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Stereoselective inhibition of muscarinic receptor subtypes by the enantiomers of hexahydro-difenidol and acetylenic analogues

*R. Feifel, *M. Wagner-Röder, †C. Strohmann, †R. Tacke, §M. Waelbroeck, §J. Christophe, *E. Mutschler & *,[1]G. Lambrecht

*Department of Pharmacology, University of Frankfurt, Theodor-Stern-Kai 7, Gebäude 75A, D-6000 Frankfurt/M., Federal Republic of Germany; †Institute of Inorganic Chemistry, University of Karlsruhe, Engesserstraße, D-7500 Karlsruhe, Federal Republic of Germany; §Department of Biochemistry and Nutrition, Medical School, Free University of Brussels, Boulevard of Waterloo 115, B-1000 Brussels, Belgium

1 The affinities of the (R)- and (S)-enantiomers of hexahydro-difenidol (*1*) and its acetylenic analogues hexbutinol (*2*), hexbutinol methiodide (*3*) and *p*-fluoro-hexbutinol (*4*) (stereochemical purity > 99.8%) for muscarinic receptors in rabbit vas deferens (M_1), guinea-pig atria (M_2) and guinea-pig ileum (M_3) were measured by dose-ratio experiments.

2 The (R)-enantiomers consistently showed higher affinities than the (S)-isomers. The stereoselectivity ratios [(R)/(S)] were greatest with the enantiomers of *1* (vas deferens: 550; ileum: 191; atria: 17) and least with those of the *p*-Fluoro-analogue *4* (vas deferens: 34; ileum: 8.5; atria: 1.7).

3 The enantiomeric potency ratios for compounds *1*–*4* were highest in rabbit vas deferens, intermediate in guinea-pig ileum and much less in guinea-pig atria. Thus, these ratios may serve as a predictor of muscarinic receptor subtype identity.

4 (S)-*p*-Fluoro-hexbutinol [(S)-*4*] showed a novel receptor selectivity profile with preference for M_3 receptors: $M_3 > M_2 \geqslant M_1$.

5 These results do not conform to Pfeiffer's rule that activity differences between enantiomers are greater with more potent compounds.

Introduction

A large body of evidence derived from both functional and radioligand binding studies suggests that there are at least three pharmacological muscarinic receptor subtypes [M_1, M_2 ($M_{2\alpha}$) and M_3 ($M_{2\beta}$)] (Eglen & Whiting, 1986; Mitchelson, 1988; Giraldo *et al.*, 1988; Waelbroeck *et al.*, 1988a; 1989; Lambrecht *et al.*, 1989d). This subclassification was recently confirmed by cloning, sequencing and expression of complementary DNA encoding five muscarinic receptors (m1–m5) (Kerlavage *et al.*, 1987; Peralta *et al.*, 1987; Akiba *et al.*, 1988; Brann *et al.*, 1988). The antagonist binding properties of m1–m3 and their patterns of expression in various tissues closely correspond to those of the M_1, M_2 and M_3 receptors (Peralta *et al.*, 1987; Akiba *et al.*, 1988; Maeda *et al.*, 1988; Buckley *et al.*, 1989). The selective muscarinic antagonists pirenzepine (Hammer *et al.*, 1980; Lambrecht *et al.*, 1988a; Waelbroeck *et al.*, 1988a; 1989), methoctramine (Melchiorre *et al.*, 1987; Wess *et al.*, 1988), AF-DX 116 (Giachetti *et al.*, 1986), hexahydro-(sila-)difenidol (Mutschler & Lambrecht, 1984; Lambrecht *et al.*, 1988a; 1989d; Waelbroeck *et al.*, 1988a; 1989; Akiba *et al.*, 1988; Buckley *et al.*, 1989) and *p*-fluoro-hexahydro-sila-difenidol (Lambrecht *et al.*, 1988a; 1989b,d) have proved to be useful tools in this subclassification.

Among these selective muscarinic antagonists, racemic hexahydro-difenidol [(R/S)-*1*; Figure 1] has been shown to have high affinity for M_1 receptors in neuronal tissues as well as for M_3 receptors in exocrine glands and smooth muscles, but a much lower affinity for cardiac M_2 receptors (Mutschler & Lambrecht, 1984; Eltze *et al.*, 1988; Lambrecht *et al.*, 1988c; Waelbroeck *et al.*, 1989). The main aim of the present study was to characterize the structural demands, including stereochemical aspects, for potency and selectivity of some chiral acetylenic analogues of hexahydro-difenidol (*1*) (Figure 1). Since a conformationally rigid acetylenic moiety is present in some selective muscarinic agonists such as McN-A-343 (Roszkowski, 1961; Lambrecht *et al.*, 1986; Eltze *et al.*, 1988; Wess *et al.*, 1988) and arecaidine propargyl ester (Mutschler & Hultzsch, 1973; Mutschler & Lambrecht, 1984; Moser *et al.*, 1989), it would be of interest to investigate whether the acetylenic analogues of hexahydro-difenidol are also selective for muscarinic receptor subtypes.

In the last few years, data have accumulated that muscarinic receptors can be differentiated on the basis of their stereoselectivity to chiral antagonists such as procyclidine (Lambrecht & Mutschler, 1986; Tacke *et al.*, 1986; Waelbroeck *et al.*, 1988b), trihexyphenidyl (Lambrecht *et al.*, 1988b; 1989d), phenglutarimide (Lambrecht *et al.*, 1989a), biperiden (Eltze & Figala, 1988) and telenzepine (Eveleigh *et al.*, 1989). Hexahydro-difenidol (*1*) and its analogues hexbutinol (*2*), hexbutinol methiodide (*3*) and *p*-fluoro-hexbutinol (*4*) (Figure 1) possess a centre of chirality and therefore exist in two enantiomers. We took advantage of this by determining the antagonist affinities of the individual enantiomers of these compounds at muscarinic receptor subtypes. The results were compared with those obtained for the selective reference drugs hexahydro-sila-difenidol ($M_3 \geqslant M_1 > M_2$) and *p*-fluoro-hexahydro-sila-difenidol ($M_3 > M_1 > M_2$). The receptors studied were presynaptic M_1 heteroreceptors in rabbit vas deferens (Eltze, 1988; Eltze *et al.*, 1988; Lambrecht *et al.*, 1988a,b), cardiac M_2 receptors present in guinea-pig atria and smooth muscle M_3 receptors present in guinea-pig ileum.

Some of the present results have been briefly presented elsewhere (Feifel *et al.*, 1988; Lambrecht *et al.*, 1989c; Tacke *et al.*, 1989).

Methods

Rabbit isolated vas deferens

Experiments on rabbit isolated vas deferens were carried out according to Eltze (1988) and Eltze *et al.* (1988). Male New Zealand white rabbits (2.5–3.0 kg) were killed by i.v. injection of $120\,mg\,kg^{-1}$ pentobarbitone sodium. Vasa deferentia were excised, dissected free of connective tissues and divided into four segments of approximately 1.5 cm length. The preparations were set up in 7 ml organ baths containing modified

[1] Author for correspondence.

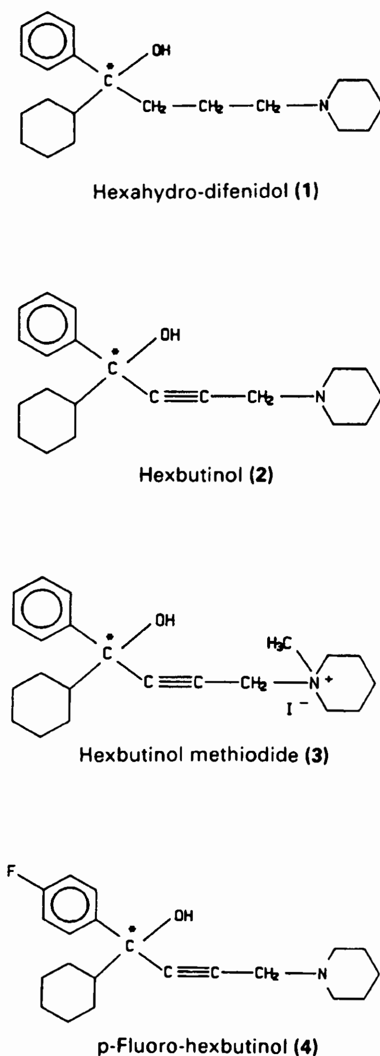

Figure 1 Chemical structures of the enantiomers of hexahydro-difenidol (*1*), hexbutinol (*2*), hexbutinol methiodide (*3*) and *p*-fluoro-hexbutinol (*4*). The asterisk denotes the centre of chirality.

Krebs buffer which consisted of (mM): NaCl 118.0, KCl 4.7, $CaCl_2$ 2.5, $MgSO_4$ 0.6, KH_2PO_4 1.2, $NaHCO_3$ 25.0 and (+)-glucose 11.1; 1 μM yohimbine was included to block α_2-adrenoceptors. The bathing fluid was maintained at 31°C and aerated with 95% O_2/5% CO_2. A basal tension of 750 mg was applied and after a 30 min period of initial equilibration isometric twitch contractions were elicited by electrical field stimulation (0.05 Hz, 0.5 ms, 30 V) with platinum electrodes. The contractions were measured isometrically by a force-displacement transducer connected to a Hellige amplifier and a Rikadenki polygraph. These effects were concentration-dependently inhibited by the M_1 receptor agonist 4-(4-chlorophenylcarbamoyloxy)-2-butynyltrimethylammonium iodide (4-Cl-McN-A-343) (Eltze *et al.*, 1988).

Guinea-pig isolated left atria and ileal longitudinal muscle

Guinea-pigs (300–400 g) of either sex were killed by cervical dislocation. The organs required were removed and set up in 6 ml organ baths, under 500 mg tension, in oxygenated (95% O_2/5% CO_2) Tyrode solution (32°C) composed of (mM): NaCl 137.0, KCl 2.7, $CaCl_2$ 1.8, $MgCl_2$ 1.05, $NaHCO_3$ 11.9, NaH_2PO_4 0.42 and (+)-glucose 5.6. Arecaidine propargyl ester (Mutschler & Hultzsch, 1973; Mutschler & Lambrecht, 1984; Moser *et al.*, 1989) was used as an agonist. Left atria were paced electrically (2 Hz, 3 ms, 5 V) by means of platinum electrodes. Negative inotropic effects to the agonist were measured as changes in isometric tension. Responses of ileal longitudinal muscle strips (Paton & Zar, 1968) to arecaidine propargyl ester were measured as isotonic contractions. The effects in atria and ileum were recorded as with the rabbit isolated vas deferens.

Antagonist affinities

After a 1 h equilibration period, concentration-response curves were constructed by adding doses of the agonists cumulatively, according to the method of Van Rossum (1963). When these responses were constant, concentration-response curves were repeated in the presence of antagonists. At least three concentrations of antagonists with log intervals of 0.5 were tested 3 to 5 times (see Table 1) in the three tissues. Each concentration of antagonist was allowed to equilibrate for 15 to 30 min (ileum) and 30 min (atrium) in guinea-pig preparations, respectively, and 30 [(S)-isomers] to 60 min [(**R**)-isomers] in rabbit vas deferens. Preliminary experiments indicated that these intervals were sufficient for equilibration of the antagonist concentrations used. No preparation was exposed to more than three concentrations of antagonists. EC_{50} values of agonists in the absence and presence of antagonists were determined graphically for calculation of dose-ratios. The slopes of the Arunlakshana-Schild plots (Arunlakshana & Schild, 1959) were determined by linear regression by the method of least squares. pA_2 values were estimated as the intercept on the abscissa scale by fitting to the data the best straight line with a slope of unity (Tallarida *et al.*, 1979).

Data analysis

All data are presented as means ± s.e.mean of 9–17 experiments. Differences between mean values were tested for statistical significance by Student's *t* test; $P < 0.05$ was accepted as being significant. Linear regression analyses were carried out by the method of least squares (Tallarida *et al.*, 1979).

Drugs

Pirenzepine dihydrochloride was obtained from Boehringer Ingelheim (F.R.G.). 4-(4-Chlorophenylcarbamoyloxy)-2-butynyltrimethylammonium iodide (4-Cl-McN-A-343) (Nelson *et al.*, 1976), arecaidine propargyl ester (Mutschler & Hultzsch, 1973), racemic hexahydro-sila-difenidol hydrochloride (Tacke *et al.*, 1985), (**R**)- and (S)-hexahydro-difenidol hydrochloride [(**R**)-*1*·HCl and (S)-*1*·HCl] (Tacke *et al.*, 1989) as well as (**R**)- and (S)-hexbutinol [(**R**)-*2* and (S)-*2*] (Tacke *et al.*, 1989) were synthesized in our laboratories according to the literature. The enantiomeric excess (ee) of the enantiomers of hexahydro-difenidol and hexbutinol was >99.8%, determined by calorimetric analysis as described by Tacke *et al.* (1987). Racemic *p*-fluoro-hexahydro-sila-difenidol hydrochloride was prepared by analogy to the synthesis of hexahydro-sila-difenidol (Tacke *et al.*, unpublished results). The enantiomers of *p*-fluoro-hexbutinol [(**R**)-*4* and (S)-*4*; enantiomeric purity: ee > 99.8%; calorimetric analysis] were synthesized by analogy to (**R**)- and (S)-hexbutinol (Tacke *et al.*, 1989). The other chemicals not described under 'Synthetic chemistry' were of reagent grade and were used as purchased.

Synthetic chemistry

The enantiomers of hexbutinol methiodide [(**R**)-*3* and (S)-*3*] were prepared as follows:

Freshly distilled methyl iodide (18 mmol) was added under an atmosphere of dried nitrogen to a solution of (**R**)- or (S)-hexbutinol (*2*) (8 mmol) in dry ethanol (50 ml). The reaction mixture was stirred for 3 h at 30°C, dry n-pentane (100 ml) was added, and the mixture was stirred for 1 h at 20°C. Thereafter, the precipitate was collected by filtration and then recry-

Table 1 pA_2 values and slopes of Arunlakshana-Schild plots (in parentheses) for muscarinic antagonists at M_1, M_2 and M_3 receptors

Compound	*Rabbit vas deferens* (M_1)	*Guinea-pig atria* (M_2)	*Guinea-pig ileum* (M_3)
HHSiD[a]	7.92 ± 0.07	6.53 ± 0.05	7.96 ± 0.03
p-F-HHSiD[a]	6.68 ± 0.12	6.01 ± 0.06	7.84 ± 0.03
Hexahydro-difenidol (*1*)			
(R)-*1*	8.71 ± 0.05	7.03 ± 0.06[b]	8.35 ± 0.04[b]
	[*n* = 17; 6]	[*n* = 9; 8]	[*n* = 14; 11]
	(1.26 ± 0.09)*	(0.83 ± 0.30)	(0.92 ± 0.07)
(S)-*1*	5.97 ± 0.04	5.80 ± 0.07[b]	6.07 ± 0.05[b]
	[*n* = 13; 9]	[*n* = 9; 8]	[*n* = 9; 6]
	(0.87 ± 0.10)	(0.96 ± 0.19)	(0.87 ± 0.17)
Hexbutinol (*2*)			
(R)-*2*	8.78 ± 0.05	7.77 ± 0.04[b]	8.78 ± 0.04[b]
	[*n* = 13; 6]	[*n* = 10; 10]	[*n* = 13; 10]
	(1.16 ± 0.11)	(1.09 ± 0.07)	(1.10 ± 0.06)
(S)-*2*	6.75 ± 0.07	6.84 ± 0.05[b]	7.14 ± 0.04[b]
	[*n* = 17; 8]	[*n* = 9; 9]	[*n* = 10; 5]
	(0.84 ± 0.15)	(1.03 ± 0.13)	(0.99 ± 0.07)
Hexbutinol methiodide (*3*)			
(R)-*3*	9.43 ± 0.06	8.62 ± 0.02	8.85 ± 0.04
	[*n* = 9; 5]	[*n* = 13; 13]	[*n* = 17; 16]
	(0.92 ± 0.11)	(1.07 ± 0.03)	(1.14 ± 0.06)
(S)-*3*	7.83 ± 0.05	7.40 ± 0.02	7.26 ± 0.02
	[*n* = 12; 4]	[*n* = 12; 4]	[*n* = 12; 7]
	(0.92 ± 0.07)	(0.91 ± 0.04)	(0.96 ± 0.03)
p-Fluoro-hexbutinol (*4*)			
(R)-*4*	8.08 ± 0.06	6.97 ± 0.04	8.50 ± 0.04
	[*n* = 13; 5]	[*n* = 12; 12]	[*n* = 16; 8]
	(1.31 ± 0.10)*	(0.84 ± 0.06)	(0.84 ± 0.06)
(S)-*4*	6.55 ± 0.08	6.75 ± 0.05	7.57 ± 0.04
	[*n* = 17; 6]	[*n* = 9; 9]	[*n* = 12; 12]
	(0.85 ± 0.18)	(1.03 ± 0.09)	(0.99 ± 0.06)

[a] Data taken from Lambrecht *et al.* (1988a).
[b] Data taken from Tacke *et al.* (1989).
The parameters shown represent the mean ± s.e.mean. The slopes of Arunlakshana-Schild plots were determined by linear regression analysis (Tallarida *et al.*, 1979). pA_2 values were obtained after the unity constraint had been imposed. The numbers of total data points (*n*) and tissues used are given in square parentheses. The slopes shown are not significantly different from unity ($P > 0.05$), except those for (R)-*1* and (R)-*4* at M_1 receptors (marked with an asterisk).
HHSiD = racemic hexahydro-sila-difenidol; p-F-HHSiD = racemic *p*-fluoro-hexahydro-sila-difenidol.

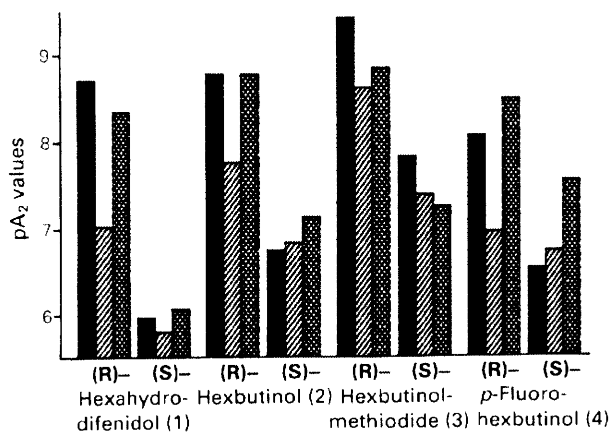

Figure 2 Affinity profiles of the enantiomers of compounds *1–4* at muscarinic M_1 receptors in rabbit vas deferens (solid columns), M_2 receptors in guinea-pig atria (diagonally-hatched columns) and M_3 receptors in guinea-pig ileum (cross-hatched columns).

Table 2 Receptor selectivity and stereoselectivity [(R)/(S)] ratios for chiral muscarinic antagonists

	Receptor selectivity			*Stereoselectivity*		
	M_1/M_2	M_3/M_1	M_3/M_2	M_1	M_2	M_3
Hexahydro-difenidol (*1*)						
(R)-*1*	48	0.44	21			
				550	17	191
(S)-*1*	1.5	1.3	1.9			
Hexbutinol (*2*)						
(R)-*2*	10	1.0	10			
				107	8.5	44
(S)-*2*	0.81	2.5	2.0			
Hexbutinol methiodide (*3*)						
(R)-*3*	6.5	0.26	1.7			
				40	17	39
(S)-*3*	2.7	0.27	0.72			
p-Fluoro-hexbutinol (*4*)						
(R)-*4*	13	2.6	34			
				34	1.7	8.5
(S)-*4*	0.63	10.5	6.6			

The values shown represent the antilogs of the differences between corresponding mean pA_2 values (Table 1) determined at M_1 receptors in rabbit vas deferens as well as at atrial M_2 and ileal M_3 receptors of guinea-pigs.

stallized from ethanol/diethylether. After drying the crystals *in vacuo*, analytically pure products were obtained [characterized by ^1H NMR, ^{13}C NMR, FAB MS measurements and calorimetric analysis (data not given) as well as by elemental analyses].

(R)-*3*: $C_{22}H_{32}INO$ (453.4), yield 92%, m.p. 174–175°C, $[\alpha]^{27}_{586} = -3$ (c = 0.5, $CHCl_3$), ee > 99.8%. Found: C, 58.3; H, 7.1; N, 2.9. Calculated: C, 58.28; H, 7.11; N, 3.09. (S)-*3*: $C_{22}H_{32}INO$ (453.4), yield 94%, m.p. 174–175°C, $[\alpha]^{27}_{586} = 3$ (c = 0.5, $CHCl_3$), ee > 99.8%. Found: C, 58.3; H, 7.1; N, 3.1. Calculated: C, 58.28; H, 7.11; N, 3.09.

Results

Twitch contractions of rabbit vas deferens elicited by electrical field stimulation were inhibited by the M_1 receptor agonist, 4-Cl-McN-A-343 (EC_{50} = 250 nM). This effect was concentration-dependently antagonized by the (R)- and (S)-enantiomers of compounds *1–4*. Similarly, all stereoisomers antagonized the negative inotropic responses in guinea-pig atria (EC_{50} = 7 nM) and ileal contractions (EC_{50} = 25 nM) of arecaidine propargyl ester. In the three tissues, parallel shifts of the agonists concentration-response curves without any appreciable changes of basal tension or reduction of maximum responses were obtained and Arunlakshana-Schild plots were linear through the concentration range tested for each antagonist, indicating competitive antagonism. Slopes (Table 1) were not significantly different from unity ($P > 0.05$), except for compounds (R)-*1* and (R)-*4* at M_1 receptors. The pA_2 values of (R)-*1* and (R)-*4* in rabbit vas deferens (8.71 and 8.08, respectively; Table 1) might therefore be regarded as a purely experimental quantity. However, the binding affinities of (R)-*1* and (R)-*4* to M_1 receptors in NB-OK 1 cells (pK_i = 8.6 and 8.1, respectively; M. Waelbroeck, unpublished results) were very similar to that obtained in rabbit vas deferens (Table 1). In these radioligand binding studies, competition curves with (R)-*1* and (R)-*4* did not deviate significantly from results expected for competitive inhibition of [^3H]-N-methyl scopolamine ([^3H]-NMS) binding.

The (R)- and (S)-enantiomers of compounds *1–4* showed quite wide variations in their affinities for the muscarinic receptor subtypes, their pA_2 values differing by more than three orders of magnitude (Table 1). Introduction of a triple bond into the basic side chain of the parent compound

hexahydro-difenidol (*1*) (→hexbutinol, *2*), quaternization of *2* (→hexbutinol methiodide, *3*) and *p*-fluoro-substitution of the phenyl ring of *2* (→*p*-fluoro-hexbutinol, *4*) changed the affinities of these compounds for M_1, M_2 and M_3 receptors differently. Thus compounds with qualitatively and/or quantitatively different receptor selectivity profiles were obtained (Table 2 and Figure 2).

At each of the three muscarinic receptor subtypes, the (**R**)-enantiomer of compounds *1–4* was more potent than the (S)-configurated isomer. The difference in potencies between the enantiomers of compounds *1–4* was greatest at the M_1, less at M_3 and least at M_2 receptors (Table 2). The degree of stereoselectivity (Table 2) was also dependent on the structure of the compounds (M_1 and M_3: $1 > 2 > 3 > 4$; M_2: $1 = 3 > 2 > 4$).

Discussion

Structural variations in the (**R**)- and (S)-hexahydro-difenidol (*1*) molecules led to muscarinic antagonists that exhibited a qualitatively or quantitatively different spectrum of receptor selectivity to the parent stereoisomers (**R**)-*1* and (S)-*1* (Table 2, Figure 2). These observed selectivities did not appear to be associated in general with high affinity and absolute configuration (Figure 2). For example, (S)-*p*-fluoro-hexbutinol [(S)-*4*] was a relatively weak compound but it had a novel receptor selectivity profile: $M_3 > M_2 \geqslant M_1$.

The results of the present study confirm and extend previous findings that rabbit vas deferens (M_1 receptors) as well as guinea-pig atria (M_2 receptors) and ileum (M_3 receptors) possess different muscarinic receptor subtypes (Eltze, 1988; Eltze *et al.*, 1988; Lambrecht *et al.*, 1988a; Waelbroeck *et al.*, 1989). Furthermore, our findings provide additional evidence that these subtypes can be identified on the basis of their stereoselectivity. There appears to be a consistent trend showing that the degree of stereoselectivity is always greatest at M_1, intermediate at M_3 and lowest at M_2 receptors (Lambrecht & Mutschler, 1986; Tacke *et al.*, 1986; Eltze & Figala, 1988; Lambrecht *et al.*, 1988b; 1989a,c,d).

Receptor selectivity of (R)-enantiomers

(**R**)-Hexahydro-difenidol [(**R**)-*1*] was found to be a potent muscarinic antagonist exhibiting high affinity for M_1 receptors in rabbit vas deferens as well as for M_3 receptors in guinea-pig ileum, whereas its affinity for M_2 receptors in guinea-pig atria was lower by factors of 48 and 21, respectively (Table 2, Figure 2). The resulting affinity profile of (**R**)-*1* ($M_1 \geqslant M_3 > M_2$) is qualitatively very similar to that found for the racemic hexahydro-difenidol (Waelbroeck *et al.*, 1989) and its racemic silicon analogue hexahydro-sila-difenidol (Table 1).

The introduction of a triple bond into the (**R**)-hexahydro-difenidol molecule [→(**R**)-hexbutinol; (**R**)-*2*] increased the affinity for M_3 and M_2 receptors by factors of 2.7 and 5.5, respectively (Table 1), whereas the affinity for M_1 receptors was not significantly different. Thus, (**R**)-hexbutinol shows a receptor selectivity profile that is qualitatively similar to that of the parent compound (**R**)-hexahydro-difenidol [(**R**)-*1*]. However, the selectivity for M_1 and M_3 over M_2 receptors is lower than that of (**R**)-*1*. This might be explained by differences in the electronic structures and/or the conformational behaviour of (**R**)-hexahydro-difenidol and (**R**)-hexbutinol. The triple bond in (**R**)-hexbutinol should make it more difficult for this molecule to adopt the active conformation at all subtypes, thus creating selectivity (Barlow *et al.*, 1988). On the other hand, the carbon-carbon triple bond might contribute to affinity and thus counteract any selectivity creating effect of rigidity.

N-methylation of (**R**)-hexbutinol [→(**R**)-hexbutinol methiodide; (**R**)-*3*] increased the affinity for M_1 receptors in rabbit vas deferens, as well as for M_2 receptors in guinea-pig atria by factors of 4.5 and 7.1, whereas the affinity for M_3 receptors in guinea-pig ileum was not affected (Table 1). Thus, N-methylation of the tertiary amine (**R**)-*2* changed the receptor selectivity pattern. The following affinity rank order for (**R**)-hexbutinol methiodide [(**R**)-*3*] was observed: $M_1 > M_3 \geqslant M_2$ (Figure 2).

A comparison of the antimuscarinic potencies of (**R**)-*p*-fluoro-hexbutinol [(**R**)-*4*] and (**R**)-hexbutinol [(**R**)-*2*] outlines the effect of fluoro-substitution in the phenyl ring on antimuscarinic potency. The *p*-fluoro substituent reduced the affinity for the muscarinic receptors up to 6 fold (Table 1). This decrease in affinity was the least pronounced at the ileal M_3 receptors. Thus, as a result (**R**)-*p*-fluoro-hexbutinol [(**R**)-*4*] showed a small preference for M_3 over M_1 receptors, but the selectivity of M_3 over M_2 receptors was enhanced (Table 2, Figure 2).

Receptor selectivity of (S)-enantiomers

In general, the (S)-enantiomers of compounds *1–4* were less potent than the corresponding (**R**)-configurated isomers (Table 1, Figure 2). However, introduction of a triple bond into the hexahydro-difenidol molecule (*1*) as well as quaternization and fluoro-substitution of hexbutinol (*2*) had different effects on affinity for the (S)-isomers in comparison to the (**R**)-enantiomers at the three muscarinic receptor subtypes. Thus, the (S)-enantiomers showed affinity profiles which were qualitatively and/or quantitatively different from those obtained for the (**R**)-isomers.

(S)-Hexahydro-difenidol [(S)-*1*] was a very weak muscarinic antagonist showing no muscarinic receptor selectivity (Table 2, Figure 2). Introduction of a triple bond into (S)-hexahydro-difenidol [→(S)-hexbutinol; (S)-*2*] increased the affinity for M_1, M_2 and M_3 receptors by factors of 6, 11 and 12, respectively (Table 1). Thus, (S)-hexbutinol [(S)-*2*] showed at most a 2 fold preference for the ileal M_3 receptors (Table 2, Figure 2). This is different to the situation of the (**R**)-enantiomers.

N-methylation of (S)-hexbutinol [(S)-*2*] increased the affinity for M_1 and M_2 receptors 12 and 3.6 fold, respectively, whereas the affinity for ileal M_3 receptors was nearly unchanged (Table 1). Thus the receptor selectivity profile of (S)-hexbutinol methiodide [(S)-*3*] is slightly different from that obtained for the (**R**)-enantiomer (Figure 2).

The influence of fluoro-substitution on potency and selectivity is demonstrated by comparison of (S)-hexbutinol [(S)-*2*] and (S)-*p*-fluoro-hexbutinol [(S)-*4*] (Table 1). Compared to (S)-*2* the fluoro derivative (S)-*4* exhibited a relatively higher affinity ($pA_2 = 7.57$) for M_3 receptors, whereas its antimuscarinic potency at M_1 and M_2 receptors was lower by factors of 10.5 and 6.6, respectively (Table 1). Thus, fluoro-substitution in the *para*-position of the phenyl ring of (S)-hexbutinol [(S)-*2*] enhanced its M_3-selectivity. The receptor selectivity profile of (S)-*p*-fluoro-hexbutinol ($M_3 > M_2 \geqslant M_1$) is also different from that of the corresponding (**R**)-enantiomer ($M_3 \geqslant M_1 > M_2$) and of *p*-fluoro-hexahydro-sila-difenidol ($M_3 > M_1 > M_2$; Table 1).

Stereoselectivity of muscarinic receptors

It has been suggested (Pfeiffer, 1956) that, with greater potencies of drugs, larger differences in pharmacological effects will be seen between the enantiomers of chiral compounds. The results obtained in this study (Table 1 and 2) do not substantiate the above suggestion and its implications (Lehmann, 1986). For example, at M_1 receptors the affinity constants (pA_2 values) of the more potent (**R**)-enantiomers decrease in the order (**R**)-hexbutinol methiodide (9.43) > (**R**)-hexbutinol (8.78) = (**R**)-hexahydro-difenidol (8.71) > (**R**)-*p*-fluoro-hexbutinol (8.08), whereas the stereoselectivity ratios [(**R**)/(S)] of these chiral compounds are in the order hexahydro-difenidol (550) > hexbutinol (107) > hexbutinol methiodide (40) > *p*-fluoro-hexbutinol (34). Thus, in this series of compounds the stereoselectivity ratio at, e.g. M_1 receptors,

was higher by more than one order of magnitude for hexahydro-difenidol (*1*) than for hexbutinol methiodide (*3*), although compound (**R**)-*3* was 5 fold more potent than (**R**)-*1*. A similar lack of correlation between potency of the eutomer and stereoselectivity ratios of enantiomers was obtained at M_2 and M_3 receptors (Tables 1 and 2). The findings of the present study confirm and extend previous results obtained with the enantiomers of biperiden (Eltze & Figala, 1988), trihexyphenidyl (Lambrecht *et al.*, 1988b; 1989d) and phenglutarimide (Lambrecht *et al.*, 1989a).

In 1982, Robert *et al.* stated a corollary of Pfeiffer's rule: "When different receptor subtypes interact with the enantiomers of chiral drugs, their stereoselectivity should increase as a function of affinity of the more potent enantiomer (= eutomer; Lehmann, 1986) for the respective subtypes". However, when the magnitude of receptor subtype stereoselectivity (difference in pA_2 values of the (**R**)- and (**S**)-enantiomers = eudismic index; Lehmann, 1986) was plotted against the pA_2 value of the more potent isomer for that particular receptor subtype, a strong correlation (correlation coefficient $r = 0.995$) was only observed for hexahydro-difenidol (Figure 3). The stereoselectivity and the affinity of the eutomer of hexahydro-difenidol was greatest at M_1, intermediate at M_3 and lowest at M_2 receptors. On the other hand the enantiomers of compounds *2–4* did not fulfill the predictions made by Robert *et al.* (1982). However, the interesting finding of this study is that the stereoselectivity ratios of all the chiral compounds *1–4* consistently show the same order: $M_1 > M_3 > M_2$. This implies that the stereochemical demands made by the muscarinic receptor subtypes are different for the enantiomers of compounds *1–4* being most stringent at M_1 receptors. Similar results have been obtained with the enantiomers of telenzepine (Eveleigh *et al.*, 1989), biperiden (Eltze & Figala, 1988), trihexyphenidyl and its methiodide (Lambrecht *et al.*, 1988b) and procyclidine (Lambrecht & Mutschler, 1986; Waelbroeck *et al.*, 1988b).

In conclusion, the present study shows that the antimuscarinic potencies and receptor subtype selectivities of the enantiomers of hexahydro-difenidol (*1*) and the acetylenic analogues *2–4* (Figure 1) depend on different structural parameters including absolute configuration. The M_1, M_2 and M_3 receptors make qualitatively and quantitatively different stereochemical demands for the (**R**)- and (**S**)-enantiomers, resulting in different receptor selectivity profiles. It is interesting to note that, of the enantiomers investigated in this study, (**S**)-*p*-fluoro-hexbutinol shows a novel receptor selectivity profile: $M_3 > M_2 \geqslant M_1$ (Figure 2). There was a variation in stereoselectivity ratios on the three receptor subtypes (Table 2): $M_1 > M_3 > M_2$. These results indicate that stereoselectivity ratios can be successfully used as a parameter to characterize muscarinic receptor subtypes providing information that racemates cannot give. However, the stereoselectivity ratios do not conform to the predictions of the 'classical' Pfeiffer's rule (Pfeiffer, 1956), and that of its corollary (Robert *et al.*, 1982).

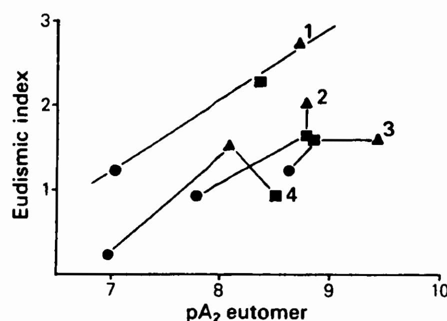

Figure 3 Plot of receptor stereoselectivity [eudismic index (EI) = difference of pA_2 values of the (**R**)- and (**S**)-enantiomers; Lehmann, 1986] versus pA_2 values of the more potent isomer [eutomer (EU)] for compounds *1–4* (see Figure 1) at muscarinic M_1 receptors in rabbit vas deferens (▲) as well as at M_2 (●) and M_3 receptors (■) in guinea-pig atria and ileum. A strong linear correlation was only observed for compound *1*: EI = 0.87 (±0.08) pA_2^{EU} − 4.91 (±0.68), $r = 0.995$, s.d. = 0.182, $n = 3$. The correlation coefficient of 0.995 is significant at the $P < 0.05$ level.

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