Pharmacological Characterization of a New Antimuscarinic Agent, Solifenacin Succinate, in Comparison with Other Antimuscarinic Agents

Akiyoshi Ohtake,* Chikashi Saitoh, Hironori Yuyama, Masashi Ukai, Hiroko Okutsu, Yukiko Noguchi, Toshiki Hatanaka, Masanori Suzuki, Shuichi Sato, Masao Sasamata, and Keiji Miyata

Pharmacology Research Labs., Drug Discovery Research, Astellas Pharma Inc.; 21 Miyukigaoka, Tsukuba, Ibaraki, 305–8585, Japan.

Solifenacin succinate [YM905; (3*R*)-1-azabicyclo[2.2.2]oct-3-yl(1*S*)-1-phenyl-3,4-dihydroisoquinoline-2(1*H*)-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 antimuscarinic properties of solifenacin and to compare the results with those obtained for tolterodine, oxybutynin, darifenacin, propiverine and atropine. In radioligand receptor binding assay, K_i values of solifenacin for human muscarinic M_1, M_2, M_3, M_4 and M_5 receptors were 26, 170, 12, 110 and 31 nM, respectively. In isolated rat urinary bladder, solifenacin competitively antagonized carbachol-induced contractions, with a pA_2 value of 7.44 ± 0.09. In these *in vitro* studies, the antimuscarinic action of solifenacin was more potent than that of propiverine and less potent than those of tolterodine, oxybutynin, darifenacin and atropine. In anesthetized rats, solifenacin and oxybutynin increased the maximum bladder capacity in a dose-dependent manner and also decreased the maximum intravesical pressure. The dosages required to produce a 30% increase in maximum bladder capacity (ED_{30} values) of solifenacin and oxybutynin were 0.35 and 0.30 mg/kg i.v., respectively, indicating approximately equal efficacies. These results support the fact that solifenacin, similarly to currently used antimuscarinic agents, is an effective agent in the treatment of overactive bladder symptoms such as urinary frequency and urge incontinence.

Key words   solifenacin; muscarinic receptor; urinary bladder; bladder capacity

Urinary bladder smooth muscle is innervated by both sympathetic and parasympathetic nerves. Acetylcholine released from postganglionic parasympathetic nerve terminals activates postjunctional muscarinic receptors in urinary bladder, which modulate urinary bladder contraction during the voiding phase and control detrusor tone during the filling phase. Five muscarinic receptor subtypes (M_1—M_5) have been identified by both molecular biological and pharmacological investigations.[1] The urinary bladder smooth muscle contains a mixed population of muscarinic M_2 and M_3 receptors.[2] Although muscarinic M_2 receptors are numerically predominant, muscarinic M_3 receptors are considered to predominate in the mediation of bladder contraction.[3,4] An important functional role of the muscarinic M_3 receptor in mediating bladder contraction has also been suggested in experiments using mutant mice lacking the muscarinic M_3 receptor gene.[5]

Overactive bladder is characterized by symptoms of urgency and urinary frequency with or without urge incontinence. It has a profoundly negative effect on the quality of life of those affected. Muscarinic receptor antagonists are the most widely used therapy for overactive bladder.[6—8] Solifenacin succinate [YM905; (3*R*)-1-azabicyclo[2.2.2]oct-3-yl(1*S*)-1-phenyl-3,4-dihydroisoquinoline-2(1*H*)-carboxylate monosuccinate] is a new muscarinic receptor antagonist developed for the treatment of overactive bladder. Affinity constants (K_i values) of this drug for human muscarinic M_1, M_2 and M_3 receptors only have been reported, along with its antagonism of the contractile effect of carbachol in isolated guinea pig urinary bladder.[9] The present study was therefore undertaken to investigate the affinity of solifenacin for all human muscarinic receptor subtypes (M_1—M_5) and its functional muscarinic M_3 receptor antagonism in rats, and to compare the results with those for tolterodine, oxybutynin, darifenacin, propiverine and atropine. Additionally, we also investigated the effect of solifenacin on voiding function in anesthetized rats.

MATERIALS AND METHODS

Materials   Solifenacin succinate (YM905, Vesicare®), tolterodine tartrate, darifenacin and propiverine hydrochloride were prepared by Astellas Pharma Inc. (Tokyo, Japan). Oxybutynin chloride, atropine sulfate and carbachol (carbamylcholine chloride) were purchased from Sigma-Aldrich (St. Louis, MO, U.S.A.). Darifenacin was dissolved in dimethyl sulfoxide and the others were dissolved in dimethyl sulfoxide, Krebs–Henseleit solution or physiological saline.

Animals   Male Wistar rats and male Sprague-Dawley rats were purchased from Charles River Laboratories Japan (Kanagawa, Japan) and Japan SLC (Shizuoka, Japan), respectively. In *in vitro* studies, rats were sacrificed by exsanguination under ether anesthesia. All animal experiments were performed in compliance with the regulations of the Institutional Animal Ethical Committee of Astellas Pharma Inc.

Radioligand Receptor Binding Assay   Membranes of Chinese hamster ovary (CHO)-K1 cells expressing human muscarinic receptors were purchased from PerkinElmer Life Science (Wellesley, MA, U.S.A.). Briefly, 50 μl of drug solution and 100 μl of [^{3}H]*N*-methyl scopolamine (final concentration of 0.25 nM) were mixed with 50 μl of membrane suspension in 50 mM Tris/HCl (pH 7.4) buffer containing 1 mM EDTA and 10 mM $MgCl_2$ in a final volume of 200 μl. This mixture was incubated at room temperature for 120 min. Reactions were terminated by filtration through UniFilter GF/C (Packard Instrument Co., Meriden, CT, U.S.A.) and the filter was washed with ice-cold 50 mM Tris/HCl (pH 7.4) buffer

∗ To whom correspondence should be addressed.   e-mail: akiyoshi.ohtake@jp.astellas.com

containing 0.9% NaCl. Radioactivity retained on the filter was counted with a TopCount™ microplate scintillation counter (Packard Instrument Co.) using a scintillation cocktail (MicroScinti-20™, Packard Instrument Co.). Nonspecific binding was determined with the reaction mixture including 5 μM atropine, and total binding was determined with the reaction mixture without test drugs, respectively. A total of 10 drug concentrations were used, appropriately chosen from the range 3×10^{-11} to 1×10^{-5} M using a common ratio of approximately 3. Further, we performed saturation binding studies to yield the dissociation constants (K_d values) of [³H]N-methyl scopolamine for each muscarinic receptor under the same conditions as above.

Carbachol-Induced Contraction of Urinary Bladder Strips The urinary bladder was isolated from male Wistar rats weighing 420—510 g and its dome and base were excised to make a cylindrical preparation. The tissue was then cut into strips of 0.3 cm width and 1 cm length. These strips were vertically suspended with 1.0 g tension in a 10 ml organ bath containing Krebs–Henseleit-buffered solution (NaCl, 118.4 mM; KCl, 4.7 mM; KH_2PO_4, 1.2 mM; $MgSO_4$, 1.2 mM; $CaCl_2$, 2.5 mM; $NaHCO_3$, 25.0 mM; glucose, 11.1 mM), maintained at 37 °C and gassed with a mixture of 95% O_2 and 5% CO_2. The strips were attached to isometric force-displacement transducers (TB-611T; Nihon Kohden, Tokyo, Japan) connected to a recorder. After equilibration, bladder contractions were elicited by 10 μM carbachol three times and a cumulative concentration–response curve for carbachol was constructed by increasing bath concentration of the agonist approximately 3-fold. After the strips were allowed to equilibrate again, the concentration-responses to carbachol were reconstructed by serially increasing the concentration of the agonist in the absence or presence of antagonists following 30 min incubation. Each antagonist was examined at three different concentrations in the same preparation.

Cystometry in Anesthetized Rats Male Sprague-Dawley rats weighing 350—490 g were anesthetized intraperitoneally (i.p.) with urethane (1.2 g/kg). A midline abdominal incision was made, and then a catheter (PE-50; Becton, Dickinson & Co., NJ, U.S.A.) was inserted into the bladder through its superior aspect. A three-way tap was attached to the other end of the catheter, and a pressure transducer (TP-400; Nihon Kohden), for the measurement of intravesical pressure, and an infusion pump (STC525; Terumo, Tokyo, Japan), for the infusion of physiological saline, were also attached. Intravesical pressure was recorded continuously on a recorder. A catheter was placed in a femoral vein for the administration of the test drugs. Following a stabilization period after surgery of at least 30 min, physiological saline warmed to about 37 °C was infused continuously into the bladder at the rate of 4.2 ml/h in order to induce the micturition reflex. The volume of physiological saline infused until micturition was defined as maximum bladder capacity. Cystometry was performed four times, with the test drugs intravenously (i.v.) administered 5 min before the fourth cystometry. The mean values of the parameters measured in the second and third cystometrograms were designated the initial values. The difference between the initial values and the post-administration values was used in the evaluation of pharmaceutical efficacy.

Data Analysis All data are expressed as the mean±

S.E.M. or the mean with 95% confidence interval. In radioligand binding experiments, specific binding was calculated as total binding minus nonspecific binding. The concentration of each test drug required to reduce the specific binding of [³H]N-methyl scopolamine by 50% (IC_{50} value) was obtained by non-linear regression analysis. A K_d value of [³H]N-methyl scopolamine for each muscarinic receptor subtype was yielded by Scatchard plot analysis. K_i values were calculated from the formula $K_i = IC_{50}/(1 + [[³H]N$-methyl scopolamine]$/K_d)$.[10] In the isolated rat bladder experiments, the 50% effective concentration (EC_{50}) of carbachol was estimated by non-linear regression. For calculation of pA_2 values, Schild plot analysis was performed in each preparation using these EC_{50} values,[11] and the result was expressed as an apparent pA_2 when the Schild slopes were different from unity using Student's t-test. In *in vivo* study, ID_{30} values, the doses produce a 30% increase in maximum bladder capacity, was determined by linear regression analysis. Statistical differences were analyzed using Dunnett's multiple comparison test.

All data analyses were performed using the SAS statistical software (SAS Institute, NC, U.S.A.). Differences with a $p < 0.05$ were considered statistically significant.

RESULTS

Radioligand Receptor Binding Assay Solifenacin, tolterodine, oxybutynin, darifenacin and propiverine inhibited the specific binding of [³H]N-methyl scopolamine to each muscarinic receptor subtype in a concentration-dependent manner (Fig. 1). K_i values are summarized in Table 1. The affinity of solifenacin for the muscarinic M_3 receptor was 2.2-, 15-, 9.1-, and 2.6-fold higher than those for M_1, M_2, M_4 and M_5 receptors, respectively. The affinities of oxybutynin, darifenacin and propiverine for the muscarinic M_3 receptor were 1.8- to 5.9-, 4.2- to 53-, and 1.4- to 3.8-fold higher than those for other muscarinic receptor subtypes, respectively, but tolterodine was devoid of any M_3 receptor subtype selectivity. The affinities of oxybutynin and tolterodine for muscarinic M_1—M_5 receptors and those of darifenacin for M_2—M_5 receptors were higher than those of solifenacin. On the other hand, propiverine showed about one-order lower affinities for all muscarinic receptor subtypes compared with solifenacin.

Carbachol-Induced Contraction of Urinary Bladder Strips In isolated rat urinary bladder strips, solifenacin (30—300 nM), tolterodine (3—30 nM), oxybutynin (30—300 nM), darifenacin (3—30 nM), propiverine (1—10 μM) and atropine (3—30 nM) shifted the concentration–contraction curves of carbachol to the right in a concentration-dependent manner (Fig. 2). In addition, darifenacin at 30 nM and propiverine at 10 μM depressed the maximum contraction to a value less than 80%. Affinity estimates (pA_2 values) and Schild slopes are summarized in Table 2. Schild slopes of solifenacin and tolterodine were not significantly different from unity, whereas those of oxybutynin, darifenacin, propiverine and atropine were significantly different from unity.

Cystometry in Anesthetized Rats When physiological saline was infused continuously at 4.2 ml/h into the bladders in anesthetized rats, intravesical pressure increased gradually and micturition was observed about 10 min after start of the
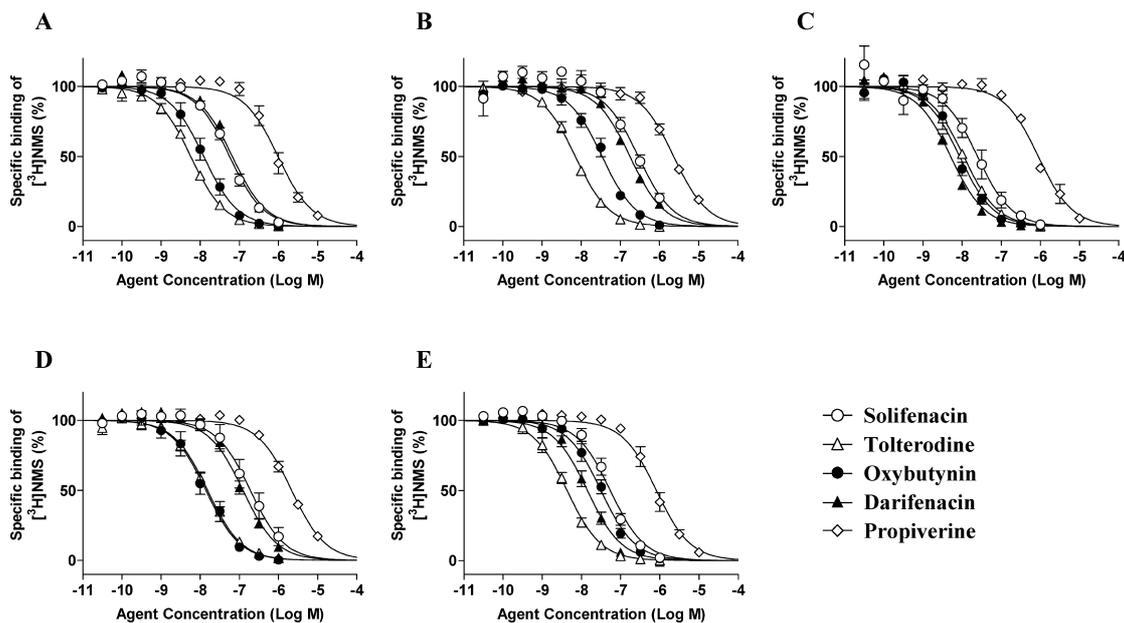
Fig. 1. Inhibition of [^{3}H]N-Methyl Scopolamine Binding to Human Muscarinic M_1 (A), M_2 (B), M_3 (C), M_4 (D) and M_5 (E) Receptors by Solifenacin, Tolterodine, Oxybutynin, Darifenacin and Propiverine

Each point represents the mean±S.E.M. of four separate experiments performed in duplicate.

Table 1. Affinities of Solifenacin and Other Muscarinic Receptor Antagonists for Membrane Preparations from Recombinant Cells Expressing Human Muscarinic Receptor Subtypes

Antagonist	K_i (nM)				
	M_1	M_2	M_3	M_4	M_5
Solifenacin	26±2.0	170±37	12±4.4	110±45	31±6.3
Tolterodine	2.7±0.23	4.2±0.51	4.4±0.45	6.6±1.7	2.5±0.49
Oxybutynin	6.1±1.5	21±3.6	3.4±0.65	6.6±2.7	18±4.0
Darifenacin	31±2.6	100±14	2.0±0.21	52±15	8.2±1.7
Propiverine	490±110	1400±220	350±53	900±200	490±120

Each value represents the mean±S.E.M. of four separate experiments performed in duplicate.

infusion. The initial values of maximum bladder capacity and maximum intravesical pressure were approximately 700 μl and 40 cm H_2O, respectively. There were no significant differences among the all groups in these initial values. Both solifenacin (0.1—1 mg/kg i.v.) and oxybutynin (0.1—1 mg/kg i.v.) increased the maximum bladder capacity in a dose-dependent manner and also decreased maximum intravesical pressure (Fig. 3). The ED_{30} values of solifenacin and oxybutynin (95% confidence interval) were 0.35 (0.17—0.83) and 0.30 (0.21—0.43) mg/kg i.v., respectively.

DISCUSSION

Overactive bladder is a condition which encompasses a number of urinary symptoms, including urgency, excessive frequency of micturition, nocturia and urge incontinence. The generation of abnormal bladder contractions in disease states as well as in normal physiological voiding is critically dependent on acetylcholine-induced stimulation of muscarinic receptors in the bladder smooth muscle. Antimuscarinic therapy is thus the mainstay of pharmacological treatment for the symptoms of overactive bladder. The aim of the present study was to evaluate the antimuscarinic properties

of solifenacin, a new muscarinic receptor antagonist developed for the treatment of overactive bladder, and to compare the results with those obtained for other muscarinic antagonists, namely tolterodine, oxybutynin, darifenacin and propiverine.

The results of the *in vitro* studies demonstrate that solifenacin is a potent and competitive muscarinic receptor antagonist. In radioligand receptor binding assay, solifenacin showed the highest affinity for the muscarinic M_3 receptor, which mediates the urinary bladder contraction, but its affinity for the M_3 receptor was only marginal over those for the M_1 and M_5 receptor subtypes. The affinity of solifenacin for the muscarinic M_3 receptor was 30-fold higher than that of propiverine, but 2.7- to 6.0-fold lower than those of tolterodine, oxybutynin and darifenacin. The affinities of oxybutynin and propiverine for the muscarinic M_3 receptor were at most 5.9-fold higher than those for other muscarinic receptor subtypes, and tolterodine was devoid of any muscarinic M_3 receptor subtype selectivity. On the other hand, the affinity of darifenacin for the muscarinic M_3 receptors was 4.2- to 53-fold higher than those for other muscarinic receptor subtypes. These results are markedly similar to those reported previously.[9,12,13]

A

B

C

D

E

F
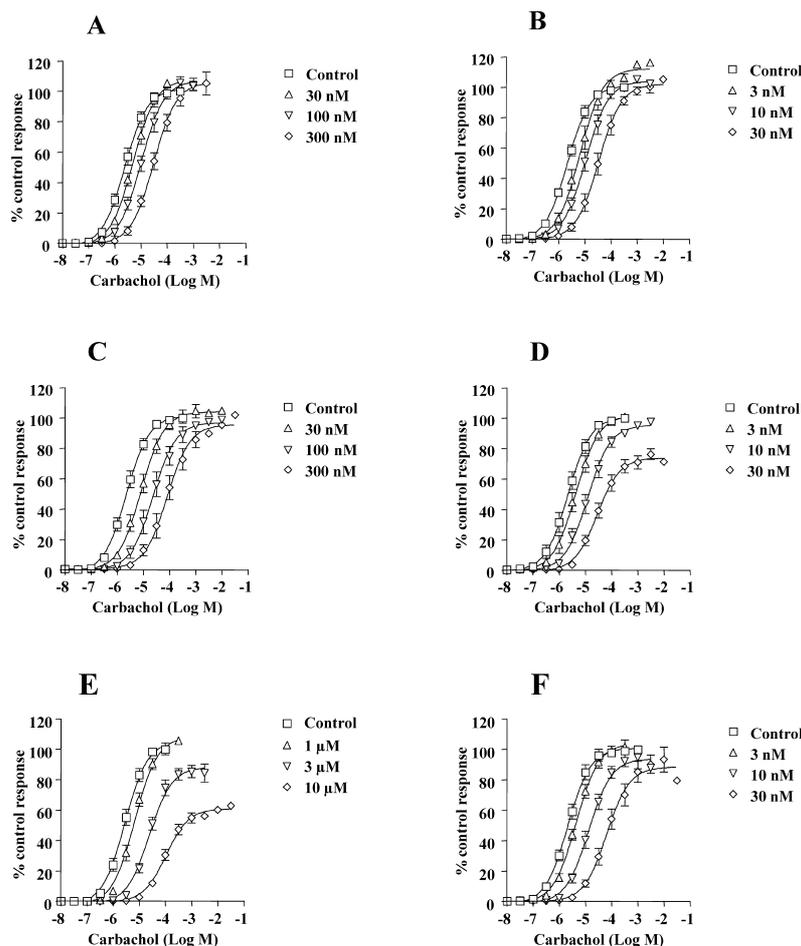
Fig. 2. Effect of Solifenacin (A), Tolterodine (B), Oxybutynin (C), Darifenacin (D), Propiverine (E) and Atropine (F) on Carbachol-Induced Contraction in Isolated Rat Urinary Bladder

Each point represents the mean±S.E.M. of five preparations.

Table 2. Potency Estimates and Schild Slopes for Solifenacin and Other Muscarinic Receptor Antagonists in Carbachol-Induced Contraction in Isolated Rat Urinary Bladder

Antagonist	pA_2	Slope
Solifenacin	7.44±0.09	1.11±0.06
Tolterodine	8.61±0.13	1.03±0.07
Oxybutynin	7.84±0.06	1.22±0.07[a]
Darifenacin	8.50±0.03	1.50±0.15[a]
Propiverine	6.03±0.04	2.14±0.12[a]
Atropine	8.47±0.06	1.73±0.16[a]

Each value represents the mean±S.E.M. of five preparations. a) Schild slope is significantly different from unity (Student's t-test).

In isolated rat urinary bladder, solifenacin shifted the concentration–contraction curves of carbachol to the right in a concentration-dependent manner, and the Schild slope was not significantly different from unity, suggesting that the antagonistic effect on the muscarinic M_3 receptor is competitive. The antimuscarinic action of solifenacin was more potent than that of propiverine, and less potent than those of tolterodine, oxybutynin and darifenacin. These results were considered to nearly reflect the rank order of affinity for the muscarinic M_3 receptor. However, the antagonistic potencies of solifenacin, oxybutynin and propiverine were weaker than their affinities for the muscarinic M_3 receptor. This discrep-

A

B
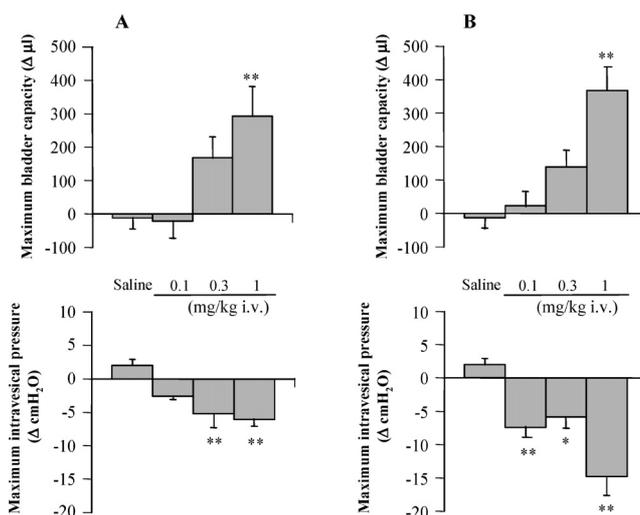
Fig. 3. Effects of Solifenacin (A) and Oxybutynin (B) on Maximum Bladder Capacity and Maximum Intravesical Pressure in Anesthetized Rats

Each column represents the mean±S.E.M. of six animals. *$p<0.05$, **$p<0.01$, significant difference from the physiological saline group (Dunnett's multiple comparison test).

ancy was probably caused by restricted drug diffusion into structured tissues, which hinders equilibrium conditions. Schild slopes for oxybutynin, darifenacin and propiverine

were significantly different from unity. A steep Schild plot is thought to be attributable to the presence of a saturable antagonist removal process in the tissue.[14] Further, since propiverine is reported to have Ca^{2+} channel blocking action,[15] it is likely that its action contributed to the observed decrease in maximum contraction, resulting in the steep slope.

In anesthetized rats, solifenacin and oxybutynin increased the maximum bladder capacity in a dose-dependent manner and also decreased the maximum intravesical pressure. These effects are considered to be based on the muscarinic M_3 receptor antagonizing action in urinary bladder. At highest dose of solifenacin, the increase in maximum bladder capacity was 47%, and the decrease in maximum intravesical pressure was only 16%. In conscious cerebral infarcted rats, solifenacin was demonstrated to increase the maximum bladder capacity and voided volume without influencing maximum intravesical pressure.[16] The present study was conducted under urethan anesthesia. Since urethan anesthesia is considered to induce a weak inhibition for neural reflex, the influence of the anesthesia on micturition reflex could not be role out. In fact, urethan anesthesia inhibited somewhat micturition reflex.[17] It was thus likely that solifenacin was more easy to decrease maximum intravesical pressure in the present study compared with previous study under conscious condition.

In the previous studies, solifenacin exhibited greater selectivity for urinary bladder over salivary gland than tolterodine, oxybutynin, darifenacin and atropine.[18,19] Since the muscarinic M_3 receptor mediates both bladder contraction and salivary secretion,[3—5,12,20] the bladder selectivity demonstrated for solifenacin can clearly not be attributable to selectivity for a single muscarinic M_3 receptor subtype. Recently, muscarinic M_1 and M_5 receptors as well as M_3 receptors have been reported to play a partial albeit important role in salivary gland, and mutant mice lacking the muscarinic M_1 and M_5 receptor gene have reduced pilocarpine-induced salivary secretion.[21—24] However, solifenacin showed only 2.2- and 2.6-fold higher affinity for the muscarinic M_3 receptor than for the M_1 and M_5 receptors, respectively. On the other hand, oxybutynin showed 1.8- and 5.1-fold higher affinity for the muscarinic M_3 receptor than for the M_1 and M_5 receptors. Darifenacin showed 16- and 4.2-fold higher affinity for the muscarinic M_3 receptor than for the M_1 and M_5 receptors, respectively. Interestingly, however, these two antagonists showed no bladder selectivity.[18] The bladder selectivity demonstrated for solifenacin is thus not considered to be attributable to its low affinity for the muscarinic M_1 and M_5 receptors. Therefore, selectivity for the other non-M_3 muscarinic receptor subtypes is not considered to contribute to the bladder selectivity. Thus, a great deal of investigation would be required to clarify the mechanism of bladder selectivity of solifenacin.

In conclusion, solifenacin is a potent and competitive muscarinic receptor antagonist and increases the maximum bladder capacity. These findings support the fact that solifenacin is useful in the treatment of overactive bladder, similarly to currently used antimuscarinic agents. In this regard, we note with interest that solifenacin at 5 and 10 mg/man has been shown in clinical studies to be more effective than placebo in improving overactive bladder symptoms.[25—28]

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