Mallinckrodt Inc.). The Tyrode solution had the following composition (mM): NaCl 137, $NaHCO_3$ 12, glucose 5.0, KCl 2.7, $MgSO_4.7H_2O$ 1.0, NaH_2PO_4 0.4 and $CaCl_2$ 1.8.

Preparation of compounds

The syntheses of the racemate (Lindgren, Lindquist, Lindeke, Svensson, Karlen, Dahlbom & Blair, 1973) and the enantiomers (Lindquist, Ringdahl, Svensson & Dahlbom, 1976) of compound 2 have been described. The enantiomers of compound 4 were prepared through the Mannich reaction from the **R**- and **S**-isomers of *N*-methyl-*N*-(1-methyl-2-propynyl)acetamide (7), *para*formaldehyde and pyrrolidine in dioxane in the presence of small amounts of CuCl as described for the racemate (Resul *et al.*, 1982). The compounds **R**- and **S**-7, required as starting material for the Mannich reaction, were obtained through acetylation of the enantiomers of 1-methyl-2-propynylamine (Lindquist *et al.*, 1976), affording **R**- and **S**-8, followed by *N*-methylation using methods described for the racemate (Resul *et al.*,

1982). The enantiomers of compound 5 were prepared by the following method. **R**- and **S**-*N*-(4-diethylamino-1-methyl-2-butynyl)- *N*-methylacetamide (9) were prepared through the Mannich reaction from **R**- and **S**-7, *para*formaldehyde and diethylamine using our standard method (Resul *et al.*, 1982). This Mannich base was treated with cyanogen bromide according to a previously described procedure (Resul, Ringdahl & Dahlbom, 1979) to give the enantiomers of *N*-(4-bromo-1-methyl-2-butynyl)-*N*-methylacetamide (10). Treatment of **R**- and **S**-10 with excess of dimethylamine in dioxane at room temperature for 1 h and working up according to standard procedures afforded **R**- and **S**-5, respectively. The racemate and enantiomers of compound 6 were prepared by addition of methyl iodide to a solution of the corresponding tertiary amine in acetone. The physical properties of the new compounds are listed in Table 1.

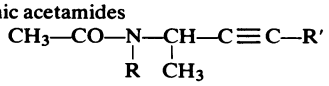
Results

The enantiomers of compounds 4 and 5 were tested for tremorogenic and tremorolytic activity in intact mice. The results are summarized in Table 2 which also includes previously published data for the racemates of 4 and 5 (Resul *et al.*, 1982) and for the enantiomers as well as racemate of 2 (Dahlbom *et al.*, 1974). The enantiomers of 2 and 4–6 and their corresponding racemates were investigated for muscarinic and antimuscarinic activity on guinea-pig isolated ileal preparations (Table 2).

The **R**-enantiomer of 4 was considerably more potent than the **S**-enantiomer in inhibiting oxotremorine-induced tremor. It was about twice as potent as the corresponding racemate. These results are qualitatively similar to those obtained for the structurally related compound 2 (Dahlbom *et al.*, 1974). Only the **R**-enantiomer of 5 produced tremor, the tremorogenic dose being about half of that previously reported (Resul *et al.*, 1982) for the racemate, i.e. about 24 times that of oxotremorine. **S**-5 was inactive as a tremorogenic or tremorolytic agent.

On the guinea-pig isolated ileum compound 2 behaved as a competitive antagonist to carbachol. **R**-2 was 257 times more potent than **S**-2 (Table 2). As previously shown for the racemate (Resul *et al.*, 1982), the enantiomers of 4 behaved in a manner typical of a partial agonist. The **R**-isomer was 13 times more potent than the **S**-isomer in causing contractions of the ileum. However, the maximal response obtained with the **R**-isomer (about 75% of that of carbachol) was lower than that of the **S**-isomer (about 85% of that of carbachol) in three different ileal preparations examined. The dose-response

Table 1 Physical data for new acetylenic acetamides



Compound	*R*	*R'*	*Derivative**	*b.p. (mmHg) or m.p. °C*	*$[\alpha]_D^{22}$†*	*Yield (%)*	*Formula‡*
R-4	CH_3	CH_2—N⟨ ⟩	Base	122 (0.5)	+ 84.3	62	$C_{12}H_{20}N_2O$
			Sesquioxalate	88–90	+ 60.8		$C_{12}H_{20}N_2O \cdot 1.5C_2H_2O_4 \cdot 0.5H_2O$
S-4			Base	122 (0.5)	− 83.9	62	
			Sesquioxalate	87–89	− 58.9		
R-5	CH_3	CH_2—$N(CH_3)_2$	Base	96–97 (0.6)	+ 110.7	80	$C_{10}H_{18}N_2O \cdot C_2H_2O_4$
			Oxalate	104–105	+ 75.8		
S-5			Base	85 (0.15)	− 108.7	83	
			Oxalate	105–106	− 73.6		
R-6	CH_3	CH_2—$N^+(CH_3)_3$	Iodide	130–131	+ 62.0	70	$C_{11}H_{21}IN_2O$
S-6				130–132	− 61.8	75	
RS-6				144–145		75	
R-7	CH_3	H		42 (0.1)	+ 84.1	71	$C_7H_{11}NO$
S-7				54 (0.8)	− 82.9	83	
R-8	H	H		86–87	+ 133.6	59	C_6H_9NO
S-8				85–87	− 130.8	65	
R-9	CH_3	CH_2—$N(C_2H_5)_2$	Base	110 (0.6)	+ 95.9	74	$C_{12}H_{22}N_2O$
S-9				90 (0.1)	− 94.2	73	
R-10	CH_3	CH_2—Br		§	+ 109.8	73	$C_8H_{12}BrNO$
S-10				§	− 107.1	86	

* Oxalates, sesquioxalates and methiodides were recrystallized from ethanol-ether, compound 8 from ligroin.
† Optical rotations were measured in ethanol (*c* 0.6–1.6).
‡ All compounds were analysed for C, H and N, and the results were within ± 0.4% of the theoretical values.
§ The oily product was purified by chromatography on a silica gel column using ether as eluent.

Table 2 Pharmacological properties of some chiral oxotremorine analogues

Compound	*Guinea-pig isolated ileum*		*Tremorogenic effect in mice*	In vivo *dose (μmol/kg) in mice required to produce oxotremorine blockade* *
	pD_2	pA_2	ED_{50} (μmol/kg)	
R-(+)-2		7.55 ± 0.02(5)		0.26
S-(−)-2		5.14 ± 0.01(6)		Inactive
(±)-2		7.28 ± 0.02(4)		0.51
R-(+)-4	6.63 ± 0.07(6)†			0.35
S-(−)-4	5.51 ± 0.05(6)†			10
(±)-4	6.38 ± 0.07(6)†			0.6
R-(+)-5	6.50 ± 0.05(6)		12	
S-(−)-5	5.29 ± 0.04(6)		Inactive	
(±)-5	6.27 ± 0.05(6)		22	
R-(+)-6	6.80 ± 0.07(6)			
S-(−)-6	5.28 ± 0.02(8)			
(±)-6	6.42 ± 0.05(6)			
Oxotremorine	7.50 ± 0.05(8)		0.5	
Carbachol	7.15 ± 0.04(8)			

pD_2 and pA_2 values given ± s.e. Number of estimates in parentheses.
*Dose required to double the dose of oxotremorine inducing a predetermined tremor intensity in 50% of the mice.
† Partial agonist.

curves of the racemates and the enantiomers of compounds 5 and 6 as well as carbachol were essentially parallel and attained the same maximum response value. The **R**-enantiomers of both 5 and 6 were about twice as potent as their corresponding racemates and 16 and 33 times, respectively, more potent than the **S**-enantiomers. Interestingly, the quaternary derivative 6 was more potent than the corresponding tertiary amine (5).

Hexamethonium chloride (3×10^{-4} M) had no appreciable effect on the dose-response curves of 5 and 6, while atropine sulphate (10^{-8} M) caused a parallel shift to the right of the curves, indicating a predominantly muscarinic mode of action of the compounds.

Discussion

Compounds 2 and 4 were about equipotent in inhibiting oxotremorine-induced tremor and showed the same stereoselectivity pattern in this respect. However, their actions on the guinea-pig ileum differed since 2 was a competitive antagonist and 4 was a partial agonist. The dimethylamino analogue of 4, i.e. 5, was a muscarinic agonist capable of producing tremor, while the dimethylamino analogue of 2 showed parasympathomimetic activity *in vitro*, although it failed to produce tremor (Svensson, Dahlbom & Blair, 1975). It thus appears that the introduction of a methyl group in the butynyl chain of agonists related to 3 is less detrimental to muscarinic activity than it is in agonists related to oxotremorine (see above).

Compound 5 and 3-acetoxyquinuclidine are the only potent tertiary muscarinic agonists known that have been resolved into their enantiomeric forms. A recent study (Ringdahl *et al.*, 1982) showed **S**-3-acetoxyquinuclidine to have about 1/5 of the peripheral parasympathomimetic and 1/22 of the tremorogenic activity of oxotremorine. The **R**-isomer of 5 appears to be almost equipotent with **S**-3-acetoxyquinuclidine as a central muscarinic agonist but only half as potent in its peripheral parasympathomimetic activity. As previously suggested for oxotremorine (Ringdahl *et al.*, 1982), the higher central specificity of **R**-5 is probably due mainly to its lower base strength as compared to 3-acetoxyquinuclidine.

A few semi-rigid tertiary muscarinic agonists including oxotremorine, 3-acetoxyquinuclidine and arecoline, in which the basic nitrogen is part of a ring system, are more potent than their *N*-methyl quaternary salts (Hanin, Jenden & Cho, 1966; Cho, Jenden & Lamb, 1972). In contrast, for muscarinic agents in which the nitrogen is contained on a flexible aliphatic chain, the reverse is true (Brimblecombe & Rowsell, 1969; Cho *et al.*, 1972). Thus the trimethylammonium analogue of oxotremorine is a more potent muscarinic agent than the parent dimethylamino derivative (Bebbington, Brimblecombe & Shakeshaft, 1966). The observation that the quaternary salt 6 was more potent than the tertiary amine 5 (Table 2) is in agreement with these findings. Since the less active **S**-isomers of 5 and 6 were equipotent, the higher potency of the quaternary derivative as compared to the tertiary amine is due to the two fold increase in

potency on *N*-methylation of the more active **R**-isomer of 5. In contrast, the potency of the more active **S**-isomer of 3-acetoxyquinuclidine was *reduced* about 1000 fold, whereas the potency of the weaker **R**-isomer was reduced only two fold on *N*-methylation leading to an inversion of the stereoselectivity for the receptor (Barlow & Casy, 1975; Lambrecht, 1976; Ringdahl *et al.*, 1982).

For the closely related compounds included in this study the **R**-isomer is more potent than the **S**-isomer both *in vivo* and *in vitro*, regardless of whether the compounds are agonists (5, 6), partial agonists (4), or competitive antagonists (2). Thus in this series of compounds the stereochemical requirements for muscarinic and antimuscarinic activity appear to be similar. This finding, together with the close structural similarity between agonists and antagonists, suggest that in the oxotremorine series agonists and antagonists interact with a common receptor site. In contrast, the binding of classical muscarinic antagonists is dominated by the interaction of large hydrophobic moieties with accessory receptor areas differing from but located close to the agonist binding site (Ellenbroek *et al.*, 1965; Ariens & Simonis, 1967; Triggle & Triggle, 1976; Ariens *et al.*, 1979).

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