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# In vitro and in vivo tissue selectivity profile of solifenacin succinate (YM905) for urinary bladder over salivary gland in rats

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**Abstract**

Solifenacin succinate [YM905; (+)-(1*S*,3′*R*)-quinuclidin-3′-yl 1-phenyl-1,2,3,4-tetrahydroisoquinoline-2-carboxylate monosuccinate] is a new muscarinic receptor antagonist developed for the treatment of overactive bladder. The aim of the present study was to evaluate the in vitro and in vivo bladder selectivity profile of solifenacin over salivary gland in the same animal species, and to compare the results with those obtained for tolterodine, oxybutynin, darifenacin and atropine. Solifenacin and the other antimuscarinic drugs inhibited carbachol-induced increases in intracellular $Ca^{2+}$ levels in bladder smooth muscle cells and salivary gland cells isolated from rats in a concentration-dependent manner. The inhibitory effect of solifenacin for bladder smooth muscle cells ($pK_i = 8.12$) was 3.6-fold more potent than that for salivary gland cells ($pK_i = 7.57$). In contrast, the inhibitory effects of the other antimuscarinic drugs for bladder smooth muscle cells were 1.7- to 2.2-fold more potent than those for salivary gland cells. In anesthetized rats, solifenacin dose-dependently inhibited carbachol-induced intravesical pressure elevation and salivary secretion, and exhibited functional selectivity (3.7- to 6.5-fold) for urinary bladder over salivary gland. Tolterodine was also 2.2- to 2.4-fold more selective in inhibition of bladder response. In contrast, oxybutynin, darifenacin and atropine did not show functional selectivity for urinary bladder. These results indicate that solifenacin exerts greater selectivity for urinary bladder over salivary gland than tolterodine, oxybutynin, darifenacin and atropine, and may consequently provide symptomatic benefit in the treatment of overactive bladder with less dry mouth than currently used antimuscarinic drugs.


*Keywords:* Solifenacin; Urinary bladder; Salivary gland; Bladder selectivity

## 1. Introduction

Urinary bladder smooth muscle is predominantly innervated by parasympathetic cholinergic nerves. Acetylcholine, released from postganglionic cholinergic nerve terminals, activates postjunctional muscarinic receptors in urinary bladder. Muscarinic receptors mediate urinary bladder contraction during the voiding phase, and control detrusor tone during the filling phase. The urinary bladder smooth muscle contains a mixed population of muscarinic $M_2$ and $M_3$ receptors (Wang et al., 1995). Although muscarinic $M_2$ receptors are numerically predominant, muscarinic $M_3$ receptors, which constitute the minor population, are considered to be predominant in the mediation of bladder contraction (Longhurst et al., 1995; Hegde et al., 1997). In addition, the muscarinic $M_3$ receptor is the predominant subtype in submandibular gland, and regulates salivary secretion (Ishikawa et al., 1998; Moriya et al., 1999). An important functional role for the muscarinic $M_3$ receptor in mediating bladder contraction and salivary secretion has also been suggested in experiments using mutant mice lacking the muscarinic $M_3$ receptor gene (Matsui et al., 2000). These mutant mice display greatly reduced detrusor contraction to carbachol, and have impaired salivary secretion to cholinergic stimulation. Male mutant mice also develop urinary retention, highlighting the importance of the muscarinic $M_3$ receptor for micturition.

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Overactive bladder is characterized by symptoms of frequency and urgency with or without urge incontinence. It has a profoundly negative effect on the quality of life of those affected. With regard to treatment, muscarinic receptor antagonists are currently used for urinary urge incontinence and other symptoms of overactive bladder. However, although the effectiveness of available drugs such as oxybutynin has been demonstrated in several clinical studies, the relatively high incidence of adverse effects with these drugs often leads to the discontinuation of treatment (Moore et al., 1990; Thuroff et al., 1991). The most common adverse effect is dry mouth (xerostomia) due to blockade of muscarinic $M_3$ receptors in salivary gland. Thus, new drugs that have a better balance between efficacy and tolerability as a consequence of greater selectivity for urinary bladder are desired. Recently, new antimuscarinic drugs such as tolterodine, a non-selective muscarinic receptor antagonist (Nilvebrant et al., 1997; Clemett and Jarvis, 2001), and darifenacin, a muscarinic $M_3$ receptor selective antagonist (Nunn et al., 1996), have been developed for the treatment of overactive bladder. These exhibit modest bladder selectivity over salivary gland as compared with oxybutynin in rats (Williamson et al., 1997) and cats (Nilvebrant et al., 1997; Gillberg et al., 1998).

Solifenacin succinate [YM905; (+)-(1*S*,3′*R*)-quinuclidin-3′-yl 1-phenyl-1,2,3,4-tetrahydroisoquinoline-2-carboxylate monosuccinate] is a new muscarinic receptor antagonist developed for the treatment of overactive bladder. The affinity constants ($pK_i$) of solifenacin for recombinant human muscarinic $M_1$, $M_2$ and $M_3$ receptors are 7.6, 6.9 and 8.0, respectively (Ikeda et al., 2002). Hence, the muscarinic $M_3$ receptor subtype selectivity of solifenacin over the muscarinic $M_2$ receptor subtype is readily apparent, but is only marginal over the muscarinic $M_1$ receptor subtype. In the same study, solifenacin and oxybutynin inhibited muscarinic $M_3$ receptor-mediated intracellular $Ca^{2+}$ mobilization in bladder smooth muscle cells isolated from guinea pigs and in submandibular gland cells isolated from mice (Ikeda et al., 2002). However, direct comparison of the antimuscarinic potencies of solifenacin between urinary bladder and submandibular gland in this study was not appropriate, owing to differences in the two assays in species and experimental design. The present study was therefore undertaken to evaluate the in vitro and in vivo bladder selectivity profile of solifenacin in the same animal species, and to compare the results with those of tolterodine, oxybutynin, darifenacin and atropine.

## 2. Materials and methods

### 2.1. Drugs used

Solifenacin succinate (YM905), tolterodine tartrate and darifenacin were synthesized by Yamanouchi Pharmaceutical Co., Ltd (Tokyo, Japan). Oxybutynin chloride, atropine sulfate and carbachol (carbamylcholine chloride) were purchased from Sigma-Aldrich (St. Louis, MO, USA). Darifenacin was dissolved in dimethyl sulfoxide or 0.1 N HCl and the others were dissolved in physiological saline.

### 2.2. In vitro study

#### 2.2.1. Preparation of dispersed bladder smooth muscle cells and submandibular gland cells

All animal experiments were performed in compliance with the regulations of the Institutional Animal Ethical Committee of Yamanouchi Pharmaceutical Co. Ltd., Male Wistar rats (aged 11–13 weeks, Charles River Japan, Kanagawa, Japan) were sacrificed by exsanguination under anesthesia with diethylether, and the urinary bladder and submandibular glands were immediately removed. Bladder smooth muscle cells and submandibular gland cells were prepared according to previously reported methods (Ikeda et al., 2002) with minor modification. Urinary bladders were collected from 24 rats per experiment. After removal of the epithelium, the isolated bladder tissues were minced into small pieces and gently agitated in 40 ml of $Ca^{2+}$/$Mg^{2+}$-free Hanks' balanced salt solution (HBSS, pH 7.4; Invitrogen, Carlsbad, CA) supplemented with 0.8 mM $MgSO_4$, 20 mM HEPES, 0.1% (w/v) bovine serum albumin, 75 mg papain (from Papaya Latex; Sigma-Aldrich), 24 mg hyaluronidase (from bovine testis; Sigma-Aldrich) and 15 mg dithiothreitol for 30 min at 37 °C after oxygenation of the solution for 10 s. The bladder tissue was washed in phosphate-buffered saline (PBS) and then digested in 40 ml of $Ca^{2+}$/$Mg^{2+}$-free HBSS supplemented with 0.8 mM $MgSO_4$, 20 mM HEPES, 0.1% (w/v) bovine serum albumin and 50 mg collagenase S-I (from *Streptomyces parvulus* subsp. *citrinus.*; Nitta Gelatin, Osaka, Japan) for an additional 50 min at 37 °C. The dispersed single smooth muscle cells were yielded by filtration through a 250-μm nylon mesh. The digestion was terminated by adding 250 mM EGTA solution to give a final concentration of 2.5 mM. Undigested bladder tissue fragments were again dispersed in a collagenase solution by the same procedure. This step was repeated several times until the remaining tissues were almost completely dispersed. Finally, the collected smooth muscle cells were washed in PBS and suspended in 15–30 ml of phenol red-free HBSS containing 20 mM HEPES and 0.1% (w/v) bovine serum albumin (HBSS-H/B).

Submandibular glands were collected from 2 rats per experiment. They were minced and then dispersed in 20 ml of HBSS-H/B supplemented with 20 mg collagenase, 6 mg hyaluronidase and oxygen gas for 50 min at 37 °C with gentle agitation. The dispersed gland cells were collected by filtration through a 100-μm nylon mesh. The undigested gland tissues were once again dispersed by the same procedure. The collected gland cells were washed and resuspended in HBSS-H/B. The prepared bladder smooth muscle cells and submandibular gland cells were stored at 4 °C, and used for the studies within 25 h of preparation.

*2.2.2. Measurement of intracellular $Ca^{2+}$ mobilization*

The protocol was in accordance with that reported previously (Ikeda et al., 2002) with minor modifications. For bladder smooth muscle cells, an aliquot of the suspension was incubated in HBSS-H/B suspended with 4 μM Fura 2-acethoxymethyl ester (Fura 2-AM, Dojindo Laboratories, Kumamoto, Japan) for 30 min at 37 °C. The bladder cells were washed and then incubated in HBSS-H/B for 20 min at 37 °C. Submandibular gland cells were exposed to Fura 2-AM at the same concentration for 60 min at 37 °C. The gland cells were then washed and resuspended in HBSS-H/B. For measurement of intracellular $Ca^{2+}$ concentration, the Fura-2 fluorescence of a 490 μl aliquot of the cell suspension in a glass cuvette maintained at 37 °C was monitored as the ratio of fluorescence intensities excited at wavelengths of 340 and 380 nm (R340/380) using a $Ca^{2+}$ analyzer, CAF-100 (JASCO, Tokyo, Japan), with an emission wavelength of 500 nm. Intracellular $Ca^{2+}$ mobilization was induced by adding 5 μl of a carbachol solution to the cells. In each cell preparation, the carbachol concentration–response relationship was investigated. Muscarinic receptor antagonists were injected into the cuvette in a volume of 5 μl and incubated for 2 min before stimulation with 10 μM carbachol, which produces a submaximal $Ca^{2+}$ response.

*2.3. In vivo study*

Female Wistar rats (Charles River Japan) weighing 240–315 g were anesthetized intraperitoneally (i.p.) with pentobarbital sodium (60 mg/kg). To control the physiological condition of the rat, blood pressure was measured with a pressure amplifier (AP-601G, Nihon Kohden, Tokyo, Japan) via a pressure transducer (TP-400T, Nihon Kohden) connected to a polyethylene catheter (PE-50) inserted into the carotid artery, and heart rate was measured with a tachometer (AT-601G, Nihon Kohden) triggered by the blood pressure pulse wave. A polyethylene catheter (PE-50) with a side-hole near the tip was inserted into the bladder via the urethra. The catheter was secured by a purse-string suture around the urethral orifice. The bladder was emptied by drainage of urine through the catheter, then less than 1 ml of physiological saline was infused into the bladder and the intravesical pressure was measured with a pressure amplifier (AP-601G, Nihon Kohden) via a pressure transducer (TP-400T, Nihon Kohden) connected to the catheter. The tail vein was cannulated with an injection needle (27 gauge) to allow injection of carbachol and muscarinic receptor antagonists. Saliva was collected using sections of filter paper (8 × 50 mm, Advantec Toyo, Tokyo, Japan) inserted into the oral cavity. Following a stabilization period after surgery of at least 30 min, intravenous (i.v.) administration of carbachol (0.01 mg/kg) producing a submaximal response was performed three times at about 15 min intervals. The maximal increase in intravesical pressure and amount of saliva collected for 10 min after administration of carbachol were evaluated. The average value of the second and third carbachol responses was defined as the baseline. Thereafter, physiological saline (2 ml/kg) or a muscarinic receptor antagonist was given by i.v. administration 10 min prior to the i.v. administration of carbachol (0.01 mg/kg). Two doses (10-fold ratio) of each antagonist except solifenacin (one or two doses) were administered to each rat at about 25 min intervals. The filter papers were weighed using an electronic balance (ER-182A, A&D, Tokyo, Japan), and the weight of salivary secretion was calculated as the difference in the weight of the paper before and after the experiment.

*2.4. Statistical analysis*

In vitro responses to carbachol were obtained as the peak increase in R340/380 ratio following carbachol stimulation. $EC_{50}$ and $IC_{50}$ values, the concentration required to induce 50% stimulatory and inhibitory responses, were calculated from carbachol concentration–response and antagonist concentration–inhibition curves fitted to a logistic equation (DeLean et al., 1978), respectively. The affinity constant ($pK_i$) was calculated using the Cheng–Prusoff equation (Cheng and Prusoff, 1973; Lazareno and Birdsall, 1993):

$$pK_i = -log(IC_{50}/([\text{carbachol}(1 \times 10^{-5}\ \text{M})]/EC_{50} + 1))$$

All data are expressed as the mean ± S.E.M. $pK_i$ values obtained from bladder smooth muscle cells and submandibular gland cells were compared using Student's *t*-test.

The effects of muscarinic receptor antagonists on carbachol-induced intravesical pressure elevation and salivary secretion are expressed as percentage inhibition of the respective baseline carbachol responses. $ID_{30}$ and $ID_{50}$ values, the doses required to induce 30% and 50% inhibition of the baseline value, respectively, were determined by linear regression analysis. Statistical differences of the inhibitory effect of antagonists from control (physiological saline) were analyzed using Student's *t*-test with actual measurement values. In addition, statistical differences between the inhibitory effects exerted by the antagonist on intravesical pressure elevation and salivary secretion were analyzed for each dose level using the paired Student's *t*-test with percentage inhibition values.

All data analyses were performed using the SAS statistical software (SAS Institute, Cary, NC, USA). Differences with a $P < 0.05$ were considered statistically significant.

## 3. Results

*3.1. In vitro study*

As shown in Figs. 1A and 2A, carbachol (0.03–100 μM) induced increases in intracellular $Ca^{2+}$ levels in bladder smooth muscle cells and submandibular gland cells isolated from rats in a concentration-dependent manner. $EC_{50}$ values

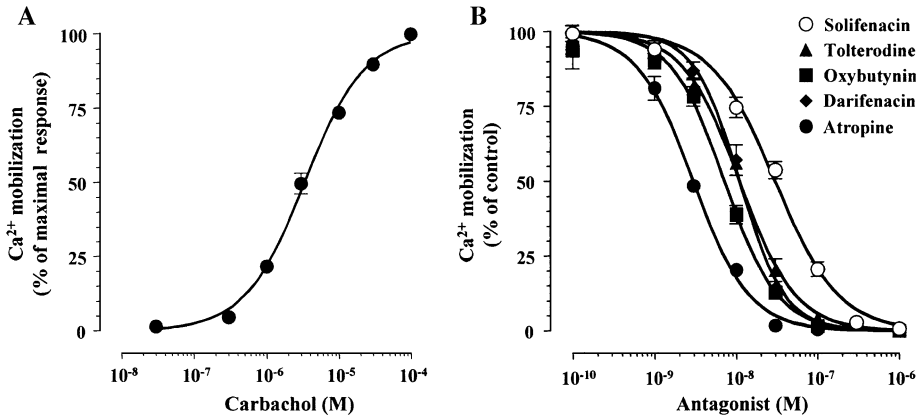


Fig. 1. (A) Concentration–response relationship for carbachol-induced intracellular $Ca^{2+}$ mobilization in bladder smooth muscle cells isolated from rats. Each point represents the mean ± S.E.M. of five separate experiments. (B) Effects of solifenacin, tolterodine, oxybutynin, darifenacin and atropine on intracellular $Ca^{2+}$ mobilization induced by 10 μM carbachol in bladder smooth muscle cells isolated from rats. Each point represents the mean ± S.E.M. of five separate experiments.

of carbachol for bladder smooth muscle cells and submandibular gland cells were 3.4 ± 0.26 and 2.3 ± 0.14 μM, respectively. In these cells, solifenacin, tolterodine, oxybutynin, darifenacin and atropine inhibited $Ca^{2+}$ mobilization induced by 10 μM carbachol in a concentration-dependent manner (Figs. 1B and 2B). p$K_i$ values of solifenacin, tolterodine, oxybutynin, darifenacin and atropine in bladder smooth muscle cells and submandibular gland cells are shown in Table 1. The inhibitory potencies of all muscarinic receptor antagonists for bladder smooth muscle cells were significantly greater than those for submandibular gland cells. Solifenacin showed a 3.6-fold bladder selectivity, calculated as the ratio of affinity constants in urinary bladder over submandibular gland.

### 3.2. In vivo study

Intravesical pressure elevation and salivary secretion were immediately induced after administration of carbachol (0.01 mg/kg i.v.) in anesthetized rats. Salivary secretion had almost completely ceased at 5 min after administration of carbachol. The baseline values of carbachol-induced intravesical pressure elevation and salivary secretion in each group ranged from 12.6 to 14.5 mm Hg and from 216 to 244 mg, respectively. Bladder and salivary responses in the control group were relatively stable during the experimental period (change less than ± 7%).

The effects of solifenacin, tolterodine, oxybutynin, darifenacin and atropine on carbachol-induced intravesical pressure elevation and salivary secretion are shown in Table 2 and Fig. 3. Solifenacin (0.03–1 mg/kg i.v.) dose-dependently and significantly suppressed increases in intravesical pressure. Although solifenacin had no effect on salivary secretion at doses up to 0.1 mg/kg i.v., it showed more than about 50% inhibition at 0.3 mg/kg i.v. However, solifenacin showed significantly more potent inhibition of bladder responses over salivary responses at all doses tested, with $ID_{30}$ and $ID_{50}$ values indicating 6.5- and 3.7-fold greater selectivity for urinary bladder, respectively. Tolterodine (0.01–0.1 mg/kg i.v.) also suppressed intravesical pressure

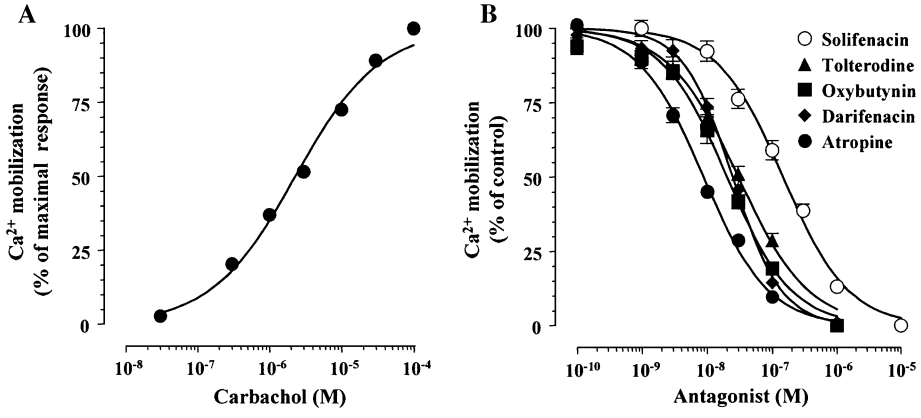


Fig. 2. (A) Concentration–response relationship for carbachol-induced intracellular $Ca^{2+}$ mobilization in submandibular gland cells isolated from rats. Each point represents the mean ± S.E.M. of five separate experiments. (B) Effects of solifenacin, tolterodine, oxybutynin, darifenacin and atropine on intracellular $Ca^{2+}$ mobilization induced by 10 μM carbachol in submandibular gland cells isolated from rats. Each point represents the mean ± S.E.M. of five separate experiments.

Table 1
Antagonist affinity constants ($pK_i$) of solifenacin, tolterodine, oxybutynin, darifenacin and atropine for carbachol-induced intracellular $Ca^{2+}$ mobilization in bladder smooth muscle cells and submandibular gland cells isolated from rats

| Antagonist | $pK_i$ | | Selectivity ratio (S/B) |
|---|---|---|---|
| | Bladder smooth muscle cells (B) | Submandibular gland cells (S) | |
| Solifenacin | $8.12 \pm 0.04^b$ | $7.57 \pm 0.06$ | 3.6 |
| Tolterodine | $8.55 \pm 0.05^b$ | $8.26 \pm 0.07$ | 2.0 |
| Oxybutynin | $8.75 \pm 0.05^b$ | $8.43 \pm 0.08$ | 2.1 |
| Darifenacin | $8.57 \pm 0.05^a$ | $8.35 \pm 0.06$ | 1.7 |
| Atropine | $9.13 \pm 0.03^b$ | $8.79 \pm 0.04$ | 2.2 |

Each value represents the mean ± S.E.M. of five separate experiments. Significant differences between $pK_i$ values of bladder smooth muscle cells and submandibular gland cells were seen for each drug tested ($^aP<0.05$, $^bP<0.01$; Student's *t*-test).

elevation and salivary secretion in a dose-dependent fashion. Tolterodine showed significantly more potent inhibition of bladder responses at doses of 0.01 and 0.1 mg/kg i.v. The $ID_{30}$ and $ID_{50}$ values indicated that tolterodine was selective for urinary bladder by 2.4- and 2.2-fold, respectively. In contrast, oxybutynin (0.03–0.3 mg/kg i.v.), darifenacin (0.03–0.1 mg/kg i.v.) and atropine (0.003–0.03 mg/kg i.v.) dose-dependently suppressed intravesical pressure elevation and salivary secretion, but did not show obvious functional selectivity for urinary bladder. The estimated rank order of bladder selectivity was therefore solifenacin>tolterodine>oxybutynin = darifenacin = atropine.

## 4. Discussion

The generation of abnormal bladder contractions in disease states as well as normal physiological voiding are both critically dependent on acetylcholine-induced stimulation of muscarinic receptors in the bladder smooth muscle. Although muscarinic $M_2$ receptors are the predominant subtype in urinary bladder (Wang et al., 1995), muscarinic $M_3$ receptors, which are the minor population, are considered to be predominant in the mediation of bladder contraction (Longhurst et al., 1995; Hegde et al., 1997). In submandibular gland, muscarinic $M_3$ receptors also play a predominant role in salivary secretion (Ishikawa et al., 1998; Moriya et al., 1999). It has been reported that muscarinic receptors are coupled to several signaling systems (Fukuda et al., 1989). In general, activation of muscarinic $M_1$ and $M_3$ receptors preferentially mediates intracellular $Ca^{2+}$ mobilization by augmenting phosphoinositide hydrolysis, whereas activation of muscarinic $M_2$ receptors inhibits adenylate cyclase activity (Ashkenazi et al., 1989). In the urinary bladder, activation of muscarinic $M_3$ receptors induces phosphoinositide hydrolysis (Andersson et al., 1991; Harriss et al., 1995), and the generation of inositol phosphates in turn leads to increases in intracellular $Ca^{2+}$ levels. In contrast, activation of muscarinic $M_3$ receptors leads to phosphoinositide hydrolysis and adenylate cyclase inhibition in the submandibular gland (Laniyonu et al., 1990; Dai et al., 1991), resulting in intracellular $Ca^{2+}$ mobilization followed by salivary secretion. Consequently, since intracellular $Ca^{2+}$ mobilization is a key event in both detrusor contraction and salivary secretion, the tissue selectivity of muscarinic receptor antagonists can be evaluated by estimating intracellular $Ca^{2+}$ mobilization in these cells. In a previous study, solifenacin inhibited carbachol-induced intracellular $Ca^{2+}$ mobilization in guinea pig bladder smooth muscle cells and murine submandibular gland cells (Ikeda et al., 2002). However, a direct comparison of potency between the two tissues could not be made, because species and experimental design in the two assays were different.

In the present study, the antimuscarinic effects of solifenacin were tested using bladder smooth muscle cells and submandibular gland cells isolated from rats. Although it might be thought that the time period used for incubation with muscarinic receptor antagonists (2 min) may result in incomplete equilibrium conditions, this period was selected not to allow the observation of Fura 2-AM leakage from cells over time. Since the $pK_i$ values of all muscarinic receptor antagonists in this study agree with their reported affinity constants for muscarinic $M_3$ receptors, the present results are considered satisfactory (Ikeda et al., 2002; Nilvebrant et al., 1997). Tissue preference could be estimated by direct comparison of affinity constants between the two tissues. The $pK_i$ value

Table 2
Inhibitory effects of solifenacin, tolterodine, oxybutynin, darifenacin and atropine on carbachol-induced intravesical pressure elevation and salivary secretion in anesthetized rats

| Antagonist | $ID_{30}$ value (95% confidence limits) | | Selectivity ratio (S/B) | $ID_{50}$ value (95% confidence limits) | | Selectivity ratio (S/B) |
|---|---|---|---|---|---|---|
| | Intravesical pressure elevation (B) | Salivary secretion (S) | | Intravesical pressure elevation (B) | Salivary secretion (S) | |
| Solifenacin | 0.023 (0.010–0.039) | 0.15 (0.11–0.24) | 6.5 | 0.11 (0.08–0.14) | 0.41 (0.33–0.52) | 3.7 |
| Tolterodine | 0.010 (0.008–0.014) | 0.024 (0.016–0.047) | 2.4 | 0.026 (0.021–0.033) | 0.058 (0.044–0.083) | 2.2 |
| Oxybutynin | 0.027 (0.015–0.045) | 0.030 (0.024–0.038) | 1.1 | 0.067 (0.050–0.090) | 0.065 (0.056–0.077) | 0.97 |
| Darifenacin | 0.0098 (0.0064–0.0150) | 0.012 (0.009–0.017) | 1.2 | 0.024 (0.018–0.033) | 0.025 (0.020–0.031) | 1.0 |
| Atropine | 0.0022 (0.0015–0.0030) | 0.0022 (0.0017–0.0026) | 1.0 | 0.0056 (0.0043–0.0071) | 0.0048 (0.0040–0.0058) | 0.86 |

$ID_{30}$ and $ID_{50}$ values (mg/kg i.v.) are the doses required to induce 30% and 50% inhibition from baseline values respectively, and were determined by linear regression analysis ($n=6$).

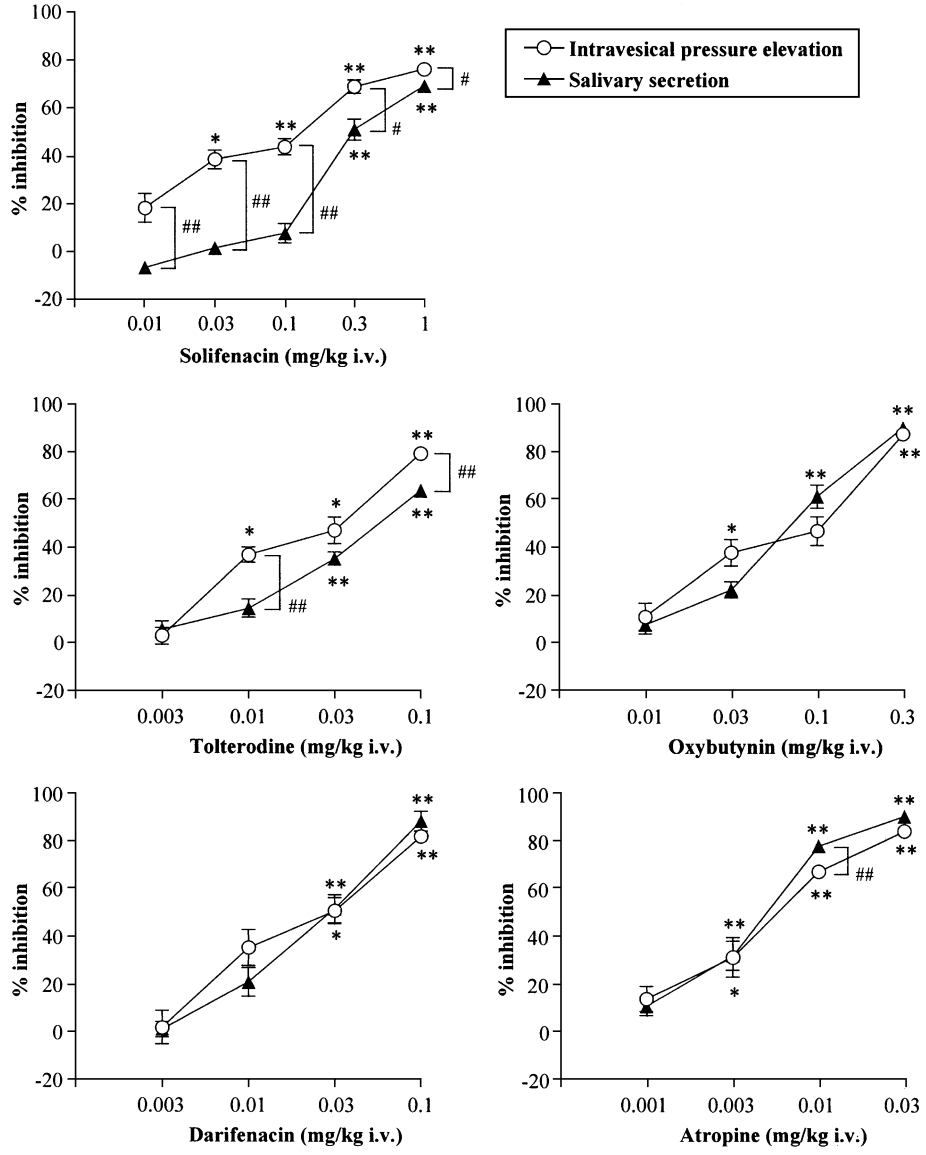


Fig. 3. Effects of solifenacin, tolterodine, oxybutynin, darifenacin and atropine on carbachol-induced intravesical pressure elevation and salivary secretion in anesthetized rats. Data are expressed as percentage inhibition of the baseline carbachol (0.01 mg/kg i.v.) response. Each point represents the mean ± S.E.M. of six rats. $^{*}P<0.05$, $^{**}P<0.01$, significant difference from control (Student's *t*-test with actual measurement values). $^{\#}P<0.05$, $^{\#\#}P<0.01$, significant difference between inhibition of intravesical pressure elevation and salivary secretion (paired Student's *t*-test).

of solifenacin for bladder smooth muscle cells ($pK_i = 8.12$) was significantly greater than that for submandibular gland cells ($pK_i = 7.57$), showing 3.6-fold greater selectivity for urinary bladder. The present in vitro results, obtained by direct comparison of solifenacin using tissues of the same animal species, provide the first evidence that solifenacin possesses bladder selectivity. Given that solifenacin, oxybutynin and darifenacin have higher affinity for muscarinic $M_3$ receptors than other subtypes (Ikeda et al., 2002; Nilvebrant et al., 1997; Gillberg et al., 1998) whereas tolterodine is non-selective (Nilvebrant et al., 1997), it is unlikely that differences in subtype selectivity among muscarinic receptors is the only factor determining bladder selectivity.

One possible explanation for bladder selectivity is heterogeneity among muscarinic $M_3$ receptors, notwithstanding that only one muscarinic $M_3$ receptor has been identified using molecular sequencing (Eglen and Watson, 1996). In support of this, the previous finding that carbachol-induced $Ca^{2+}$ mobilization was sensitive to the L-type $Ca^{2+}$ channel blocker nicardipine in bladder smooth muscle but not in submandibular gland suggests a difference between the two cell types in $Ca^{2+}$ entry pathway (Ikeda et al., 2002). In addition, muscarinic $M_3$ receptors are coupled to a single signal transduction pathway in the urinary bladder (Andersson et al., 1991; Harriss et al., 1995), but to two pathways in the submandibular gland (Laniyonu et al., 1990; Dai et al., 1991). The bladder selectivity of solifenacin may

therefore result from differences in the mode of muscarinic $M_3$ receptor antagonism and cell physiology.

In anesthetized rats, solifenacin dose-dependently inhibited carbachol-induced intravesical pressure elevation and salivary secretion. The effect of solifenacin on salivary response was observed at higher doses only, while that on bladder response was shown at a wide dose range. For this reason, the efficacy of muscarinic receptor antagonists was compared by calculating both $ID_{30}$ and $ID_{50}$ values. The bladder selectivity ratio (6.5-fold) calculated from $ID_{30}$ values was greater than that (3.7-fold) from $ID_{50}$ values. Because solifenacin showed only modest bladder selectivity (3.6-fold) over salivary gland in the present in vitro study, these results cannot fully explain the more outspoken effects observed in the in vivo study. It was thought that bladder selectivity of solifenacin might not be reflective of its plasma half-life, because intravenously administered solifenacin was eliminated from plasma with a half-life of about 1 h in rats (data not shown). With respect to the in vivo study, the results might be explained by differences in the distribution and elimination profiles of solifenacin in the target tissues. Further, tolterodine showed somewhat more potent inhibition of the bladder response than the salivary response, although to a lesser extent than that seen with solifenacin. In contrast, oxybutynin, darifenacin and atropine did not show functional selectivity for urinary bladder, although tolterodine, oxybutynin, darifenacin and atropine had almost the same selectivity ratio in the present in vitro study. These findings therefore indicate differences in pharmacokinetics among these muscarinic receptor antagonists. In previous studies, tolterodine exhibited modest selectivity for urinary bladder over salivary gland in anesthetized cats, while oxybutynin, darifenacin and atropine were equipotent in these tissues (Nilvebrant et al., 1997; Gillberg et al., 1998). These data accord with the present in vivo results. On the other hand, it has been reported that darifenacin decreased micturition pressure at lower doses than those required to inhibit methacholine-induced salivary secretion in conscious rats (Williamson et al., 1997). The respective evaluations for bladder and salivary responses, however, were performed in different rats. In addition, salivary secretion was subjectively evaluated by a scoring system with possibly low sensitivity.

It was recently reported that muscarinic $M_2$ receptors play a functional role in bladder contraction (Gillberg et al., 1998). Both prejunctional facilitatory ($M_1$) and inhibitory ($M_2$) muscarinic receptors (Somogyi et al., 1994; Braverman et al., 1998) have been shown to be present on cholinergic nerves in urinary bladder. However, their participation could not be confirmed in the present study.

In conclusion, these findings suggest that solifenacin is a muscarinic receptor antagonist with functional selectivity for urinary bladder over salivary gland. The mechanism by which solifenacin exhibits bladder selectivity remains to be elucidated, however. Solifenacin may provide symptomatic benefit in the treatment of overactive bladder with less dry mouth than currently prescribed muscarinic receptor antagonists. In this regard we note with interest that in clinical studies, solifenacin at 5 and 10 mg/man was shown to be more effective than placebo in improving overactive bladder symptoms with a low incidence of dry mouth (Chapple et al., 2002; Cardozo et al., 2003).

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