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Characterization of the interaction of zamifenacin at muscarinic receptors in vitro

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Abstract

The interaction of zamifenacin ((3*R*)-(+)-diphenylmethoxy-1-(3,4)-methylenedioxyphenethyl)piperidine) at muscarinic receptor subtypes was studied using radioligand binding and functional techniques, in vitro. In radioligand binding studies, zamifenacin acted as a competitive antagonist, with the following pK_i values; rat cerebral cortex (M_1) 7.90 ± 0.08, myocardium (M_2) 7.93 ± 0.13, submaxillary gland (M_3) 8.52 ± 0.04 and rabbit lung (M_4) 7.78 ± 0.04. In functional studies zamifenacin acted as a surmountable antagonist, exhibiting the following apparent affinity values; canine saphenous vein (putative M_1) 7.93 ± 0.09, guinea-pig left atria (M_2) 6.60 ± 0.04, guinea-pig ileum (M_3) 9.31 ± 0.06, guinea-pig oesophageal muscularis mucosae (M_3) 8.84 ± 0.04, guinea-pig trachea (M_3) 8.16 ± 0.04, and guinea-pig urinary bladder (M_3) 7.57 ± 0.15. Therefore, zamifenacin is selective for muscarinic M_3 receptors in guinea-pig ileum, oesophageal muscularis mucosae, trachea and bladder over muscarinic M_2 receptors in atria. The degree of muscarinic M_3/M_2 receptor selectivity depends upon the muscarinic M_3 receptor preparation studied.

Keywords: Zamifenacin; Muscarinic receptor subtype

1. Introduction

Molecular biological techniques have identified five distinct, but related, genes encoding for muscarinic receptors. Four of these genes m1–m4 have been shown to encode for the four pharmacologically characterised M_1–M_4 receptors (see Hulme et al., 1990; Hosey, 1992; Caulfield, 1993 for reviews). Pharmacological characterisation of muscarinic receptors is hampered by a lack of ligands selective for any one receptor subtype over all others (Dörje et al., 1991). Nonetheless, several antagonists have been identified with high apparent affinity at muscarinic M_3 receptors and relatively low affinities at muscarinic M_2 receptors, including 4-DAMP (4-diphenylacetoxy-*N*-methylpiperidine methiodide, Barlow et al., 1976), DAC 5945 (Micheletti et al., 1990), *p*-F-HHSiD (*para*-fluorohexahydrosiladifenidol; Lambrecht et al., 1988) and UH-AH 37 (Wess et al., 1991). Zamifenacin (UK 76,654-42, (3*R*)-(+)-diphenylmethoxy-1-(3,4)-methylenedioxyphenethyl)piperidine; Fig. 1) has also been shown to antagonise selectively muscarinic M_3 receptors over muscarinic M_2 receptors in vitro (Wallis et al., 1993) and in vivo (Quinn et al., 1993; Wallis, 1995), inhibiting gastrointestinal motility in dogs at doses that do not effect heart rate or pupil diameter (McRitchie et al., 1993). In man zamifenacin has been shown to inhibit small bowel and colonic motility in a dose-dependent fashion with little or no side effects (Wallis, 1995).

Assessment of the selectivity of novel muscarinic receptor antagonists, such as zamifenacin, for muscarinic M_3 receptors over muscarinic M_1, M_2, and M_4 receptors may depend upon the choice of bioassay employed to estimate functionally the apparent affinity. For example, the pA_2 value at guinea-pig ileal muscarinic M_3 receptors is 9.1 whereas that at guinea-pig tracheal muscarinic M_3 receptors is 8.1 (Wallis et al., 1993). This does not appear to be unique to zamifenacin, since similar differences in pA_2 values between ileal and tracheal muscarinic M_3 receptors have also been seen with *p*-F-HHSiD (Eglen et al., 1990a; Roffel et al., 1994). The reasons for these apparent differ-

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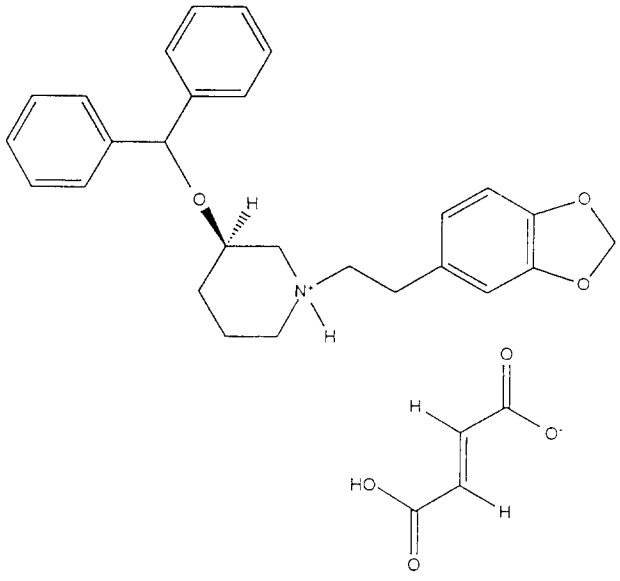

Fig. 1. Structure of zamifenacin fumarate.

ences in affinity are presently unclear although, in the case of *p*-F-HHSiD, the affinity estimation was not compromised by conditions of disequilibrium (Eglen et al., 1990b). The muscarinic M_1 receptor affinity for zamifenacin previously reported (7.4, Wallis et al., 1993) was established on electrically evoked contractions of rabbit isolated vas deferens. The prejunctional muscarinic receptors mediating inhibition of these electrically evoked contractions shows a high apparent affinity for pirenzepine and consequently has been taken to reflect activation of muscarinic M_1 receptors (Eltze, 1988, 1994; Eltze et al., 1988). However, this receptor exhibits high pA_2 values toward both pirenzepine (7.8, 8.1) and himbacine (8.0, 8.1 from Eltze et al., 1988 and Sagrada et al., 1994, respectively), which suggests a profile consistent with activation of muscarinic M_4 receptors (Caulfield, 1993).

The aim of the present study was to characterise the interaction of zamifenacin at muscarinic receptors, using both radioligand binding (M_1–M_4) and functional (M_1–M_3) assays. Since initial reports (Wallis et al., 1993) indicated variability in the affinity of zamifenacin in different muscarinic M_3 receptor preparations, several guinea-pig M_3 receptor preparations have been included in this study. Additionally, with the doubts surrounding the validity of the isolated rabbit vas deferens as a muscarinic M_1 receptor preparation (Caulfield, 1993), an M_1 receptor affinity estimate has been made in the present study, in canine saphenous vein (Eglen et al., 1990a). The data obtained support the conclusions of Wallis et al. (1993) and Wallis (1995) that zamifenacin is a selective muscarinic M_3 receptor antagonist, which exhibits differential apparent affinity values at a variety of muscarinic M_3 receptor populations in smooth muscle tissues. A preliminary account of this work has been presented elsewhere (Eglen et al., 1994).

2. Materials and methods

2.1. Radioligand binding studies

Binding studies to M_1, M_2, M_3 and putative M_4 sites in rat (Sprague-Dawley, 200–250 g) cerebral cortex, myocardium, submaxillary gland and rabbit (New Zealand White, 2–3 kg) lung, respectively, used EDTA-washed membranes (Cheung et al., 1982; Table 1). Competition binding studies in the presence of radioligand, zamifenacin and membranes were carried out in assay buffer (50 mM Tris, 0.5 mM EDTA, pH 7.4) in a final volume of 1.25 ml for 120 min at 32°C. Muscarinic M_1 binding sites were labelled with [^{3}H]pirenzepine (0.5 nM) and the remaining sites were labelled with [^{3}H]-*N*-methylscopolamine (0.1 nM).

Table 1
Preparations employed in the present study

Preparation	Receptor	Reference	Buffer	Temperature
Radioligand binding studies				
Rat cerebral cortex	M_1	Delmendo et al. (1989)		
Rat myocardium	M_2	Delmendo et al. (1989)		
Rat submaxillary gland	M_3	Delmendo et al. (1989)		
Rabbit lung	M_4	Lazareno et al. (1990)		
Functional studies				
Canine saphenous vein	M_1	Eglen et al. (1990a)	Krebs	37°C
Guinea-pig left atria [a]	M_2	Blinks (1966)	Krebs	32°C
Guinea-pig ileum	M_3	Clague et al. (1985)	Tyrode	37°C
Guinea-pig OMM [b]	M_3	Kamikawa et al. (1985)	Krebs	37°C
Guinea-pig trachea	M_3	Watson and Eglen (1994)	Krebs	37°C
Guinea-pig bladder [c]	M_3	see Methods	Krebs	37°C

[a] Electrically paced (5 Hz, 1.2 times threshold voltage, 5 ms duration). [b] Oesophageal muscularis mucosae of the guinea-pig. [c] Urinary bladder ring preparations.

Bound ligand was separated from free ligand by vacuum filtration over GF/B filtermats (pretreated with 0.1% polyethylene imine solution for 24 h) using a Brandel cell harvester. Non-specific binding was defined using atropine (1 μM).

2.2. Functional studies

Tissues were isolated from male, Dunkin-Hartley guinea-pigs (300–400 g) previously killed by CO_2 asphyxiation or female, mongrel dogs (8–12 kg) anaesthetized with sodium pentobarbital and exsanguinated. The tissues and physiological salt solutions used (see below for composition), with the exception of the urinary bladder, have been described previously (Table 1). Urinary bladder was isolated and a transverse ring (2 mm wide) was cut from the area of greatest circumference. Rings were then mounted in modified Krebs solution under 1 g resting tension.

All prepartions were equilibrated for 60 min, after which time concentration-effect curves to the non-selective muscarinic agonist (+)-*cis*-dioxolane, were constructed in a cumulative fashion, except in ileum and bladder preparations. The non-cumulative technique was used in these two tissues using a 30 s agonist exposure every 5 min or every 2 min for ileum and bladder, respectively. In isolated trachea, oesophageal muscularis mucosae, ileum, urinary bladder and atria, concentration-effect curves to (+)-*cis*-dioxolane were established first in the absence and then after a 60 min equilibration with zamifenacin. Each preparation was exposed to only one concentration of zamifenacin. In isolated saphenous vein, one concentration-effect curve was established on each preparation, since temporal changes in sensitivity to the agonist precluded construction of consecutive concentration-effect curves.

To assess the nature of the interaction between zamifenacin and the tracheal muscarinic M_3 receptor, the combination-ratio method of Paton and Rang (1965) was used. Portions of trachea were studied in parallel and after construction of the control curve, tissues were equilibrated with either atropine (10 nM), zamifenacin (0.1 μM) or both for a period of 60 min and a second concentration-effect curve was then established.

2.3. Measurement and analysis of results

In binding studies, the concentration of zamifenacin that elicited 50% displacement of specific binding (IC_{50}) was calculated by non-linear iterative curve fitting procedures and the apparent affinity was then determined by the method of Cheng and Prusoff (1973). In functional studies, tissue responses were measured as changes in isometric tension using a Grass FT04 force displacement transducer and were displayed on a Grass 79B polygraph recorder. Agonist potency (pD_2) was calculated according to the relationship of Parker and Waud (1971) using non-linear iterative curve fitting procedures (Leung et al., 1992). Apparent antagonist affinities were established by determination of the pA_2 value using the method of Arunlakshana and Schild (1959). When the Schild slope was not significantly different from unity, the pA_2 value quoted is that obtained when the slope was constrained to unity. In experiments using urinary bladder and saphenous vein, the apparent affinity of zamifenacin was estimated using a single concentration of antagonist (pK_B; Furchgott, 1972).

In all studies statistically significant differences were assessed by Student's t-test, with $P < 0.05$ being considered significant. All values quoted are the means ± S.E.M. from at least six animals unless otherwise stated.

2.4. Physiological salt solutions (mM)

Modified Krebs: NaCl 118.2, KCl 4.6, $MgSO_4$ 1.2, $CaCl_2$ 2.5, KH_2PO_4 1.2, $NaHCO_3$ 24.8, glucose 10.0. *Tyrode*: NaCl, 136.9, KCl 2.7, $MgCl_2 \cdot 7H_2O$ 1.1, $CaCl_2$ 1.8, $NaH_2PO_4 \cdot 2H_2O$ 0.4, $NaHCO_3$ 11.9, glucose 5.6. These physiological salt solutions were gassed with 5% CO_2-95% O_2 (pH 7.4) and maintained at the temperatures indicated in Table 1. Indomethacin (1.0 μM) was present in studies with bladder, oesophagus and trachea to inhibit prostaglandin-induced tone and tetrodotoxin (0.1 μM) was present in studies with bladder, oesophagus, saphenous vein and trachea to ensure that contractions were the result of activation of post-junctional receptors.

2.5. Compounds used

[^{3}H]Pirenzepine (specific activity 70–87 Ci mmol^{-1}) and [^{3}H]-*N*-methylscopolamine (specific activity 60–85 Ci mmol^{-1}) were purchased from New England Nuclear (Boston, MA, USA) and Amersham (Arlington Heights, IL, USA), respectively. Atropine and (+)-*cis*-dioxolane (L-(+)-*cis*-2-methyl-4-trimethylammonium methyl-1,3-dioxolane iodide, a 60:40 mixture of *cis:trans*) were purchased from Research Biochemicals (Natick, MA, USA). Zamifenacin ((3*R*)-(+)-diphenylmethoxy-1-(3,4)-methylenedioxyphenethyl)piperidine fumarate) was generously provided by Pfizer (Sandwich, UK).

Indomethacin was prepared as a 1 mg ml^{-1} solution in propylene glycol and solubilized by a brief period (2–3 min) of sonication. Tetrodotoxin was prepared as a 1.0 mM solution in 0.01 M acetic acid. A 10 mM solution of zamifenacin was prepared using ethanol and dilutions were made using distilled water. All other solutions were prepared in distilled water.

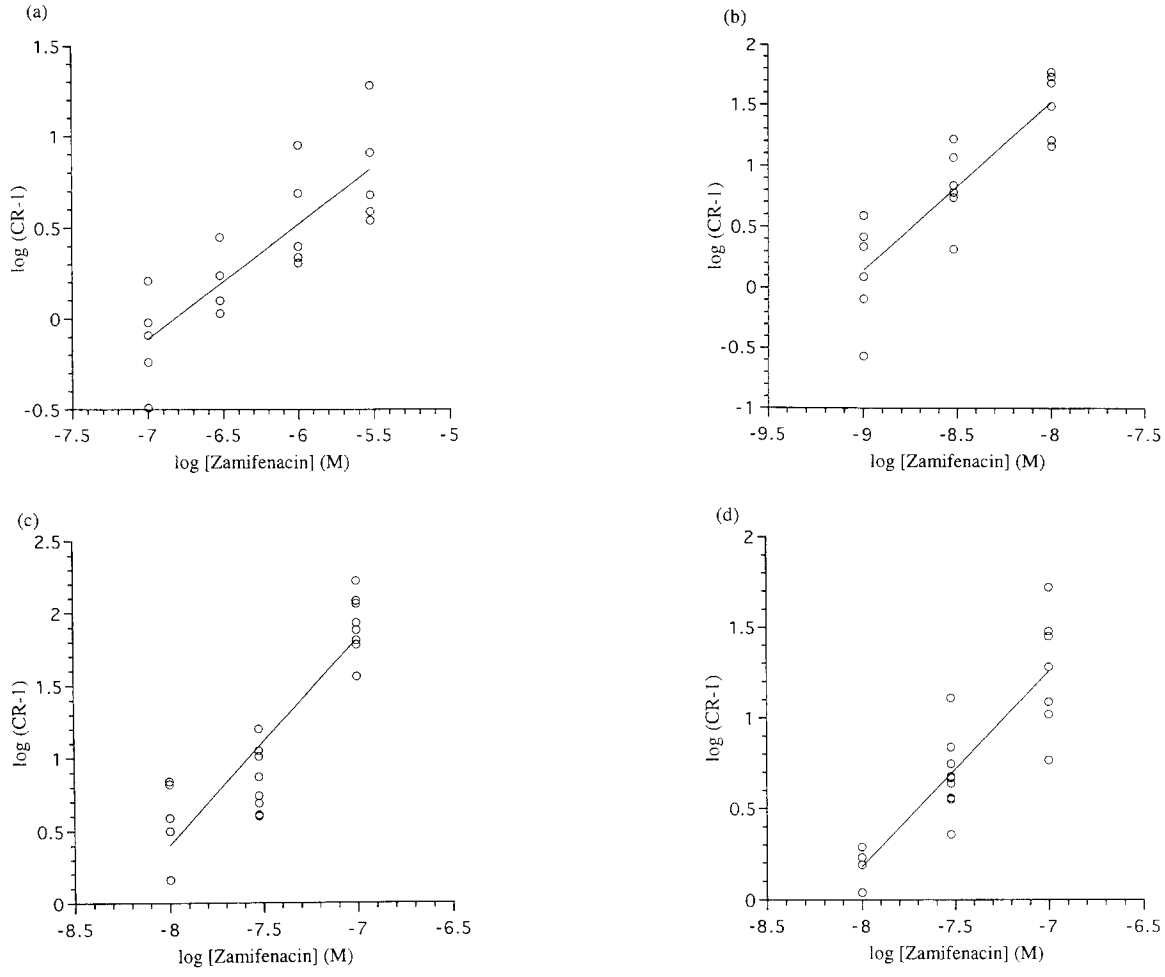

Fig. 2. Schild plots for the interaction of zamifenacin at guinea-pig (a) atria, (b) ileum, (c) oesophageal muscularis mucosae and (d) trachea.

3. Results

3.1. Radioligand binding studies

The affinities and Hill coefficients for displacement of radioligand by zamifenacin are shown in Table 2. The rank order of affinities was rat submaxillary gland (M_3) > rat cortex (M_1) = rat myocardium (M_2) > rabbit lung (M_4). These data showed upto a 4-fold selectivity of zamifenacin for muscarinic M_3 receptors in rat submaxillary gland over M_2 receptors in rat myocardium.

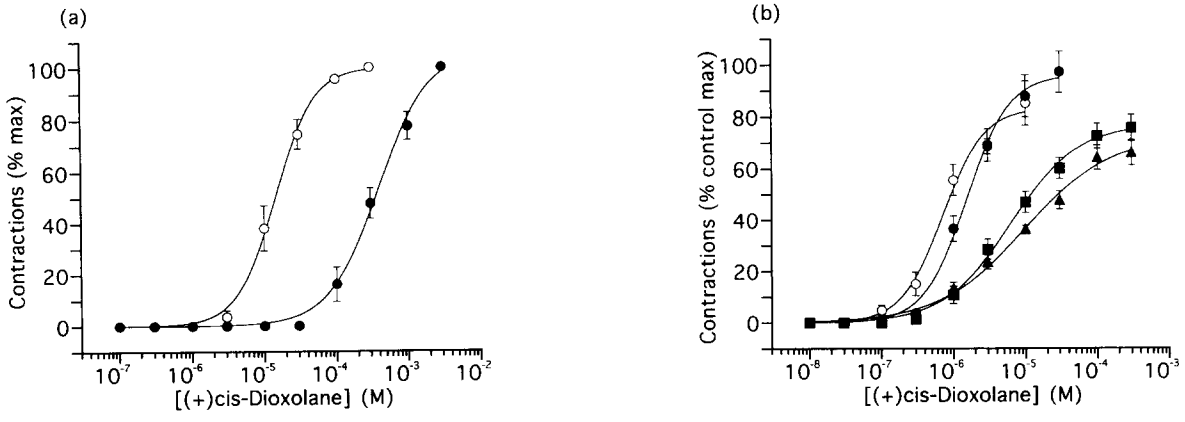

Fig. 3. Concentration-effect curves to (+)-*cis*-dioxolane in (a) canine saphenous vein in the absence (○) and presence (●) of zamifenacin (0.3 μM) and (b) guinea-pig urinary bladder, in the absence (○) and presence of zamifenacin 30 nM (●), 0.1 μM (■) and 0.3 μM (▲). Values are the means ± S.E.M. $n = 4–6$ animals.

Table 2
Apparent affinities (pK_i) and Hill coefficients (nH) for zamifenacin at muscarinic binding sites in various tissues

Preparation	pK_i	nH
Rat cerebral cortex	7.90 ± 0.08	0.96 ± 0.05
Rat myocardium	7.93 ± 0.13	0.92 ± 0.08
Rat submaxillary gland	8.52 ± 0.04	1.05 ± 0.06
Rabbit lung	7.78 ± 0.04	1.12 ± 0.11

Values are means ± S.E.M. $n = 4$ determinations.

3.2. Functional studies

The non-selective muscarinic agonist, (+)-*cis*-dioxolane, caused concentration-dependent increases in isometric tension in guinea-pig ileum, oesophageal muscularis mucosae, urinary bladder, trachea and canine saphenous vein. In guinea-pig paced left atria (+)-*cis*-dioxolane caused a concentration-dependent decrease in isometric tension (negative ionotropic response). (+)-*cis*-Dioxolane was most potent at muscarinic receptors mediating contraction of ileum and trachea and least potent at receptors mediating contraction of saphenous vein (Table 3).

Zamifenacin caused parallel rightward shifts in the concentration-effect curves to (+)-*cis*-dioxolane in all tissues studied, but had no significant effect alone in any of the preparations, at any of the concentrations tested. There was no significant difference in the maximal response to (+)-*cis*-dioxolane in the presence of zamifenacin (3 nM to 3 μM), with the exception of 0.1 and 0.3 μM zamifenacin in urinary bladder (see below). Schild analysis of data from guinea-pig ileum, oesophageal muscularis mucosae, atria and trachea revealed slopes not significantly different from unity (Fig. 2). Affinity estimates for zamifenacin in saphenous vein and urinary bladder (Table 3) were determined using single concentrations of zamifenacin (pK_B), which produced parallel shifts in the concentration-effect curves to (+)-*cis*-dioxolane with no significant change in maximum responses (Fig. 3). In urinary bladder high concentrations of zamifenacin (0.1 and 0.3 μM) produced pK_B values of 7.77 ± 0.13 and 7.56 ± 0.14, respectively, but these concentrations were associated with significant reductions in the maximum response to (+)-*cis*-dioxolane (Fig. 3).

Table 3
Potency (pD_2) of (+)-*cis*-dioxolane and apparent affinity values (pA_2) for zamifenacin in various tissues

Preparation	(+)-*cis*-Dioxolane	Zamifenacin	
	(pD_2)	(pA_2)	pA_2 [a]
Canine saphenous vein	5.15 ± 0.29	7.93 ± 0.09 [b]	nd
Guinea-pig left atria [c]	7.30 ± 0.06	6.60 ± 0.04	7.1
Guinea-pig leum	7.77 ± 0.11	9.31 ± 0.06	9.1
Guinea-pig OMM [d]	7.40 ± 0.06	8.84 ± 0.04	nd
Guinea-pig trachea	7.91 ± 0.07	8.16 ± 0.04	8.1
Guinea-pig bladder	6.32 ± 0.06	7.57 ± 0.15 [b]	nd

Values are the means ± S.E.M. $n = 5$–9. The apparent affinity values quoted are those where the slope of the Schild plot, not being significantly different from unity, was constrained to unity. [a] Data from Wallis et al. (1993). [b] Data from saphenous vein and urinary bladder represent the apparent affinity estimate obtained using single concentrations of zamifenacin (0.3 μM and 30 nM, respectively). [c] Electrically paced (5 Hz, 1.2 times threshold voltage, 5 ms duration). [d] Oesophageal muscularis mucosae.

In terms of the apparent antagonist affinity values, for zamifenacin at the tissues studied, the rank order was ileum > oesophageal muscularis mucosae > trachea > saphenous vein > urinary bladder > atria (Table 3). The muscarinic M_3/M_2 selectivity of zamifenacin was dependent upon the smooth muscle studied (i.e. ileum 502-fold, oesophageal muscularis mucosae 160-fold, trachea 40-fold and urinary bladder 10-fold, over atrial M_2 receptors).

The nature of the interaction of zamifenacin at tracheal muscarinic M_3 receptors was investigated using combination studies with atropine and zamifenacin. Atropine (10 nM) produced a concentration-ratio (CR_1) of 10.7 ± 2.3 and zamifenacin (0.1 μM) produced a concentration-ratio (CR_2) of 12.7 ± 3.1. Together zamifenacin (0.1 μM) and atropine (10 nM) caused a combined concentration-ratio (CR_3) of 21.5 ± 2.9. This value was not significantly different from the predicted concentration-ratio for two competitive antagonists interacting at the same site ($CR_3 = [CR_1 + CR_2] - 1$).

4. Discussion

This study has assessed the interaction of zamifenacin, a novel selective muscarinic M_3 receptor antagonist (Wallis et al., 1993; Wallis, 1995), at muscarinic receptors using both radioligand binding and functional techniques. This evaluation has included a range of muscarinic M_3 receptor preparations and has establish an affinity estimate for zamifenacin at muscarinic M_1 receptors in canine saphenous vein.

In radioligand binding studies, the displacement isotherms were best described by Hill slopes of unity and are therefore consistent with an interaction at a homogeneous population of receptors. The absolute pK_i value obtained at M_1 receptors in the binding studies was similar to the affinity values estimated functionally in canine saphenous vein (7.9). The absolute pK_i value obtained at M_3 receptors in binding studies in rat submaxillary gland (8.5) was in agreement with those determined functionally in guinea-pig trachea (8.2) and oesophagus (8.8), however, this was not the case with all the estimates obtained functionally in the other muscarinic M_3 receptor preparations, notably ileum and bladder. Discrepancies between values

estimated functionally at muscarinic M_3 receptors in smooth muscle and those estimated in binding studies with submaxillary glands have also been reported by others (Nilvebrandt and Sparf, 1986; Kunysz et al., 1989; see Hulme et al., 1990 for review). Additionally, binding studies in guinea-pig parotid gland using zamifenacin revealed an affinity at these M_3 receptors which is similar to that described here functionally in guinea-pig trachea (8.0; Wallis, 1995). A discrepancy was also observed between binding and functional studies at muscarinic M_2 receptors (rat myocardial $pK_i = 7.9$ and guinea-pig atrial $pA_2 = 6.6$). Although differences in ionic strength of buffers may influence antagonist apparent affinity at M_2 receptors (Hulme et al., 1990; Caulfield, 1993), the two apparent affinity values determined here, differed to a greater degree than that usually found (see Hulme et al., 1990 for review). Similar discrepancies have been noted with the muscarinic M_2 receptor antagonist, methoctramine, in these preparations (rat myocardium $pK_i =$ 8.8, Michel and Whiting, 1988; and guinea-pig atria $pA_2 = 7.9$, Melchiorre et al., 1987 and Eglen et al., 1988), while the muscarinic M_1 receptor antagonist, pirenzepine shows better agreement (rat myocardium $pK_i = 6.7$, Michel and Whiting, 1988; and guinea-pig atria $pA_2 = 6.9$, Eglen et al., 1992). The reason for the discrepancies is presently unknown, however, a consequence is that an estimation of the M_3/M_2 selectivity from binding studies is lower than that noted in the functional studies (see below).

In the present functional studies (+)-*cis*-dioxolane was used as the muscarinic agonist, while carbachol and acetylcholine were used in the earlier report (Wallis et al., 1993). The general agreement of our apparent affinity values with those of Wallis et al. (1993) supports the independence of the affinity of zamifenacin with regard to agonist structure. Zamifenacin acted in a surmountable fashion at all muscarinic receptors studied, with the exception of muscarinic M_3 receptors in guinea-pig urinary bladder. In that preparation, maximal responses were reduced in the presence of high concentrations of zamifenacin. Schild slopes in experiments using guinea-pig atria, trachea, oesophageal muscularis mucosae and ileum were not significantly different from unity. These data are consistent with (though not proof of) a competitive interaction of zamifenacin at these muscarinic M_2 and M_3 receptors. Conversely, in bladder at high concentrations of zamifenacin, the unsurmountable antagonism seen may indicate deviation from competitive antagonism in this preparation. The reason for this is unclear.

The data obtained in functional studies in guinea-pig ileum and atria, support the earlier report by Wallis et al. (1993), that zamifenacin is selective for muscarinic M_3 receptors over M_2 receptors. However, the affinity (pA_2) of zamifenacin at atrial muscarinic M_2 receptors in the present study was lower than that reported previously (Wallis et al., 1993, Table 2), making the ileal M_3/atrial M_2 selectivity greater in the present study. The affinity of zamifenacin at M_1 receptors mediating contraction of canine saphenous vein was higher than that reported previously by Wallis and colleagues in rabbit vas deferens (7.4, Wallis et al., 1993), but agrees with that reported here in binding studies in rat cortex.

Zamifenacin exhibited different pA_2 values between muscarinic M_3 receptors in smooth muscles as suggested by earlier reports (Wallis et al., 1993; Wallis, 1995). Thus, the affinity of zamifenacin at tracheal muscarinic M_3 receptors (8.2) was lower than at either ileal or oesophageal M_3 receptors (9.3 and 8.8, respectively). It is unlikely that conditions of disequilibrium contribute to the difference in affinity, since Schild analysis revealed slopes not significantly different from unity and surmountable antagonism (this study and Wallis et al., 1993). Further evidence to support a competitive interaction at muscarinic M_3 receptors in trachea, is provided by the combination concentration-ratio experiments. In these studies zamifenacin interacted competitively with atropine, a well established competitive muscarinic receptor antagonist. These data indicate that the selectivity of zamifenacin for muscarinic M_3 over M_2 receptors is contingent upon the smooth muscle preparation studied. Consequently, the M_3/M_2 selectivity was most apparent when ileal muscarinic M_3 receptors were considered, but less so when tracheal and urinary bladder M_3 receptors were considered.

There is some functional evidence showing that antagonists exhibit different pA_2 values at muscarinic M_3 receptors present in different tissues and different species. Dicyclomine, although a non-competitive antagonist, has been shown to be more potent against muscarinic M_3 receptors in rabbit and human urinary bladder than in ileum (Downie et al., 1977). *p*-F-HHSiD appears to distinguish (approx. 10-fold) between tracheal and ileal M_3 receptors, but does not discriminate between ileal and urinary bladder M_3 receptors (Eglen et al., 1990a,b). Others have reported species and tissue differences with regard to the affinities of muscarinic antagonists for smooth muscle M_3 receptors, particularly *p*-F-HHSiD, and have suggested that this may reflect important differences in amino acid sequences of the muscarinic M_3 receptors (Roffel et al., 1994).

Differences in amino acid sequences of the muscarinic M_3 receptors may account for the disparities in affinities (Roffel et al., 1994), but other factors can also influence antagonist affinity. In mouse urinary bladder and rabbit ileum the presence of an uptake system for quaternary muscarinic receptor antagonists renders assessment of antagonist affinities for such antagonists

problematic (Durant et al., 1991; Kenakin and Beek, 1987). In bovine trachea, assessment of quarternary muscarinic antagonist affinities is complicated by their action at a positive allosteric site (Roffel et al., 1991). Additional experiments are required to eliminate similar explanations for the variation in apparent affinities observed for zamifenacin. Hence, the most conservative explanation for the observed differences is that the nature of the membrane lipid environment around the muscarinic M_3 receptor influences the affinity (Eglen et al., 1990a,b).

In summary therefore, zamifenacin appears to be a competitive, surmountable antagonist at muscarinic M_3 receptors in vitro. The degree of subtype selectivity is variable, since the affinity at muscarinic M_3 receptors appears to be tissue-dependent. In the present study the ileal M_3 versus atrial M_2 receptor selectivity was greater than that previously reported (502-fold, this study versus 135-fold, Wallis et al., 1993), however the higher affinity of zamifenacin established at M_1 receptors in canine saphenous vein compared to rabbit vas deferens, reduces the M_1/ileal M_3 selectivity of this compound (25-fold, this study versus 78-fold Wallis et al., 1993).

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