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Comparison of the in vitro and in vivo profiles of tolterodine with those of subtype-selective muscarinic receptor antagonists

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Abstract

Tolterodine [(*R*)-*N*,*N*-diisopropyl-3-(2-hydroxy-5-methylphenyl)-3-phenylpropanamine] is a new potent and competitive muscarinic receptor antagonist developed for the treatment of urinary urge incontinence and other symptoms of overactive bladder. In vivo, tolterodine exhibits functional selectivity for the urinary bladder over salivary glands, a profile that cannot be explained in terms of selectivity for a single muscarinic receptor subtype. The aim of this study was to compare the in vitro and in vivo antimuscarinic profiles of tolterodine with those of muscarinic receptor antagonists with distinct receptor subtype-selectivity profiles: darifenacin [(*S*)-2-{1-[2-(2,3-dihydrobenzofuran-5-yl)ethyl]-3-pyrrolidinyl}-2,2-diphenylacetamide; selective for muscarinic M_3 receptors]; UH-AH 37 (6-chloro-5,10-dihydro-5-[(1-methyl-4-piperidinyl)acetyl]-11H-dibenzo-[*b*,*e*][1,4]diazepine-11-one; low affinity for muscarinic M_2 receptors); and AQ-RA 741 (11-({4-[4-(diethylamino)butyl]-1-piperidinyl}acetyl)-5,11-dihydro-6H-pyrido[2,3-*b*][1,4]benzodiazepine-6-one; high affinity for muscarinic M_2 receptors). The in vitro profiles of these compounds were in agreement with previous reports; darifenacin and UH-AH 37 demonstrated selectivity for muscarinic M_3/m3 over M_2/m2 receptors, while the converse was observed for AQ-RA 741. In vivo, AQ-RA 741 was more potent (1.4–2.7-fold) in inhibiting urinary bladder contraction than salivation in the anaesthetised cat (i.e., a profile similar to that of tolterodine [2.5–3.3-fold]), while darifenacin and UH-AH 37 showed the reverse selectivity profile (0.6–0.8 and 0.4–0.5-fold, respectively). The results confirm that it is possible to separate the antimuscarinic effects on urinary bladder and salivary glands in vivo. The data on UH-AH 37 and darifenacin support the view that a selectivity for muscarinic M_3/m3 over M_2/m2 receptors may result in a more pronounced effect on salivation than on bladder contraction. The data on AQ-RA 741 may indicate that muscarinic M_2/m2 receptors may have a role in bladder contraction.

Keywords: Tolterodine; Darifenacin; UH-AH 37; AQ-RA 741; Muscarinic receptor antagonist; Urinary bladder; Salivation

1. Introduction

The existence of molecularly distinct muscarinic receptor subtypes is well established (Hulme et al., 1990; Caulfield, 1993), and five genes encoding for muscarinic receptors have been cloned and expressed in cell lines. Most smooth muscles contain both muscarinic M_2 ('cardiac') and M_3 ('glandular') receptors (for review, see Eglen et al., 1994). The presence of mRNA for muscarinic m2 and m3 receptors has been demonstrated in the urinary bladder of rat, pig (Maeda et al., 1988) and human (Yamaguchi et al., 1994). Immunoprecipitation data indicate that the ratio of muscarinic m2:m3 receptors is 3:1 in the human bladder (Wang et al., 1995). Radioligand binding studies in the guinea-pig bladder (Nilvebrant and Sparf, 1986, 1988) and other smooth muscles (Eglen et al., 1994; Giraldo et al., 1987; Michel and Whiting, 1988) mainly reflect the presence of muscarinic M_2 receptors. However, data from functional in vitro studies with a number of selective antagonists indicate that the contraction of most smooth muscle is mediated only by muscarinic M_3 receptors (Caulfield, 1993; Eglen et al., 1994; Tobin and Sjögren, 1995; Wang et al., 1995). While a role for muscarinic M_2 receptors in smooth muscle contraction remains to be established, emerging evidence suggests that the functional importance of these receptors may have been underestimated (Caulfield, 1993; Chen et al., 1995; Eglen et al., 1994; Griffin and Ehlert, 1992; Reddy et al., 1995; Thomas et al., 1993; Zhang and Buxton, 1991).

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Tolterodine is a new muscarinic receptor antagonist intended for the treatment of urinary urge incontinence and other symptoms related to overactive bladder (Nilvebrant et al., 1994, 1995, 1997c). In the anaesthetised cat, tolterodine is significantly more potent in inhibiting contraction of the urinary bladder than salivation (Nilvebrant et al., 1997a), while it binds with similar affinity to all muscarinic receptor subtypes (m1–m5) in vitro (Nilvebrant et al., 1996). In contrast, oxybutynin, which has been characterised as a muscarinic M_1/M_3 selective antagonist (Nilvebrant and Sparf, 1982, 1983b, 1986; Noronha-Blob and Kachur, 1991), displays the reverse selectivity profile in vivo (Nilvebrant et al., 1997a).

The aim of this study was to compare the in vitro and in vivo antimuscarinic profiles of tolterodine with those of selective muscarinic receptor antagonists, including darifenacin (a selective muscarinic M_3 receptor antagonist) (Wallis et al., 1995), the pirenzepine derivative UH-AH 37 (which has low affinity for the muscarinic M_2/m2 receptor compared with other muscarinic receptor subtypes) (Wess et al., 1991) and AQ-RA 741 (high affinity for muscarinic M_2/m2 receptors) (Dörje et al., 1991). A preliminary report of these data was presented at the 1996 Meeting of the International Continence Society (Nilvebrant et al., 1996).

2. Materials and methods

2.1. Functional in vitro studies

Male guinea-pigs (Dunkin Hartley, 300–500 g) were used in all experiments. Antimuscarinic potency of tolterodine, darifenacin, UH-AH 37 and AQ-RA 741 was determined in guinea-pig isolated urinary bladder strips, as previously described (Nilvebrant et al., 1997a). After equilibration, the urinary bladder preparations were repeatedly exposed to a standard concentration of the muscarinic receptor agonist carbachol (3 μM; EC_{80}). Reproducible control responses were established before the effects of each antagonist were determined. Following the generation of a cumulative concentration–response curve to carbachol (control), each antagonist was added to the tissue bath for 60 min (Nilvebrant, 1986) and a second cumulative concentration–response curve to carbachol generated in the presence of the antagonist. The effects of the antagonists were studied at 4–5 different concentrations using separate tissue preparations. Responses were expressed as a percentage of the maximal contractile response elicited by carbachol in the control curve.

2.2. Radioligand binding studies

The affinities of tolterodine, darifenacin, UH-AH 37 and AQ-RA 741 for muscarinic receptors were determined by radioligand binding studies in homogenates of guinea-pig tissues (urinary bladder, parotid gland, heart and cerebral cortex) and in Chinese hamster ovary (CHO) cells expressing the human muscarinic receptor subtypes (m1–m5). The culture technique for CHO cells, the tissue/CHO cell homogenisation method and the radioligand binding assay have been described elsewhere (Nilvebrant et al., 1997a). Briefly, tissue homogenates were incubated (25°C) with the radioligand [^{3}H](−)-*l*-quinuclidinyl benzilate and different concentrations of unlabelled antagonist under conditions of equilibrium: urinary bladder, 60 min (Nilvebrant and Sparf, 1983a); parotid gland, 210 min (Nilvebrant and Sparf, 1982); and heart and cerebral cortex, 80 min (Nilvebrant and Sparf, 1986). Incubations were terminated by rapid centrifugation (Nilvebrant and Sparf, 1983a) and the radioactivity in the pellets determined by liquid scintillation spectrometry. Radioligand binding studies in CHO cells were carried out in culture plates. Briefly, cell homogenates were incubated with [^{3}H](−)-*l*-quinuclidinyl benzilate and different concentrations of unlabelled antagonist under conditions of equilibrium (37°C for 330 min). Incubations were terminated by filtration and the amount of radioactivity determined by liquid scintillation spectrometry. Total binding of [^{3}H](−)-*l*-quinuclidinyl benzilate was determined in the absence of any competing ligand, while non-specific binding was determined in the presence of unlabelled atropine (10 μM). Receptor-specific binding in each experiment was defined as total-non-specific binding.

2.3. In vivo studies

The antimuscarinic effects of tolterodine (21–2103 nmol kg^{-1} (0.01–1 mg kg^{-1})), darifenacin (1.8–1843 nmol kg^{-1} (0.001–1 mg kg^{-1})), UH-AH 37 (24–2379 nmol kg^{-1} (0.01–1 mg kg^{-1})) and AQ-RA 741 (60–5999 nmol kg^{-1} (0.03–3 mg kg^{-1})) in vivo were studied in the anaesthetised cat. Female European short-haired cats (2.4–3.5 kg; 9 ± 1 months of age) were used in the experiments, with five cats being used for each antagonist. The methods have been described in detail elsewhere (Nilvebrant et al., 1997a). Briefly, bladder contractions were elicited by intra-arterial (i.a.) administration of a submaximal dose of acetylcholine (1–2 μg kg^{-1}), while salivation was induced by submaximal electrical stimulation (6 V, 2 ms, 5 Hz) of the parasympathetic chorda-lingual nerve over 2 min. Acetylcholine was administered before and approximately 9 and 16 min after each dose of antagonist or saline control, which was administered by intravenous (i.v.) infusion in the right femoral vein at a rate of 1 ml kg^{-1} min^{-1}. Electrical stimulation of the chorda-lingual nerve was performed before and approximately 7 min after each dose of antagonist or saline control.

2.4. Data analysis

All data are expressed as mean ± S.E.M. The concentration of carbachol that produced 50% of the maximal

contractile response (EC_{50}) in the guinea-pig isolated urinary bladder was determined in the absence and presence of each antagonist, respectively. From this, concentration ratios (r) were obtained for the concentration of antagonist used [A], and the antagonist affinity (K_B) calculated in each experiment using the formula $K_B = [A]/(r-1)$.

In radioligand binding experiments, the concentration of antagonist that inhibited [^{3}H](−)-*l*-quinuclidinyl benzilate binding by 50% (IC_{50}) was determined from the experimental concentration–inhibition curves. Dissociation constants (K_i) were calculated by correcting the IC_{50} values for the radioligand-induced parallel shift and the differences in receptor density, using the method described by Jacobs et al. (1975); see also Nilvebrant and Sparf (1982, 1983b) for details.

Differences between the inhibitory effect exerted by each antagonist on acetylcholine-induced urinary bladder contraction and electrically induced salivation were analysed for each dose using a paired Student's *t*-test. ID_{30} (threshold dose) and ID_{50} values of each antagonist for inhibition of bladder contraction and salivation were also determined. P values < 0.05 were considered statistically significant.

2.5. Drugs and chemicals

The following drugs and chemicals were used: tolterodine [(*R*)-*N*,*N*-diisopropyl-3-(2-hydroxy-5-methylphenyl)-3-phenylpropanamine, as the hydrogen L-(+)-tartrate salt] and darifenacin [(*S*)-2-{1-[2-(2,3-dihydrobenzofuran-5-yl)ethyl]-3-pyrrolidinyl}-2,2-diphenylacetamide, as the fumarate salt] (Pharmacia&Upjohn, Sweden); UH-AH 37 (6-chloro-5,10-dihydro-5-[(1-methyl-4-piperidinyl)acetyl]-11H-dibenzo-[*b*,*e*][1,4]diazepine-11-one, as the hydrochloride salt) and AQ-RA 741 (11-({4-[4-(diethylamino)butyl]-1-piperidinyl}acetyl)-5,11-dihydro-6H-pyrido[2,3-b][1,4]benzodiazepine-6-one, as the hydrochloride salt) (gifts from Karl Thomae, Germany); acetylcholine chloride, atropine sulphate, carbachol (carbamylcholine chloride) and phenyl methyl sulphonyl fluoride (Sigma, USA); [^{3}H](−)-*l*-quinuclidinyl benzilate, specific activity 32–52 Ci mmol^{-1} (1.18–1.94 TBq/mmol) (Amersham, UK or Du Pont NEN Research Products, USA); pentobarbital sodium (Apoteksbolaget, Sweden); α-choralose (Merck KGaA, Germany); Dulbecco's modified Eagle's medium and HAM's F12 medium (National Veterinary Institute, Sweden); foetal bovine serum albumin (HyClone Lab, USA); L-glutamine and penicillin/streptomycin (ICN Biomedicals, USA). Other chemicals used (analytical grade) were purchased from general commercial sources.

[^{3}H](−)-*l*-quinuclidinyl benzilate was diluted in absolute ethanol. Fresh solutions of tolterodine, darifenacin, UH-AH 37, AQ-RA 741, carbachol, atropine and acetylcholine were prepared for each experiment; for functional in vitro studies drugs were dissolved and diluted in double-distilled water, while for in vivo studies drugs were dissolved and diluted in saline.

3. Results

3.1. Functional in vitro studies

Tolterodine (10–500 nM), darifenacin (1–100 nM), UH-AH 37 (50–1000 nM) and AQ-RA 741 (100–5000 nM) all produced concentration-dependent, parallel, rightward shifts in the concentration–response curve to carbachol in guinea-pig urinary bladder strips. None of the compounds tested depressed the maximum response to carbachol within the concentration ranges studied. The dissociation constants (K_B) were determined (Table 1), and Schild plot analysis for the antagonists revealed slopes that were linear and close to unity.

3.2. Radioligand binding studies in tissue homogenates

Tolterodine, darifenacin, UH-AH 37 and AQ-RA 741 all caused concentration-dependent inhibition of [^{3}H](−)-*l*-quinuclidinyl benzilate binding in homogenates of guinea-pig urinary bladder and parotid gland (Fig. 1). Tolterodine, darifenacin and AQ-RA 741 concentration–inhibition curves of [^{3}H](−)-*l*-quinuclidinyl benzilate binding were parallel and had Hill coefficients (n_H) close

Table 1
In vitro affinity of tolterodine, darifenacin, UH-AH 37 and AQ-RA 741 at muscarinic receptors in guinea-pig tissues

Antagonist	Urinary bladder			Cerebral cortex		Heart		Parotid gland	
	K_B (nM)	K_i (nM)	n_H	K_i (nM)	n_H	K_i (nM)	n_H	K_i (nM)	n_H
Tolterodine	3.0 ± 0.2	2.7 ± 0.2	1.02 ± 0.03	0.75 ± 0.01	1.05 ± 0.03	1.6 ± 0.04	1.04 ± 0.06	4.8 ± 0.3	1.04 ± 0.03
Darifenacin	0.87 ± 0.10	77.7 ± 15.7	0.93 ± 0.06	8.3 ± 0.3	0.90 ± 0.05	44.1 ± 12.9	0.99 ± 0.05	1.7 ± 0.4	0.89 ± 0.01
UH-AH 37	5.2 ± 0.7	82.1 ± 5.7	1.02 ± 0.03	3.7 ± 0.2	0.82 ± 0.03[a]	50.0 ± 5.4	1.25 ± 0.08	26.4 ± 4.4	0.94 ± 0.03
AQ-RA 741	140 ± 17	11.7 ± 1.6	1.00 ± 0.08	10.2 ± 0.7	0.93 ± 0.03	2.4 ± 0.1	0.93 ± 0.03	167.9 ± 5.5	1.03 ± 0.06

Affinity was determined from functional (K_B) and radioligand binding (K_i) studies.
[a] High and low affinity binding sites were identified (K_i 2.4 ± 0.3 nM and 41 ± 15 nM, respectively; $n = 6$).
n_H, Hill coefficient. K_B values are mean ± S.E.M. of 8–17 separate experiments, K_i and n_H values are mean ± S.E.M. of 3–6 separate experiments.

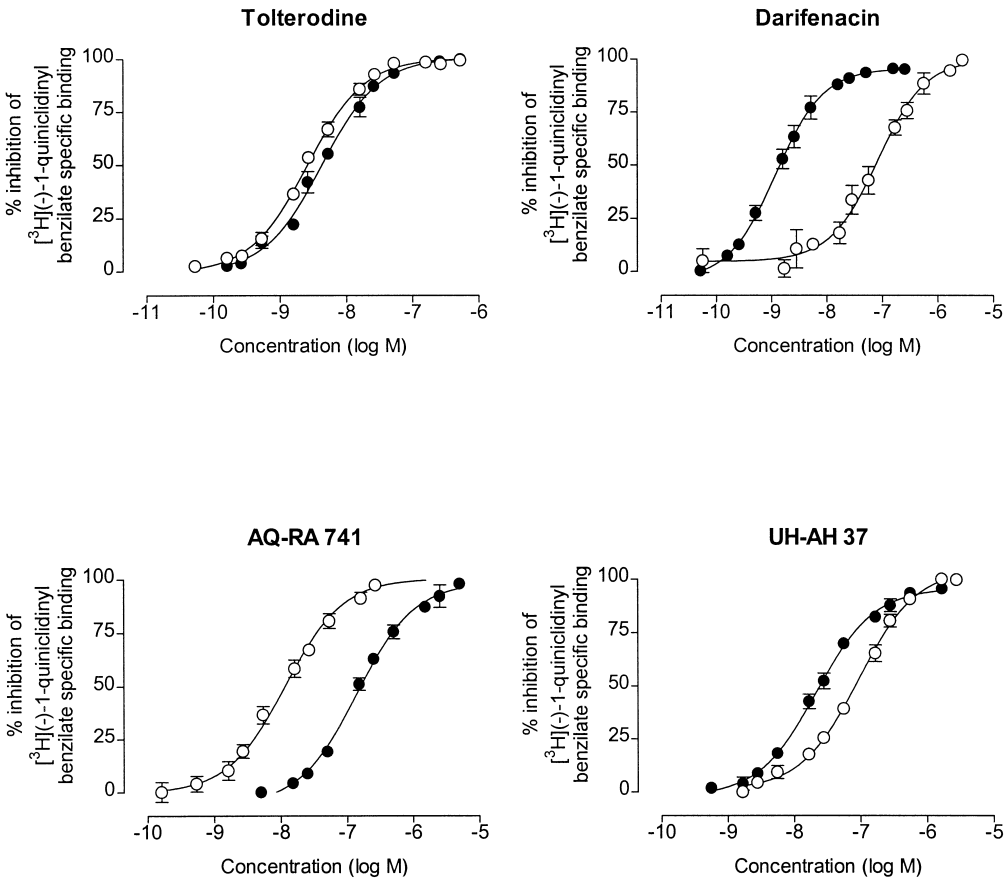

Fig. 1. Concentration-dependent inhibition of [^{3}H](−)-*l*-quinuclidinyl benzilate binding by tolterodine, darifenacin, UH-AH 37 and AQ-RA 741 and in homogenates of guinea-pig urinary bladder (○) and parotid gland (●). Results are expressed as percentage inhibition of the total amount of specific [^{3}H](−)-*l*-quinuclidinyl benzilate binding, and are the mean ± S.E.M. of 4–6 separate experiments.

to unity in all tissues (Table 1), indicating that each bound to a single population of muscarinic binding sites. While n_H values for UH-AH 37 were also close to unity in the urinary bladder, heart and parotid glands (Table 1), binding for muscarinic receptors in the cerebral cortex was characterised by a n_H value that was significantly ($P < 0.05$) lower than unity. These results indicated heterogeneity of muscarinic binding sites for UH-AH 37 in the cerebral cortex—i.e., high and low affinity binding sites could be identified (Table 1). The high affinity binding sites for UH-AH 37 accounted for 70.2 ± 6.3% of the total population of muscarinic binding sites in the cerebral cortex.

Compared with tolterodine, darifenacin had higher affinity for muscarinic receptors in the parotid gland, but displayed lower affinity in the urinary bladder, cerebral cortex and heart (Table 1). UH-AH 37 bound with higher affinity to muscarinic receptors in the parotid gland compared with those in either the urinary bladder or heart, while AQ-RA 741 displayed almost the reverse binding profile, having higher affinity for muscarinic receptors in the urinary bladder and heart compared with the parotid gland (Table 1).

3.3. Radioligand binding studies in CHO cells

Tolterodine, darifenacin, UH-AH 37 and AQ-RA 741 were potent inhibitors of [^{3}H](−)-*l*-quinuclidinyl benzilate binding in homogenates of CHO cells expressing human muscarinic m1–m5 receptors (Table 2). Tolterodine displayed similar affinity for all of the subtypes of the human muscarinic receptor expressed in CHO cells. In contrast, darifenacin had highest affinity for the muscarinic m3 receptor subtype and lowest affinity for the muscarinic m2 subtype. The affinity of darifenacin for the muscarinic m3 receptor was almost 50-fold higher than for the muscarinic m2 subtype. UH-AH 37 had high affinity for all muscarinic receptor subtypes, with the exception of the muscarinic m2 receptor. AQ-RA 741 had highest affinity for the muscarinic m2 receptor, followed by the muscarinic m4 receptor, with low affinity for the muscarinic m1, m3 and m5 receptor subtypes (Table 2).

3.4. In vivo studies

Acetylcholine produced dose-dependent contraction of the urinary bladder in the anaesthetised cat, with intra-

Table 2
Dissociation constants (K_i) and Hill coefficients (n_H) for tolterodine, darifenacin, UH-AH 37 and AQ-RA 741 at human muscarinic receptors expressed in Chinese hamster ovary cells

Antagonist	Parameter	Muscarinic receptor subtype					m2:m3 ratio
		m1	m2	m3	m4	m5	
Tolterodine	K_i (nM)	3.0 ± 0.2	3.8 ± 0.7	3.4 ± 0.8	5.0 ± 0.8	3.4 ± 0.8	1.1
	n_H	1.03 ± 0.04	1.00 ± 0.04	1.06 ± 0.03	1.05 ± 0.07	1.00 ± 0.05	
Darifenacin	K_i (nM)	35.0 ± 3.2	56.0 ± 3.8	1.2 ± 0.1	18.0 ± 3.0	9.0 ± 0.6	47
	n_H	1.08 ± 0.07	1.13 ± 0.04	1.03 ± 0.08	1.12 ± 0.11	1.03 ± 0.03	
UH-AH 37	K_i (nM)	2.4 ± 0.3	49.0 ± 2.0	7.2 ± 1.9	4.0 ± 1.0	5.4 ± 0.4	6.8
	n_H	0.98 ± 0.06	1.10 ± 0.03	1.05 ± 0.04	1.00 ± 0.02	1.09 ± 0.09	
AQ-RA 741	K_i (nM)	62.0 ± 13.0	4.4 ± 0.9	55.0 ± 8.0	15.0 ± 2.0	732.0 ± 94.0	0.08
	n_H	1.05 ± 0.09	0.90 ± 0.03	1.02 ± 0.08	0.96 ± 0.06	0.98 ± 0.09	

Results are expressed as mean ± S.E.M. of 4–6 separate experiments.

arterial administration of 1–2 μg kg^{-1} producing a reproducible submaximal response (data not shown). Electrical stimulation of the chorda-lingual nerve induced mean salivation of 329 ± 22 μl/2 min.

As previously reported for tolterodine (Nilvebrant et al., 1997a), darifenacin and UH-AH 37 also produced dose-dependent inhibition of acetylcholine-induced urinary bladder contraction and electrically stimulated salivation (Fig. 2 and Table 3). Unlike tolterodine, however, darifenacin was equipotent for both responses at low doses and significantly more potent ($P < 0.01$) at inhibiting salivation at high doses. Similarly, UH-AH 37 was significantly ($P < 0.05$) more potent for inhibition of salivation than urinary bladder contraction. At low doses the effect of AQ-RA 741 was essentially similar to that of tolterodine, i.e., it was significantly more potent ($P < 0.01$) at inhibiting urinary bladder contraction than salivation (Fig. 2 and Table 3). In the in vivo model, no significant changes in heart rate were

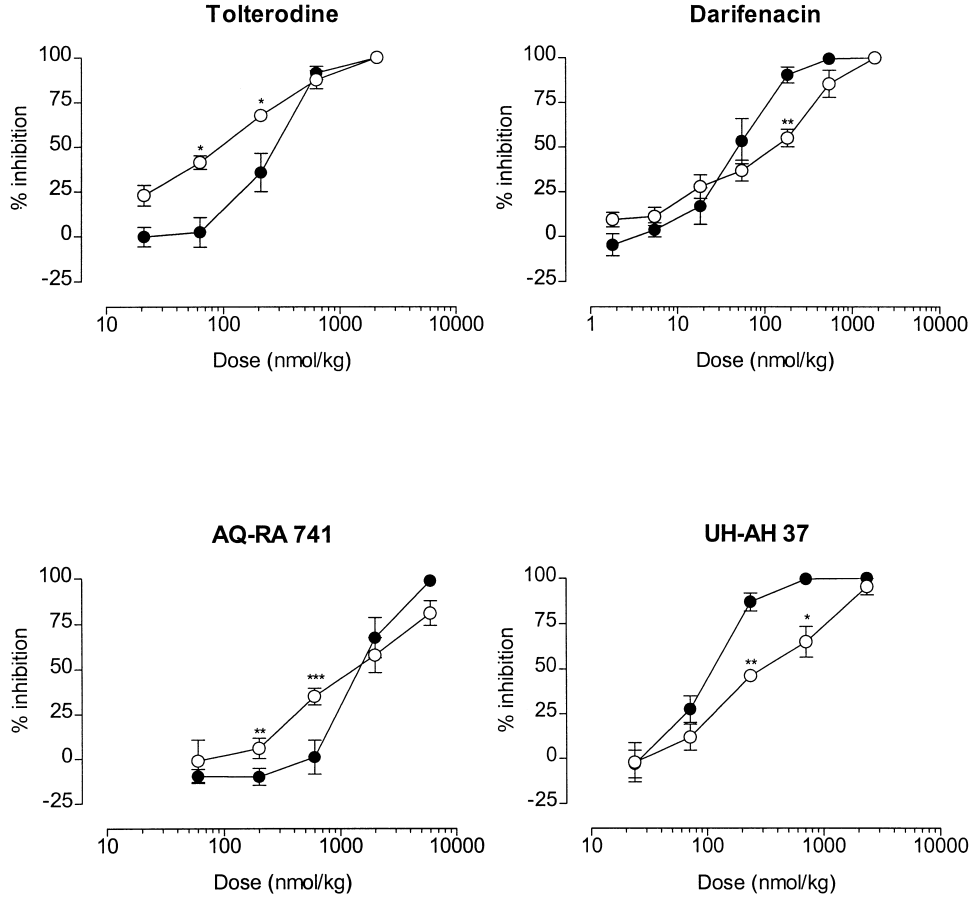

Fig. 2. Effect of tolterodine, darifenacin, UH-AH 37 and AQ-RA 741 on acetylcholine-induced urinary bladder contraction (○) and electrically stimulated salivation (●) in the anaesthetised cat. Results are expressed as percentage inhibition of the maximum response in each experiment, and are the mean ± S.E.M. of 4–5 separate experiments. * $P < 0.05$, ** $P < 0.01$ and *** $P < 0.001$ vs. inhibition of salivation (paired Student's t-test).

Table 3
ID_{30} and ID_{50} values of antagonists of acetylcholine-induced urinary bladder contraction and electrically induced salivation in the anaesthetised cat

Antagonist	ID_{30} (nmol kg^{-1})		ID_{50} (nmol kg^{-1})	
	Bladder contraction	Salivation	Bladder contraction	Salivation
Tolterodine	40 ± 8^a	132 ± 29	101 ± 8^a	257 ± 44
Darifenacin	48 ± 10	30 ± 11	119 ± 26	99 ± 33
UH-AH 37	124 ± 18^a	62 ± 5	311 ± 67^a	120 ± 19
AQ-RA 741	374 ± 71^a	1028 ± 230	1060 ± 189^a	1536 ± 275

Results are mean ± S.E.M. of 4–5 separate experiments.
[a] $P < 0.05$ vs. effect on salivation (paired Student's *t*-test).

observed for tolterodine, darifenacin, UH-AH 37 or AQ-RA 741 at the doses investigated (data not shown).

Administration of saline had only minor effects on basal bladder contraction and salivation compared with all other compounds used in the studies (data not shown).

4. Discussion

Most smooth muscles, including the urinary bladder, contain a mixed population of muscarinic M_2 and M_3 receptors (see e.g., Eglen et al., 1994, 1996). However, based on functional in vitro data on subtype-selective antagonists it is generally believed that smooth muscle contraction is mediated only by muscarinic M_3 receptors (Caulfield, 1993; Eglen et al., 1994, 1996; Tobin and Sjögren, 1995; Wang et al., 1995). The function of the large population of muscarinic M_2 receptors remains to be clarified, but emerging evidence from studies on intestinal smooth muscle and gall bladder suggest that these receptors may have a functional role in smooth muscle contraction (Zhang and Buxton, 1991; Griffin and Ehlert, 1992; Caulfield, 1993; Thomas et al., 1993; Eglen et al., 1994, 1996; Chen et al., 1995; Reddy et al., 1995). With respect to the urinary bladder, the effects of a series of selective antagonists in vitro and in vivo have recently been reported by Hegde et al. (1997). Their results clearly suggested that both muscarinic M_2 and M_3 receptors can cause contraction of the bladder in vitro, and may also mediate reflex bladder contraction in vivo.

Tolterodine is a new muscarinic receptor antagonist intended for the treatment of urinary urge incontinence and other symptoms related to overactive bladder. In previous studies we have reported that both tolterodine and its major active metabolite (DD 01, PNU-200577) are significantly more potent at inhibiting urinary bladder contraction than salivation in vivo, while oxybutynin shows the reverse selectivity (Nilvebrant et al., 1997a,b,c). The selectivity for the urinary bladder in vivo cannot be explained in terms of selectivity for a particular muscarinic receptor subtype, since both tolterodine and the metabolite are non-selective with respect to human muscarinic m1–m5 receptors expressed in CHO cells (Nilvebrant et al., 1996, 1997a,b,c). Both tolterodine and PNU-200577 show a high specificity for muscarinic receptors, as compared to other potential cellular targets. Thus, it is important to note that the more pronounced effect on the bladder as compared to the salivary glands cannot be attributed to, for example, calcium channel-blocking properties (Nilvebrant et al., 1994; Gillberg et al., 1996). Based on the combined in vitro and in vivo data on tolterodine, PNU-200577 and oxybutynin (which shows a 10-fold selectivity for muscarinic m3 over m2 receptors) we have previously suggested that the different selectivity profiles in vivo may be related to the difference in relative affinity for muscarinic M_3 and M_2 receptors. Thus, the data may indicate either that a selectivity for muscarinic M_3 receptors is associated with a more pronounced effect on glands, or that the muscarinic M_2 receptors may also be involved in bladder contraction (Nilvebrant et al., 1997a,b,c). The aim of the present study was to compare the in vitro and in vivo antimuscarinic profiles of tolterodine with those of some well-known subtype-selective muscarinic receptor antagonists, i.e., darifenacin (muscarinic M_3/m3 receptor selective; Newgreen et al., 1995; Wallis et al., 1995), UH-AH 37 (low affinity at the muscarinic M_2/m2 receptor compared with other muscarinic receptor subtypes; Wess et al., 1991) and AQ-RA 741 (muscarinic M_2/m2 receptor selective; Dörje et al., 1991).

The selectivity profiles of darifenacin, UH-AH 37 and AQ-RA 741 in vitro were in agreement with previous reports (e.g., Dörje et al., 1991; Nunn et al., 1996; Wess et al., 1991; Wallis et al., 1995). For example, darifenacin and UH-AH 37 showed 47- and almost 7-fold selectivity, respectively, for muscarinic m3 over m2 receptors in CHO cells, while the K_i values of both compounds in the heart (muscarinic M_2 receptors) were much higher than those in the parotid gland (muscarinic M_3 receptors). In radioligand binding studies, AQ-RA 741 displayed high affinity for muscarinic receptors in the heart (K_i 2.4 nM) compared with the parotid gland (K_i 167.9 nM) and it had a muscarinic m2:m3 affinity ratio of 0.08 in CHO cells. The functional in vitro data for bladder contraction (K_B) determined for darifenacin and UH-AH 37 showed a good correlation with the affinities determined at the human muscarinic m3 receptor. The K_B value for AQ-RA 741 (140 nM) correlated with the K_i value determined at muscarinic m3 receptors (55 nM) rather than at muscarinic m2 receptors (4.4 nM). Thus, the present in vitro results are in line with the current belief that smooth muscle contraction is mediated only by muscarinic M_3/m3 receptors.

On the other hand, the in vivo data in the anaesthetised cat confirmed our previous finding that it is possible to separate the effects on the bladder and salivary glands in vivo, despite the fact that both smooth muscle contraction and salivation are believed to be mediated by muscarinic M_3/m3 receptors. Thus, within the dose range examined,

UH-AH 37 was more potent at inhibiting salivation than urinary bladder contraction (Fig. 2). Similarly, although darifenacin was non-selective at lower doses, the effect on glands was the most pronounced at the higher doses required to achieve more than 30% inhibition of either response. In contrast, AQ-RA 741 exhibited a selectivity for the bladder over salivary glands similar to that exhibited by tolterodine (Fig. 2). Our findings for darifenacin are in contrast to those of Newgreen et al. (1995), who reported an 8.5-fold selectivity for bladder over salivary gland in the anaesthetised dog. It is not clear whether this discrepancy may be a result of methodological differences. Thus, the selectivity of tolterodine for the bladder over salivary glands reported by Newgreen et al. (1995) was in fact also greater (5-fold) than that demonstrated by us using the anaesthetised cat model (2.5-fold).

The data on tolterodine (Nilvebrant et al., 1997a) and other muscarinic receptor antagonists (Eglen and Watson, 1996) clearly show that it is not possible to predict in vivo selectivity from in vitro affinity profiles. However, the results of the present study are in line with our previous findings with oxybutynin, i.e., that a selectivity for muscarinic M_3/m3 receptors over M_2/m2 receptors may result in a more pronounced effect on salivation (Nilvebrant et al., 1997a,b,c). Thus, like oxybutynin, both darifenacin and UH-AH 37 showed a selectivity for muscarinic M_3/m3 over M_2/m2 receptors in vitro, and both compounds tended to inhibit salivation to a greater extent than bladder contraction in vivo. In contrast, AQ-RA 741 showed a more pronounced effect on the bladder than on salivary glands in vivo. This finding may suggest that muscarinic M_2 receptors may also be involved in bladder contraction.

The muscarinic receptor function in smooth muscles is probably much more complex than previously believed, as pointed out by several authors (e.g., Caulfield, 1993; Eglen et al., 1994, 1996; Hegde et al., 1997). With respect to the urinary bladder, both prejunctional inhibitory (M_2) and facilitating (M_1) muscarinic receptors (Flood et al., 1994; Somogyi and De Groat, 1992; Somogyi et al., 1994; Tobin and Sjögren, 1995) have been demonstrated on cholinergic nerves in the bladder from different species. It has been suggested that the prejunctional facilitating muscarinic M_1 receptor may serve as an amplification mechanism during micturition (Flood et al., 1994; Somogyi and De Groat, 1992; Somogyi et al., 1994). However, the relative functional importance of the different pre- and post-junctional muscarinic receptor subtypes in the bladder remains to be delineated, particularly in vivo.

In conclusion, the results of the present study confirm that it is possible to separate antimuscarinic effects on urinary bladder and salivary glands in vivo. The data on UH-AH 37 and darifenacin support the view that a selectivity for muscarinic M_3/m3 over M_2/m2 receptors may result in a more pronounced effect on salivation than on bladder contraction. The data on AQ-RA 741 may indicate that muscarinic M_2/m2 receptors may have a role in bladder contraction, as was recently suggested also by Hegde et al. (1997). Whether this may contribute to the different selectivity profiles observed in vivo for tolterodine and PNU-200577 vs. oxybutynin, as well as darifenacin and UH-AH 37 vs. AQ-RA 741, remains to be shown. However, it is interesting to note that results from comparative clinical studies in patients with overactive bladder show that while tolterodine and oxybutynin are comparable with respect to the efficacy on the bladder, treatment with tolterodine is associated with fewer and less severe cases of dry mouth (Appell, 1997; Van Kerrebroeck et al., 1997).

References

Appell, R.A., 1997. Clinical efficacy and safety of tolterodine in the treatment of overactive bladder: a pooled analysis. J. Urol. 50 (Suppl. 6A), 90–96.

Caulfield, M.P., 1993. Muscarinic receptor-characterization, coupling and function. Pharmacol. Ther. 58, 319–379.

Chen, Q., Yu, P., De Petris, G., Biancani, P., Behar, J., 1995. Distinct muscarinic receptors and signal transduction pathways in gallbladder muscle. J. Pharmacol. Exp. Ther. 273, 650–655.

Dörje, F., Wess, J., Lambrecht, G., Tacke, R., Mutschler, E., Brann, M.R., 1991. Antagonist binding profiles of five cloned human muscarinic receptor subtypes. J. Pharmacol. Exp. Ther. 256, 727–733.

Eglen, R.M., Watson, N., 1996. Selective muscarinic receptor agonists and antagonists. Pharmacol. Toxicol. 78, 59–68.

Eglen, R.M., Reddy, H., Watson, N., Challis, R.A.J., 1994. Muscarinic acetylcholine receptors in smooth muscle. Trends Pharmacol. Sci. 15, 114–119.

Eglen, R.M., Hegde, S.S., Watson, N., 1996. Muscarinic receptor subtypes and smooth muscle function. Pharmacol. Rev. 48, 531–565.

Flood, H.D., Somogyi, G.T., Bellinger, M.F., De Groat, W.C., 1994. Muscarinic facilitation of acetylcholine release in the human bladder: a mechanism for enhancement of micturition contractions. Proceedings of the 24th Annual Meeting of the International Continence Society, August 31–September 2, 1994, Prague.

Gillberg, P.-G., Sparf, B., Nilvebrant, L., 1996. Pharmacological in vitro and in vivo profile of DD 01—a major metabolite of tolterodine. Neurourol. Urodyn. 15, 308–309.

Giraldo, E., Vigano, M.A., Hammer, R., Ladinsky, H., 1987. Characterization of muscarinic receptors in guinea pig ileum longitudinal smooth muscle. Mol. Pharmacol. 33, 617–625.

Griffin, M.T., Ehlert, F.J., 1992. Specific inhibition of isoproterenol-stimulated cyclic AMP accumulation by M2 muscarinic receptors in rat intestinal smooth muscle. J. Pharmacol. Exp. Ther. 263, 221–225.

Hegde, S.S., Choppin, A., Bonhaus, D., Briaud, S., Loeb, M., Moy, T.M., Loury, D., Eglen, R.M., 1997. Functional role of M_2 and M_3 muscarinic receptors in the urinary bladder of rats in vitro and in vivo. Br. J. Pharmacol. 120, 1409–1418.

Hulme, E.C., Birdsall, N.J.M., Buckley, N.J., 1990. Muscarinic receptor subtypes. Annu. Rev. Pharmacol. Toxicol. 30, 633–673.

Jacobs, S., Chang, K.-J., Cuatrecasas, P., 1975. Estimation of hormone receptor affinity by competitive displacement of labelled ligand. Biochem. Biophys. Res. Commun. 66, 687–692.

Maeda, A., Kubo, T., Mishima, M., Numa, S., 1988. Tissue distribution of mRNAs encoding muscarinic acetylcholine receptor subtypes. FEBS Lett. 239, 339–342.

Michel, A.D., Whiting, R.L., 1988. Methoctramine reveals heterogeneity of the M_2 muscarinic receptors in longitudinal ileal smooth muscle membranes. Eur. J. Pharmacol. 145, 305–311.

Newgreen, D.T., Anderson, C.W.P., Carter, T.J., Naylor, A.M., 1995.

Darifenacin—a novel bladder-selective agent for the treatment of urge incontinence. Neurourol. Urodyn. 14, 555–557.

Nilvebrant, L., 1986. On the muscarinic receptors in the urinary bladder and the putative subclassification of muscarinic receptors. Acta Pharmacol. Toxicol. 59 (Suppl. I), 1–45.

Nilvebrant, L., Sparf, B., 1982. Muscarinic receptor binding in the guinea pig parotid gland. Different affinities of some anticholinergic drugs between the parotid gland and ileum. Scand. J. Gastroenterol. 17, 69–77, (Suppl. 72).

Nilvebrant, L., Sparf, B., 1983a. Differences between binding affinities of some antimuscarinic drugs in the parotid gland and those in the urinary bladder and ileum. Acta Pharmacol. Toxicol. 53, 304–313.

Nilvebrant, L., Sparf, B., 1983b. Muscarinic receptor binding in the guinea pig urinary bladder. Acta Pharmacol. Toxicol. 52, 30–38.

Nilvebrant, L., Sparf, B., 1986. Dicyclomine, benzhexol and oxybutynin distinguish between subclasses of muscarinic binding sites. Eur. J. Pharmacol. 123, 133–143.

Nilvebrant, L., Sparf, B., 1988. Receptor binding profiles of some selective muscarinic antagonists. Eur. J. Pharmacol. 151, 83–96.

Nilvebrant, L., Glas, G., Jönsson, Å., Sparf, B., 1994. The in vitro pharmacological profile of tolterodine—new agent for the treatment of urinary urge incontinence. Neurourol. Urodyn. 13, 433–435.

Nilvebrant, L., Stahl, M., Andersson, K.-E., 1995. Interaction of tolterodine with cholinergic muscarinic receptors in human detrusor. Neurourol. Urodyn. 14, 523–524.

Nilvebrant, L., Sundquist, S., Gillberg, P.-G., 1996. Tolterodine is not subtype (m1–m5) selective but exhibits functional bladder selectivity in vivo. Neurourol. Urodyn. 15, 310–311.

Nilvebrant, L., Andersson, K.-E., Gillberg, P.-G., Stahl, M., Sparf, B., 1997a. Tolterodine—a new bladder-selective antimuscarinic agent. Eur. J. Pharmacol. 327, 195–207.

Nilvebrant, L., Gillberg, P-G., Sparf, B., 1997b. Antimuscarinic potency and bladder selectivity of PNU-200577, a major metabolite of tolterodine. Pharmacol. Toxicol. 81, 169–172.

Nilvebrant, L., Hallén, B., Larsson, G., 1997c. Tolterodine—a new bladder selective muscarinic receptor antagonist: preclinical pharmacological and clinical data. Life Sci. 60, 1129–1136.

Noronha-Blob, L., Kachur, J.F., 1991. Enantiomers of oxybutynin: in vitro pharmacological characterization at M1, M2 and M3 receptors and in vivo effects on urinary bladder contraction, mydriasis and salivary secretion in guinea pigs. J. Pharmacol. Exp. Ther. 256, 562–567.

Nunn, P.A., Greengrass, P.M., Newgreen, D.T., Naylor, A.M., Wallis, R.M., 1996. The binding profile of the novel muscarinic receptor antagonist darifenacin against the five cloned human muscarinic receptors expressed in CHO cells. Br. J. Pharmacol. 117 (Proc. Suppl.), 130P.

Reddy, H., Watson, N., Ford, A.P.D.W., Eglen, R.M., 1995. Characterization of the interaction between muscarinic M_2 receptors and β-adrenoceptor subtypes in guinea pig isolated ileum. Br. J. Pharmacol. 114, 49–56.

Somogyi, G.T., De Groat, W.C., 1992. Evidence for inhibitory nicotinic and facilitatory muscarinic receptors in cholinergic nerve terminals of the rat urinary bladder. J. Auton. Nerv. Syst. 37, 89–98.

Somogyi, G.T., Tanowitz, M., De Groat, W.C., 1994. M1 muscarinic receptor-mediated facilitation of acetylcholine release in the rat urinary bladder. J. Physiol. 480, 81–89.

Thomas, E.A., Baker, S.A., Ehlert, F.J., 1993. Functional role for the M_2 muscarinic receptor in smooth muscle of guinea pig ileum. Mol. Pharmacol. 44, 102–110.

Tobin, G., Sjögren, C., 1995. In vivo and in vitro effects of muscarinic receptor antagonists on contractions and release of [^{3}H]acetylcholine release in the rabbit urinary bladder. Eur. J. Pharmacol. 281, 1–8.

Van Kerrebroeck, Ph.E.V.A., Serment, G., Dreher, E., 1997. Clinical efficacy and safety of tolterodine compared to oxybutynin in patients with overactive bladder. Neurourol. Urodyn. 16, 478–479.

Wallis, R.M., Burges, R.A., Cross, P.E., MacKenzie, A.R., Newgreen, D.T., Quinn, P., 1995. Darifenacin, a selective muscarinic M_3 antagonist. Pharmacol. Res. 31 (Suppl.), 54.

Wang, P., Luthin, G.R., Ruggieri, M.R., 1995. Muscarinic acetylcholine receptor subtypes mediating urinary bladder contractility and coupling to GTP binding proteins. J. Pharmacol. Exp. Ther. 273, 959–966.

Wess, J., Lambrecht, G., Mutschler, E., Brann, M.R., Dörje, F., 1991. Selectivity profile of the novel muscarinic antagonist UH-AH 37 determined by the use of cloned receptors and isolated tissue preparations. Br. J. Pharmacol. 102, 246–250.

Yamaguchi, O., Shishido, K., Tamura, K., Ogawa, T., Fujimura, T., 1994. Evaluation of mRNA encoding muscarinic receptor subtypes in human detrusor muscle. Neurourol. Urodyn. 13, 464–465.

Zhang, L., Buxton, I.L.O., 1991. Muscarinic receptors in canine colonic circular smooth muscle: II. Signal transduction pathways. Mol. Pharmacol. 40, 952–959.