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ORIGINAL ARTICLE

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M_3 receptor antagonism by the novel antimuscarinic agent solifenacin in the urinary bladder and salivary gland

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Abstract The antimuscarinic profile of the experimental drug solifenacin/YM905 [(+)-(1*S*,3′*R*)-quinuclidin-3′-yl 1-phenyl-1,2,3,4-tetrahydroisoquinoline-2-carboxylate] for the treatment of overactive bladder was compared with the commonly prescribed agent oxybutynin. In radioligand binding assays, pK_i values of solifenacin for M_1, M_2, and M_3 receptors were 7.6, 6.9, and 8.0, respectively. These values for oxybutynin were 8.6 (M_1), 7.7 (M_2), and 8.9 (M_3). Solifenacin and oxybutynin antagonized the contractile effect of carbachol (CCh) on isolated guinea pig urinary bladder smooth muscle (detrusor), displaying the negative logarithm of antagonist apparent affinity constant (pK_b value) of 7.1 for solifenacin and 7.4 for oxybutynin. To study the tissue selectivity between bladders and salivary glands, guinea pig detrusor and mouse submandibular gland cells were stimulated with CCh and monitored for intracellular Ca^{2+}, as determined by Fura 2 fluorescence. Ca^{2+} mobilization of detrusor cells was inhibited equipotently by solifenacin (pK_i=8.4) and oxybutynin (pK_i =8.6), whereas that of the gland cells was antagonized less potently by solifenacin (pK_b=7.4) than by oxybutynin (pK_b=8.8), although the M_3 subtype mediated both cell responses. In anesthetized rats, solifenacin (63–2100 nmol kg^{-1} or 0.03–1 mg kg^{-1}) dose-dependently inhibited CCh-stimulated increases in urinary bladder pressure, while its inhibitory effects on salivation and bradycardia were apparent only at a dose of 2100 nmol kg^{-1}. In contrast, oxybutynin within a dose range of 77–770 nmol kg^{-1} (0.03–0.3 mg kg^{-1}) inhibited responses of the bladder and salivary gland slightly more potently than that of the heart. In addition, inhibitory effects of darifenacin indicated a major role of M_3 receptors in the bladder and salivary gland. Therefore, M_3 receptor antagonism by solifenacin could be bladder-selective. This selectivity remains to be elucidated and may provide new approaches to the pharmacotherapy of overactive bladder.

Keywords YM905 · Solifenacin · Oxybutynin · Darifenacin · Urinary bladder · Salivary glands · Muscarinic receptors

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Introduction

Acetylcholine is the primary spasmogen for the detrusor, acting via muscarinic acetylcholine receptors. Muscarinic receptors of mammalian detrusors are comprised predominantly of the M_2 subtype and a minor population of M_3 subtype (Wang et al. 1995). A large body of evidence (Eglen et al. 1996) and recent findings in M_2 and M_3 receptor knockout mice (Matsui et al. 2000; Stengel et al. 2000) suggest that the minor M_3 receptor population mediates muscarinic detrusor contraction.

Detrusor functions are divided into two phases: relaxation during bladder filling and contraction during bladder emptying. Muscarinic receptors not only mediate micturition contraction but control detrusor tone during the filling phase since antimuscarinic drugs such as oxybutynin increase the bladder capacity in normal subjects and those with overactive bladder, that is, with urological disease symptoms, including urinary frequency, urgency, and incontinence (Andersson 2000).

Oxybutynin is widely used to treat overactive bladder. However, it often causes the unwanted effect of xerostomia/dry mouth due to antagonism of salivary gland M_3 receptors (Chapple 2000). To address this problem, Kaiser et al. (1993) generated an M_3 receptor antagonist selective for the bladder, although the molecular sequences of M_3 receptors are homogenous (Caulfield and Birdsall 1998). Encouraged by their finding, new antimuscarinic agents were screened by functional assays (Naito et al. 1998), and solifenacin has been chosen for clinical investigation. In this report, the antimuscarinic profiles of solifenacin in a study using laboratory animals are described.

Materials and methods

Reagents. Darifenacin, nicardipine and solifenacin monosuccinate were prepared at Yamanouchi Pharmaceutical Co., Ltd. (Tsukuba, Japan). The chemical structure of solifenacin/YM905 was reported previously by Eglen et al. (1999). Methoctramine tetrahydrochloride and 4-diphenylacetoxy-*N*-methylpiperidine methiodide (4-DAMP) were purchased from Research Biochemicals Inc. (Mass., USA), Fura 2-acetoxymethyl ester (Fura 2-AM) was from Dojindo Laboratories (Kumamoto, Japan), collagenase (290 units/mg) was from Nitta Gelatin (Osaka, Japan), and [^{3}H]*N*-methyl scopolamine was from New England Nuclear (Del., USA). Oxybutynin chloride and other reagents were obtained commercially from Sigma Chemical Co. (Mo., USA). Darifenacin, 4-DAMP, Fura 2-AM and nicardipine were dissolved in dimethyl sulfoxide then freshly diluted in appropriate physiological salt solutions. Other chemicals were dissolved in aqueous solutions and diluted.

Animals. Balb/c mice of both sexes and male Hartley guinea pigs were purchased from Charles River Japan (Kanagawa, Japan) and female Wistar rats from SLC (Shizuoka, Japan). The experimental protocol was approved by our Institutional Ethics Review Committee for animal use. Mice and rats were humanely killed by cervical dislocation and guinea pigs were killed by exsanguination through cardiac puncture under ether anesthesia.

Radioligand receptor binding assay. Membranes of Sf-9 cells expressing human muscarinic receptors (New England Nuclear; Del., USA) were suspended in 50 mM Tris-HCl (pH 7.4) buffer supplemented with 1 mM EDTA and 10 mM $MgCl_2$, and were incubated in the 540 µl reaction mixture containing approximately 0.2 nM [^{3}H]*N*-methyl scopolamine at 27 °C for 1 h. Nonspecific binding was determined by including 1 µM atropine in the reaction mixture. Reactions were terminated by filtration through a Whatman GF/B filter and the radioactivity retained on the filter was counted by means of liquid scintillation. Nonspecific binding was less than 10% of bound radioactivity and K_d values of *N*-methyl scopolamine for these M_1, M_2, and M_3 receptors were 0.20, 0.21, and 0.16 nM, respectively.

Carbachol-induced contraction of urinary bladder strips. Guinea pig urinary bladder domes were freed of mucosa and cut into longitudinal strips (5 mm in width). These strips were suspended under an initial tension of 1 g in 10 ml organ baths containing modified Krebs–Henseleit solution (composition in mM: NaCl, 118; $CaCl_2$, 2.5; KH_2PO_4, 1.2; KCl, 4.7; $MgSO_4$, 1.2; $NaHCO_3$, 25; glucose, 10) maintained at 37 °C. Tension was measured with isometric force transducers (Nihon Kohden; Tokyo, Japan). Strips were allowed to equilibrate for 1 h, then stimulated with serially increasing concentrations of CCh, and this procedure was repeated with an interval of 1 h. Antagonists were added to the bath approximately 30 min before CCh stimulation and examined at three different concentrations in a strip.

Cytosolic Ca^{2+} mobilization in detrusor cells. Cytosolic Ca^{2+} mobilization was determined in guinea pig detrusor cells as described (Ikeda et al. 1999). Briefly, single detrusor cells were prepared from epithelium-free bladders, loaded with Fura 2, and suspended in phenol red-free Hanks' balanced salt solution supplemented with 20 mM HEPES (pH=7.4) and 0.1% bovine serum albumin (HBSS-H/B). A 490 µl aliquot of the cell suspension was continuously stirred, kept at 28 °C and monitored for the ratio of fluorescence at 500 nm with excitation at 340 nm to that at 380 nm. To each aliquot, 5 µl of test drug and stimulant solutions were serially added with a 2 min interval, and the peak increase over the level just before stimulation was used for data analyses.

Cytosolic Ca^{2+} mobilization in salivary gland cells. Two lobes of the submandibular glands were taken from mice and finely minced with iridectomy scissors. These tissues were digested with 10 mg of collagenase and 2 mg of hyaluronidase (440 units/mg) dissolved in 10 ml of HBSS-H/B under continuous agitation (130 strokes/min) at 37 °C for 30 min. Then, tissues were triturated with a 5 ml plastic pipette, filtered through 100 µm nylon mesh to remove tissue chunks and briefly centrifuged (200 *g* for 10 s). Dissociated cells were washed three times and resuspended in HBSS-H/B. These procedures yielded spherical cells and their clusters. The cells were incubated with 4 µM Fura 2-AM at 37 °C for 30 min, washed three times and finally suspended in 4 ml of HBSS-H/B. Fura 2-loaded gland cells were subjected to fluorometry and treated as described for detrusor cells. To obtain the fluorescence ratio when Fura 2 was saturated with Ca^{2+} (R_{max}) and the ratio at zero Ca^{2+} (R_{min}), 0.1% Triton X100 and 5 mM EGTA were added to the aliquot. Mean R_{max} and R_{min} values (with SEM; N=28) were 5.35±0.14 and 0.64±0.0080, respectively. The experimentally determined R_{max} values were sevenfold smaller than the value in cell-free solution used to establish the equation to obtain Ca^{2+} levels (Grynkiewicz et al. 1985). Therefore, raw fluorescence ratios were used for data analysis.

Carbachol-induced bladder contraction, saliva secretion and bradycardia in rats. Female rats, weighing 150–200 g, were anesthetized with a sub-lethal dose of sodium pentobarbital (Nembutal; 50 mg kg^{-1} i.v.) and placed supine on a heating pad to maintain body temperature. A polyethylene catheter (PE-50) was cannulated to the bladder via the urethra and secured by purse-string suture around the external urethral opening. The common carotid artery and external jugular vein were catheterized for blood pressure monitoring and drug injections at 1 ml/kg, respectively. The arterial and bladder catheters were connected to pressure transducers (Nihon Kohden, Tokyo, Japan) via three-way stopcock valves. Heart rates were calculated from the arterial pressure using a Nihon Kohden heart-rate analyzing module. The bladder was emptied by drainage of urine through the catheter, distended with approximately 500 µl of physiological saline and monitored for intravesical pressure. Additionally, a spear-shaped filter paper was preweighed and placed in the sublingual space to collect saliva for 5 min after CCh injection. The amount of saliva was quantified by reweighing the filter paper. After bladder pressure stabilization, rats were injected with 56 nmol kg^{-1} (10 µg kg^{-1}) of CCh at an interval of 15 min or longer. By this procedure, bladder contractions, saliva secretion and bradycardia occurred reproducibly for 1 h without appreciable deterioration of physical condition. After two responses to CCh were obtained, the test drug was injected 15 min before another CCh stimulation. The percent inhibition of means of prior drug responses was obtained in four to seven rats per treatment group. Rats developing rhythmic bladder contractions were not used for data analysis.

Data analysis. The 50% stimulatory and inhibitory concentrations, EC_{50} or IC_{50}, were determined by sigmoidal curve fitting. For receptor binding and detrusor cell experiments, IC_{50} values were converted into K_i values based on either K_d values of *N*-methyl scopolamine or pooled EC_{50} values of CCh using the Cheng–Prusoff equation (Lazareno and Birdsall 1993). For organ bath and gland cell experiments, the dose ratio (DR) was obtained by dividing EC_{50} in the presence of antagonist by that in the absence of the antagonist, and the negative logarithm of antagonist apparent affinity constant at a single concentration (pK_b value) was calculated by the equation: pK_b=–log([antagonist]/(DR–1)). In an in vivo study, the 50% inhibitory dose (ID_{50}) was obtained by linear regression. Data are expressed as means±SEMs or with 95% confidence limits.

Results

Binding affinities of solifenacin, oxybutynin and darifenacin for M_1, M_2 and M_3 receptors are shown in Table 1, and the dose–inhibition curves for solifenacin are depicted in Fig. 1. Both solifenacin and oxybutynin were approximately one log unit selective for M_1 and M_3 recep-

Table 1 Affinity constants (pK_i) of solifenacin and reference drugs in radioligand binding assays using recombinant muscarinic receptors

	pK_i[a]		
	M_1	M_2	M_3
Solifenacin	7.6±0.056 (1.0±0.080)	6.9±0.034 (0.90±0.069)	8.0±0.021 (1.0±0.077)
Oxybutynin	8.6±0.062 (1.2±0.10)	7.7±0.049 (1.0±0.043)	8.9±0.052 (1.2±0.050)
Darifenacin	7.8±0.091 (1.1±0.024)	7.3±0.082 (0.97±0.062)	8.8±0.040 (0.97±0.040)

[a]Values represent means±SEMs of three to five experiments. The values in parentheses are Hill slopes

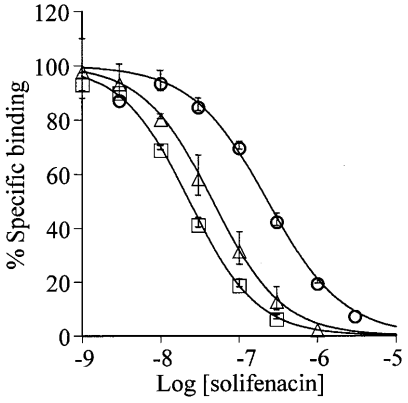

Fig. 1 Displacement of [^{3}H]*N*-methyl scopolamine binding to muscarinic M_1 (Δ), M_2 (○) and M_3 (❑) receptors by solifenacin. Data points represent means±SEMs of three to five experiments

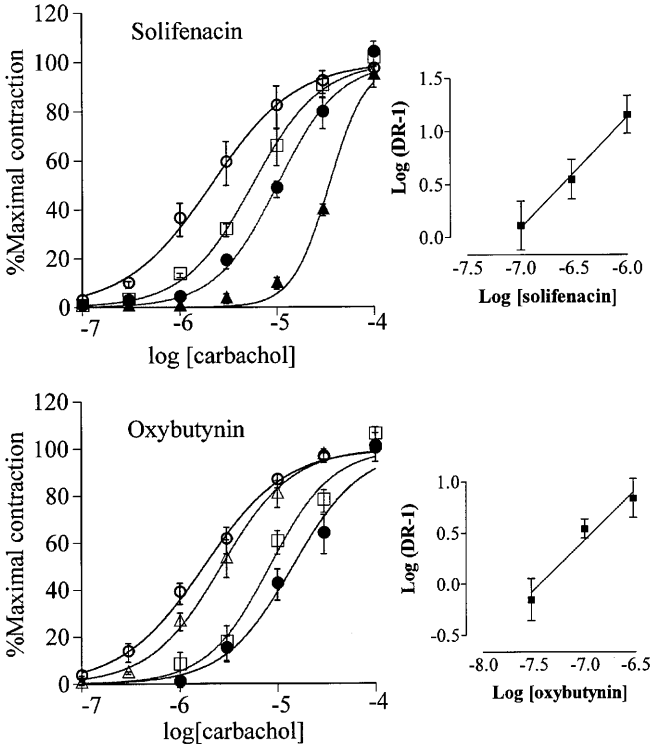

Fig. 2 Contractile effects of carbachol on detrusor strips (*left-hand-side panels*) in the absence (○) and in the presence of solifenacin (*top*) and oxybutynin (*bottom*) at 30 nM (Δ), 100 nM (❑), 300 nM (●) and 1000 nM (▲); and Schild plots of the data (*right-hand-side panels*). Schild slopes (95% confidence intervals) of solifenacin and oxybutynin were 1.0 (0.46–1.6) and 0.98 (0.44–1.5) for solifenacin and oxybutynin, respectively. Each data point represents the mean±SEM of four or five strips

tors over M_2 receptors. This subtype selectivity contrasted with that of the M_3 subtype selective muscarinic antagonist darifenacin.

In the organ bath experiments, solifenacin and oxybutynin antagonized CCh-induced contraction of guinea pig detrusor strips (Fig. 2). The pK_b values of solifenacin and oxybutynin were 7.1±0.11 (N=15) and 7.4±0.09 (N=11), respectively. The slope of Schild plots (95% confidence intervals) was 1.0 (0.46–1.6) for solifenacin and 0.98 (0.44–1.5) for oxybutynin, and pA_2 values were equal to the pK_b values. Atropine caused the dose-dependent rightward shift at 3, 10 and 30 nM and produced a pK_b value of 8.4±0.10 (N=12), a Schild slope (95% confidence intervals) of 1.3 (0.78–1.8) and a pA_2 value of 8.3.

In Fura-2-loaded cells of guinea pig detrusor and murine submandibular glands, cytosolic Ca^{2+} mobilization occurred within the concentration range of 0.1 to 100 μM. The dose–activation curve of CCh for detrusor cells is depicted in Fig. 3 and that for gland cells is shown in Fig. 4. These curves had slopes of 1.1±0.080 in detrusor cells and 0.59±0.017 in gland cells. EC_{50} values of CCh for detrusor and gland cells were 1.3±0.12 μM (N=9) and 0.63±0.050 μM (N=28), respectively.

In detrusor cells, varying doses of test drugs were examined for their inhibitory effects on Ca^{2+} responses to 10 μM CCh stimulation (Fig. 3) and IC_{50} values were converted to K_i values using the CCh EC_{50} value and the Cheng–Prusoff equation since the curve of CCh had the slope of one (Lazareno and Birdsall 1993). Solifenacin and oxybutynin displayed pK_i values of 8.4±0.13 and 8.6±0.090, respectively. Their dose–inhibition curves were not parallel: the slopes were 0.88±0.24 for solifenacin and 1.9±0.20 for oxybutynin. This Ca^{2+} mobilization was reduced by 34% with 1 μM nicardipine. Effects of 1 μM solifenacin, oxybutynin and nicardipine on 40 mM KCl-induced Ca^{2+} influx were also examined (Fig. 3). In three of the four preparations, Ca^{2+} responses were detected. Inhibitory effects of solifenacin and oxybutynin were around 20% and that of nicardipine was 80%.

In murine submandibular gland cells, the antagonistic effects of 100 nM solifenacin and oxybutynin on Ca^{2+} mobilization evoked by varying doses of CCh were examined (Fig. 4). Solifenacin did not shift the CCh dose–activation curve in a parallel manner whereas oxybutynin showed insurmountable antagonism. The pK_b values were obtained as 7.4±0.17 for solifenacin and 8.8±0.21 for oxybutynin. The inhibitory effects of atropine and subtype preferring muscarinic antagonists were also evaluated at 100 nM. The rank order of potencies was atropine (pK_b, 9.9±0.053; N=4), 4-DAMP (M_3; pK_b, 9.6±0.077; N=4) >

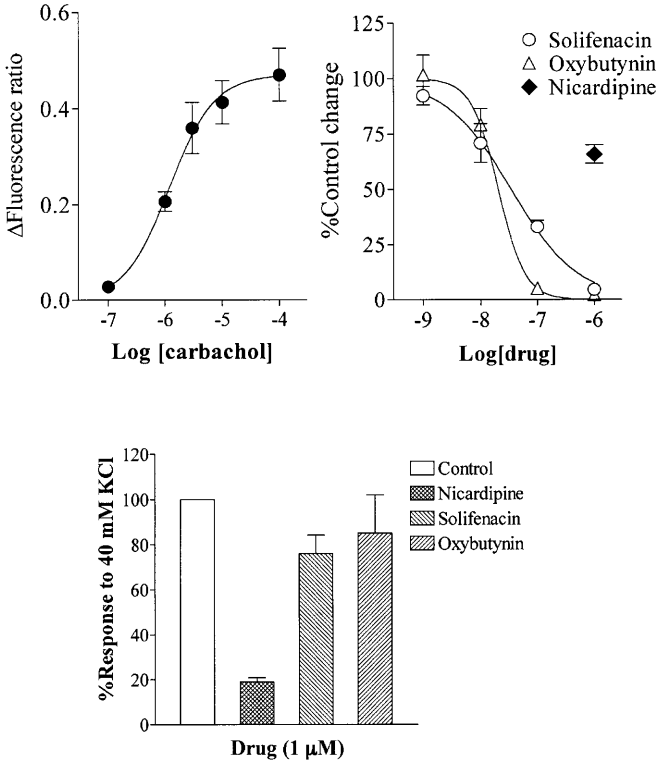

Fig. 3 Carbachol-induced Ca^{2+} mobilization, as determined by Fura 2 fluorometry, in guinea pig detrusor cells (*top left*; *N*=9; slope=1.1±0.080) and inhibitory effects of solifenacin, oxybutynin and nicardipine on the responses to 10 μM carbachol (*top right*; *N*=4). Effects of nicardipine, solifenacin and oxybutynin on KCl-evoked Ca^{2+} mobilization in detrusor cells (*bottom*; *N*=3). The control increase in fluorescence ratio by KCl was 0.087±0.023. Note nonparallel dose inhibition curves shown by solifenacin and oxybutynin

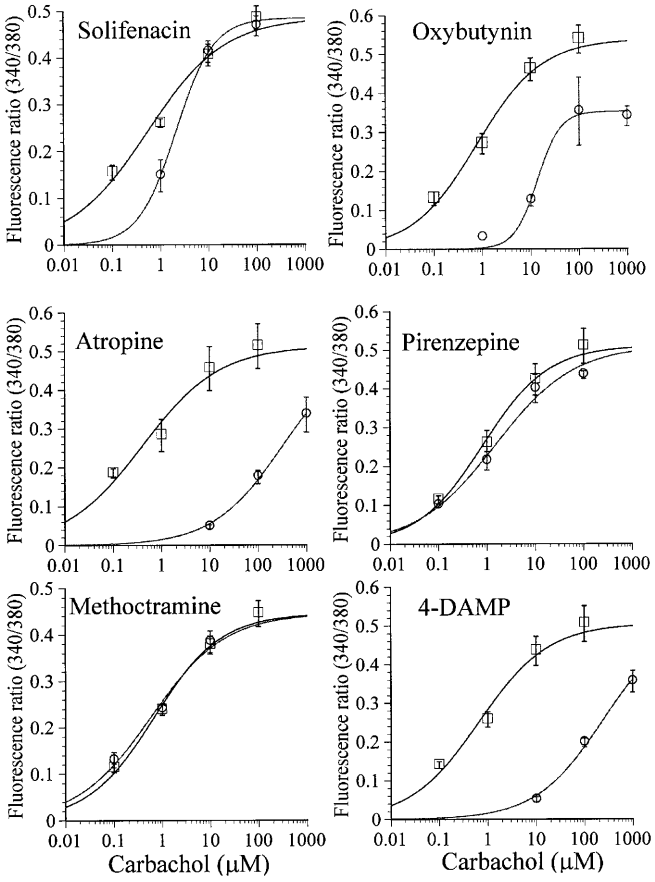

Fig. 4 Carbachol-induced Ca^{2+} mobilization, as determined by Fura 2 fluorescence, in murine submandibular gland cells in the absence (❑) or presence of 100 nM antagonist (❍). The slope of the dose–activation curves in the absence of antagonist was 0.59±0.017. Each curve was constructed using data from four independent experiments

pirenzepine (M_1; pK_b, 6.9±0.035; *N*=4) > methoctramine (M_2; pK_b, 6.1; obtained in two out of four experiments). In addition, the effect of 1 μM nicardipine on Ca^{2+} mobilization by 10 μM CCh in gland cells (*N*=3) was found to be negligible: changes in fluorescence ratios were 0.24±0.068 for control cells and 0.23±0.064 for nicardipine-treated cells.

Finally, bladder contractions, saliva secretion and decrease in heart rate were induced by 56 nmol kg^{-1} (10 μg kg^{-1}) of CCh in anesthetized rats. Typical chartgrams for solifenacin treatments are shown in Fig. 5, and Fig. 6 shows the percent inhibition of control responses by solifenacin, oxybutynin and darifenacin. Solifenacin reduced bladder responses by 40% at a dose of 210 nmol kg^{-1} (0.1 mg kg^{-1}) and abolished them at 2100 nmol kg^{-1} (1 mg kg^{-1}). In contrast, its inhibitory effects on salivary and cardiac responses were only slight at 630 nmol kg^{-1} (0.3 mg kg^{-1}), and reached 66% and 49%, respectively, at 2100 nmol kg^{-1} (1 mg kg^{-1}). At doses of 63 and 210 nmol kg^{-1} (0.03 and 0.1 mg kg^{-1}), solifenacin slightly increased saliva secretion. In contrast, oxybutynin equipotently inhibited bladder and salivary responses at doses up to 770 nmol kg^{-1} (0.3 mg kg^{-1}), leaving 41% of control responses in the heart. The ID_{50} values (Table 2) indicated that solifenacin was selective for the bladder by 3.7-fold over the salivary gland and by fivefold over the heart. By this logic, oxybutynin was 2.8-fold selective for the bladder over the heart but without any bladder over salivary gland selectivity. The M_3 receptor-selective antagonist darifenacin suppressed saliva secretion and bladder contraction at a dose of 230 nmol (0.1 mg)/kg without any effect on bradycardia. The control response in bladder pressure, saliva weight and heart rate for solifenacin were 32±2.4 cmH_2O, 46±1.7 mg and 270±9.8 beats per minute (bpm), respectively. These values were 32±2.7 cmH_2O, 49±1.8 mg and 270±14 bpm for oxybutynin; and 31±2.0 cmH_2O, 38±1.7 mg and 270±11 bpm for darifenacin.

Discussion

Blockade of M_3 receptors, which are important for bladder contraction and saliva secretion, often causes dry mouth when overactive bladder is treated with oxybutynin (Chapple 2000). The selectivity for M_3 over the M_2 subtype is implicated in dry mouth induction by oxybutynin

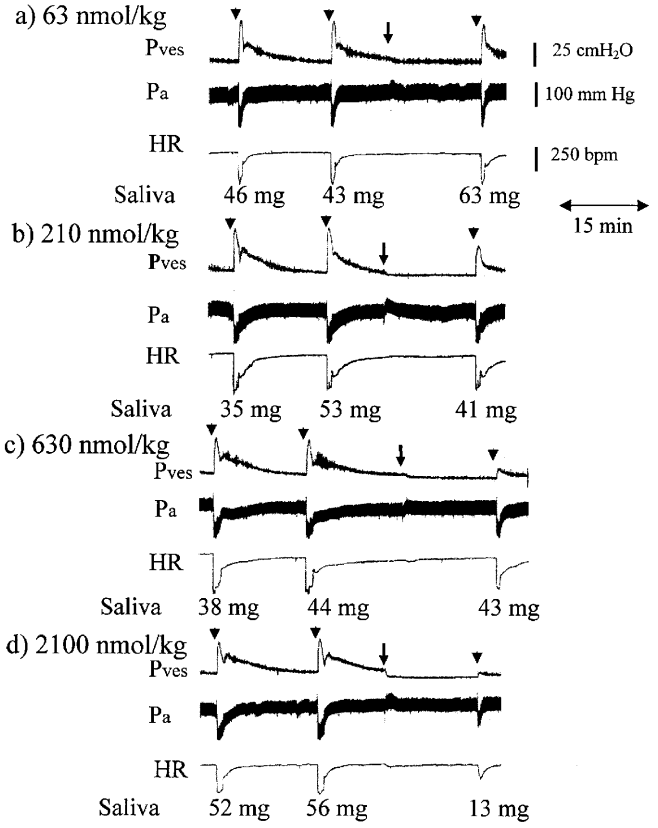

Fig. 5a–d Typical chartgrams showing the effects of different doses of solifenacin on carbachol-evoked bladder contraction, bradycardia and salivary secretion in single pentobarbital-anesthetized rats. *Pves*, *Pa* and *HR* represent traces of the bladder pressure, arterial pressure and heart rate, respectively. *Arrowheads* indicate carbachol administration and *arrows* indicate solifenacin injection. (Refer to the "Materials and methods" section for experimental protocols)

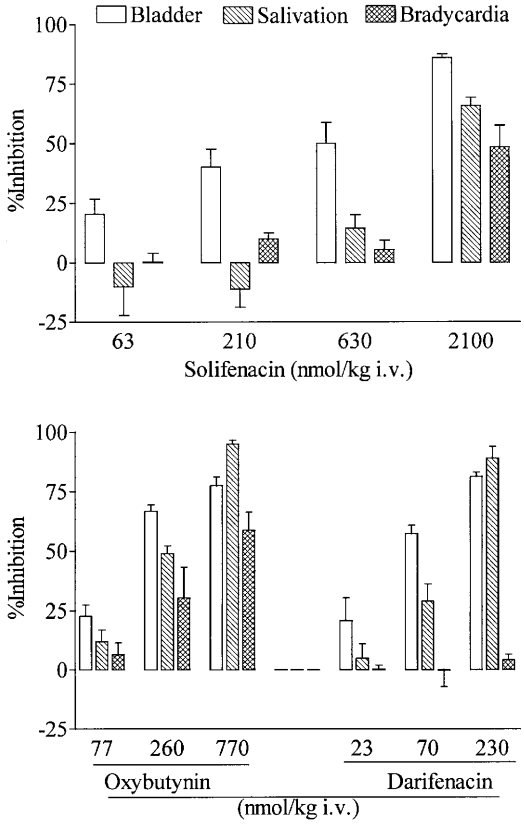

Fig. 6 Inhibitory effects of solifenacin (*top*), oxybutynin and darifenacin (*bottom*) on carbachol-induced bladder contraction, salivation and bradycardia in rats. Pentobarbital-anesthetized female rats were stimulated with 56 nmol kg^{-1} carbachol, given an antagonist and stimulated again with carbachol. Each *bar* represents the mean±SEM of four to seven rats

as tolterodine, a muscarinic antagonist without such subtype selectivity, shows preferential effects on in vivo bladder contraction to which M_2 receptors contribute (Gillberg et al. 1998). Furthermore, the incidence of dry mouth due to tolterodine treatment is low in clinical studies (Chapple 2000). On the other hand, the organ selectivity of a drug does not necessarily result from receptor selectivity due to a variety of mechanisms (Kenakin 1982). Indeed, an M_3 receptor selective antagonist was shown to be more potent against muscarinic bladder contraction (Kaiser et al. 1993). The working hypothesis for solifenacin is based on this latter thoughts.

In receptor binding assays, M_1, M_2 and M_3 subtypes were studied as their physiological roles are well documented among the five (M_1 to M_5) subtypes (Caulfield and Birdsall 1998). Solifenacin and oxybutynin were tenfold selective for M_1 and M_3 subtypes over the M_2 subtype. Against muscarinic contraction of isolated guinea pig detrusor, solifenacin and oxybutynin displayed comparable potencies. These antagonistic potencies were weaker than their binding affinities for M_3 receptors, which are thought to mediate detrusor contractility (Eglen et al. 1996). This dissociation was probably caused by restricted drug diffusion into structured tissues (Kenakin 1982), which hinders equilibrium conditions. In fact, published K_b values of oxybutynin in guinea pig detrusor vary from 4.4 to 42 nM (Kachur et al. 1988; Nilvebrant et al. 1997), so that the pK_b values range from 7.4 to 8.4. Solifenacin caused a steep CCh activation curve at 1000 nM, therefore its Schild plot with a slope of one may not exclude the possibility of noncompetitive antagonism, such as allosterism (Pöch et al. 1992).

Table 2 The 50% inhibitory doses (ID_{50}) of solifenacin, oxybytynin and darifenacin for simultaneously recorded responses to intravenous carbachol in anesthetized rats

	ID_{50} (nmol kg^{-1} i.v.)[a]		
	Bladder contraction	Saliva secretion	Bradycardia
Solifenacin	410 (240–700)	1500[b] (1100–2300)	ca. 2100
Oxybutynin	200 (160–250)	230 (200–270)	550 (330–1700)
Darifenacin	64 (48–84)	92 (75–110)	>230

[a]Data are expressed as means; the values in parentheses are 95% confidence limits
[b]Data points from 210 to 2100 nmol kg^{-1} were analyzed to obtain this ID_{50} value

Muscarinic Ca^{2+} mobilization is a key event in either detrusor contraction (Eglen et al. 1996) or fluid secretion from salivary glands (Ishikawa et al. 1998). M_3 subtypes were shown previously to mediate CCh-evoked Ca^{2+} flux of detrusor cells (Ikeda et al. 1999), from which pK_i values of atropine, 4-DAMP and methoctramine were obtained as 9.5±0.072 (N=4), 9.4±0.17 (N=4) and 6.7 (N=2), respectively. In mouse submandibular glands, the rank order of potencies of subtype-selective muscarinic antagonists clearly demonstrated the dominant role of M_3 receptors in Ca^{2+} mobilization. Although the time period (2 min) of preincubation with test drugs might cause incomplete equilibrium conditions, the results would be satisfactory. It should be noted that the potency estimates of atropine and other antimuscarinics against Ca^{2+} mobilization in this study agree with reported affinity constants of them for M_3 receptors (Caulfield and Birdsall 1998). Consequently, the organ selectivity of M_3 receptor antagonism was assessed between these cells. Since the pharmacology of muscarinic ligands is not significantly different across diverse species (Caulfield and Birdsall 1998), the current combination of cell sources was chosen among laboratory animals mainly for the following technical reasons: with regard to the Ca^{2+} mobilization of salivary gland cells, the muscarinic responses of guinea pig cells were too poor to test antagonist effects possibly due to poor incorporation of Fura 2; conversely, an enormous number of mice or rats would have been required to obtain meaningful data on the effects of antagonists on their bladders. Moreover, technical limitations, such as the number of assays run in each experiment, hampered the use of a theoretically ideal study design such as Schild analysis.

The inhibitory effect of solifenacin on detrusor cells (pK_i=8.4) was only slightly less potent than that of oxybutynin (pK_i=8.6). In contrast, the antagonistic potency of solifenacin in gland cells (pK_b=7.4) was apparently weaker than that of oxybutynin (pK_b=8.8). Although these estimates deviated only modestly from binding affinities for M_3 receptors, solifenacin had anomalous patterns of M_3 receptor blockage and appeared to be a preferential inhibitor of detrusor M_3 receptors when compared with oxybutynin. However, the quantitative difference in binding affinities for these M_3 receptors was not inferred because of genetic homogeneity of M_3 receptors (Caulfield and Birdsall 1998) and possible non-equilibrium assay conditions. A literature search revealed a marked pharmacological difference between the two types of cells: muscarinic Ca^{2+} mobilization in intestinal smooth muscle cells is partially sensitive to the L-type Ca^{2+} channel blocker nicardipine (Sato et al. 1994), whereas that in sublingual gland cells is dependent on capacitative Ca^{2+} channels (Melvin et al. 1991). In the present study, nicardipine partially reduced CCh-induced Ca^{2+} mobilization in detrusor cells but not in submandibular gland cells. Moreover, oxybutynin and other antimuscarinics indicated for overactive bladder show Ca^{2+} channel blocking effects (Wada et al. 1995). Thus, it had been hypothesized that blockade of L-type Ca^{2+} channels might make detrusor cells more susceptible to solifenacin, and the inhibitory effect on KCl-stimulated Ca^{2+} influx was examined. However, inhibitory effects of solifenacin on Ca^{2+} channels were too weak to explain its detrusor cell-selectivity. Nevertheless, the different susceptibility to nicardipine revealed that some mechanism leading to M_3 receptor-mediated Ca^{2+} mobilization differs between the two types of cells. Therefore, it is speculated that some difference in their cell physiology and the mode of M_3 receptor antagonism resulted in the detrusor selectivity of solifenacin.

In anesthetized rats, the selectivity indices indicated that solifenacin was only marginally selective for CCh-induced contraction of the bladder over salivary secretion and bradycardia. However, it should be noted that solifenacin at 630 nmol kg^{-1} suppressed bladder contraction with minor effects on the other responses in a single rat (Fig. 5). At lower doses, solifenacin slightly enhanced saliva secretion although its agonist action has not been detected. These observations were in contrast with the equal antagonism of bladder and salivary responses by oxybutynin. Then, the M_3 subtype-selective antagonist darifenacin was tested to determine the muscarinic receptor subtype involved in bladder contractions in this model as a significant role of M_2 receptors in the bladder was observed for reflex-evoked rhythmic contractions in rats (Hegde et al. 1997) and contractile responses to acetylcholine in cats (Gillberg et al. 1998). In the present study, darifenacin suppressed bladder contractions and saliva secretion equipotently without any effect on bradycardia. Muscarinic-evoked bradycardia and salivation are unanimously reported to be mediated by M_2 and M_3 receptors, respectively (Matsui et al. 2000; Stengel et al. 2000), and detrusors possess only M_2 and M_3 receptors (Wang et al. 1995). Therefore, the effects of darifenacin suggest a dominant role of M_3 receptors in CCh-induced bladder contractions in rats. The role of neuronal M_1 receptors, activation of which indirectly leads to bladder contraction via acetylcholine release (Noronha-Blob et al. 1991), could be ignored in this model as rats developing rhythmic bladder contractions, indicating activities of neurons innervating the bladder, were not analyzed. Accordingly, solifenacin inhibited bladder contraction more effectively than salivation in an in vivo model, although M_3 receptors were pivotal for both responses.

In conclusion, solifenacin showed receptor selectivity for the M_3 over M_2 subtype and tissue preference for the bladder over the salivary gland. Its exact mechanism of antimuscarinic effects remains to be clarified. Solifenacin is under investigation for its therapeutic usefulness in patients with overactive bladder. At present, the relative importance of M_2 and M_3 receptors in overactive bladder is an open question (Igawa 2000). Therefore, results of the clinical study of solifenacin may help to answer this question.

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