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RESEARCH PAPER

In vivo and *in vitro* pharmacological characterization of SVT-40776, a novel M_3 muscarinic receptor antagonist, for the treatment of overactive bladder

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Background and purpose: Highly selective M_3 muscarinic receptor antagonists may represent a better treatment for overactive bladder syndrome, diminishing side effects. Cardiac side effects of non-selective antimuscarinics have been associated with activity at M_2 receptors as these receptors are mainly responsible for muscarinic receptor-dependent bradycardia. We have investigated a novel antimuscarinic, SVT-40776, highly selective for M_3 over M_2 receptors (Ki = 0.19 nmol·L^{-1} for M_3 receptor affinity). This study reports the functional activity of SVT-40776 in the bladder, relative to its activity in atria.
Experimental approach: *In vitro* and *ex vivo* (oral dosing) inhibition of mouse detrusor and atrial contractile responses to carbachol were used to study the functional activity of SVT-40776. The *in vivo* efficacy of SVT-40776 was characterized by suppression of isovolumetric spontaneous bladder contractions in anaesthetized guinea pigs after intravenous administration.
Key results: SVT-40776 was the most potent in inhibiting carbachol-induced bladder contractions of the anti-cholinergic agents tested, without affecting atrial contractions over the same range of concentrations. SVT-40776 exhibited the highest urinary versus cardiac selectivity (199-fold). In the guinea pig *in vivo* model, SVT-40776 inhibited 25% of spontaneous bladder contractions at a very low dose (6.97 μg·kg^{-1} i.v), without affecting arterial blood pressure.
Conclusions and implications: SVT-40776 is a potent inhibitor of M_3 receptor-related detrusor contractile activity. The absence of effects on isolated atria preparations represents an interesting characteristic and suggests that SVT-40776 may lack unwanted cardiac effects; a feature especially relevant in a compound intended to treat mainly elderly patients.
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Keywords: muscarinic antagonist; urinary bladder; overactive bladder; SVT-40776; carbachol; isolated bladder tissue; atria contractions

Abbreviations: AP, arterial pressure; OAB, overactive bladder

Introduction

Overactive bladder (OAB) is a widely prevalent condition characterized by urgency, with or without urge incontinence, usually accompanied by frequency and nocturia (Abrams *et al.*, 2003). It arises from involuntary contractions of the detrusor muscle during bladder filling (Wein and Rovner, 2002). In an attempt to clarify the etiology of this bladder dysfunction, different theories endorse its either myogenic (Brading, 1997) or neural (de Groat, 1997) origin. However, in most cases, OAB is idiopathic in nature.

Muscarinic acetylcholine receptors are the predominant receptor system controlling bladder contractility (Andersson and Yoshida, 2003, Abrams *et al.*, 2006) and its pharmacological characterization mediating detrusor smooth muscle contraction has been well established in humans (Fetscher *et al.*, 2002; Tyagi *et al.*, 2006), mice (Choppin and Eglen, 2001; Choppin, 2002), rats (Longhurst *et al.*, 1995; Hegde *et al.*, 1997; Longhurst and Levendusky, 2000), guinea pigs (Wang *et al.*, 1995), rabbits (Tobin and Sjogren, 1995; Choppin *et al.*,

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1998) and monkeys (Lai *et al*., 1998). M_2 and M_3 muscarinic receptors coexist in the bladder of different mammalian species, including humans, with a major presence of the M_2 receptor (M_2 receptors account for 70–80% of the receptor population whereas the M_3 receptors comprise only 20–30%) (Eglen *et al*., 1996). However, although M_2 receptors predominate in number, numerous studies have suggested that the M_3 receptor represents the main subtype controlling bladder contractility in humans (Hegde and Eglen, 1999, Chess-Williams *et al*., 2001; Andersson, 2002a; Fetscher *et al*., 2002; Stevens *et al*., 2007), through a mechanism involving activation of phosphoinositide breakdown followed by an increase in intracellular Ca^{2+} levels (Harriss *et al*., 1995; Tran *et al*., 2006), in addition to activation of the Rho-kinase pathway (Schneider *et al*., 2004). Further evidence comes from studies using M_3 receptor knockout mice, in which bladder contractile response to carbachol is virtually abolished (Matsui *et al*., 2000). The residual (~5%) contractions that persist in those animals are completely lost in M_2/M_3 double knockout mice, revealing the occurrence of minor M_2 receptor-mediated contractions (Matsui *et al*., 2002). Moreover, M_2 receptors are capable of mediating bladder contractions by enhancing the contractile response to M_3 receptor activation, indicating a contribution of M_2 receptors in M_3 receptor-mediated contractile responses (Ehlert *et al*., 2005). Although this contribution has been suggested to become more important in diseased states (Pontari *et al*., 2004; Stevens *et al*., 2007), in both normal and overactive human bladders, direct contractile response to carbachol is mediated mainly by M_3 receptors.

Muscarinic M_2 receptors, the major muscarinic subtype present in mammalian heart (Caulfield, 1993; Hoover *et al*., 1994), have been reported to be essential for muscarinic receptor-dependent bradycardia, modulating pacemaker activity and atrioventricular conduction (Stengel *et al*., 2000; Dhein *et al*, 2001; Andersson and Olshansky, 2007). Although expression of M_1, M_3 and M_4 muscarinic receptors have been reported in guinea pig and rat intrinsic intracardiac neurons (Hassall *et al*., 1993) as well as in canine atria (Shi *et al*., 1999), their functional role is not completely understood. Also in human heart, M_1, M_3 and M_5 receptors have been localized (Hellgren *et al*., 2000; Wang *et al*., 2001; Andersson and Olshansky, 2007). However, to date, no data indicate that any other muscarinic receptor subtype, apart from M_2 receptors, mediate effects on heart rate (Andersson and Olshansky, 2007).

This study reports the functional characterization of SVT-40776, a novel quinuclidine derivative (Farrerons *et al*., 2002), highly selective for M_3 over M_2 receptors, on mouse detrusor and isolated atrial tissues, as well as in a guinea pig bladder contraction model *in vivo*. We have compared its pharmacological activity with those of the currently marketed anticholinergic agents, tolterodine, darifenacin and solifenacin. Our results demonstrated that SVT-40776 was the most potent in inhibiting carbachol-induced bladder contractions, compared with the anticholinergic agents tested, without affecting atrial contractions at the same range of concentrations. SVT-40776 exhibited the highest urinary versus cardiac selectivity (199-fold) in the animal models studied. These data may suggest that SVT-40776 could provide improved tolerability over currently available treatments for OAB.

Methods

Animal experiments

All animal procedures were according to the published guidelines on the use of animals in research (EU Directive 86/609/ECC). CD-1 male mice (25–30 g) and DH female guinea pigs (250–300 g) were purchased from Harlan Iberica (Spain). They were housed in a temperature-controlled environment (20 ± 2°C, 12 h light/dark cycle) with standard laboratory food and water freely available. All animals were fasted for 18 h prior to the experiment.

In vitro *contractile study*

Bladder detrusor preparations Mice were killed by CO_2 and the urinary bladder was isolated and placed in Krebs' solution (composition in mmol·L^{-1}: NaCl 118, KCl 4.6, $CaCl_2$ 1.5, $MgCl_2$ 1.5, KH_2PO_4 1.15, $NaHCO_3$ 25 and glucose 11). Indomethacin (30 μmol·L^{-1}) and hexamethonium (1 mmol·L^{-1}) were routinely included in the Krebs' solution to abolish prostaglandin-induced spontaneous activity and any possible nicotinic receptormediated activity respectively. One strip of tissue per animal (4 × 2 mm) was cut from the posterior region of bladder body, parallel to the longitudinal axis. Tissues were mounted in 25 mL organ baths containing Krebs' solution, maintained at 37°C and constantly aerated with 95% O_2 and 5% CO_2 (pH = 7.4). Isometric tension generated by the tissue was measured by pure isometric transducers (Cibertec) and recorded using the PowerLab® software (ADInstruments, Bella Vista, Australia). Tissues were maintained at a resting tension of 5 mN during an equilibration period of 60 min. Tension adjustments were made as necessary. Tissues were washed every 15 min. The viability of each tissue was assessed by determining the contractile response to KCl (120 mmol·L^{-1}) at the start of the experimental protocol. After washing, tissues were re-equilibrated for 15 min and allowed to regain baseline tension. Repeated contractions to carbachol (3 μmol·L^{-1}) were then induced, in order to obtain two consecutive contractions with less than 10% difference. After tissue equilibration, cumulative consecutive concentration-effect curves to carbachol were then constructed in each bladder preparation. Antagonist was incubated for a 60 min period between curves.

Atrial preparations Mice were killed by CO_2 and both atria were isolated, placed in aerated Krebs' solution (without either indomethacin or hexamethonium) with ligatures placed on the right and left atrium. Tissues were maintained at a resting tension of 1.47 mN during an equilibration period of 10 min to obtain spontaneous contractions in order to measure the beating frequency. After tissue equilibration, cumulative consecutive concentration-effect curves to carbachol were then constructed in each atrial preparation. Antagonist was incubated for a 60 min period between the first and second curve.

Ex vivo *contractile study*

Bladder and atria preparations Groups of animals (n = 4–6 per dose) received a single oral dose (0.3 to 50 mg·kg^{-1}) of antago-

nists or vehicle (three to five doses per compound). Mice were killed 3 h later and the urinary bladder and atria were excised and prepared as described before. After tissue equilibration, the viability of each strip was assessed by determining the contractile response to KCl at the start of the experimental protocol. Repeated KCl contractions were used to warm up the tissue. A unique cumulative concentration-effect curve to carbachol was then constructed in each tissue and referred to KCl effect.

In vivo *animal study*

Guinea pigs were anaesthetized with urethane 1.5 $g \cdot kg^{-1}$ i.p. (n = 4–6 animals per compound). A polyethylene catheter (PE-50) was implanted in the bladder via the urethra and the bladder was emptied. Carotid artery and jugular vein were cannulated to register arterial pressure (AP) and to administer drugs respectively. Bladder and carotid catheters were connected to pressure transducers (Transpac IV) and analysed using PowerLab® Software.

A baseline AP of 59.8 ± 1.7 mmHg was registered. The bladder was filled with 2.8 mL of saline to obtain a mean pressure of 58.5 ± 2.8 mmHg, which induced regular spontaneous contractions. After obtaining a stable response, the compound was administered by intravenous bolus followed by a cumulative consecutive dose–response protocol (15 min between doses or when stable contractions were obtained). Mean AP was measured during the first 5 min post-dose periods. Amplitude from all bladder contractions (intravesical pressure) was measured during the 15 min period between doses and a mean amplitude was calculated for baseline and for each dose. Per cent change per dose was calculated, relative to baseline effects and an ED_{25} (effective dose 25% of maximum response) was obtained for each compound.

Data analysis

In the *in vitro* study, bladder contractions were recorded as changes in tension from baseline and expressed as a percentage of the maximum response of the first agonist concentration-effect curve. Atrial beating frequency was recorded and expressed as a percentage of the initial frequency before the first agonist concentration-effect curve. Carbachol concentration-effect curves were analysed by using a non-linear fitting program (GraphPad Prism software) and pEC_{50} values were calculated. Concentration ratio (CR) was determined from pEC_{50} values in the presence and absence of different antagonist concentrations. pA_2 values were obtained from the x-axis intercept from the Schild plot and expressed as means with 95% confidence interval. The Schild plots of all antagonists were linear, with slopes close to unity. All other data are expressed as mean ± SEM.

In the *ex vivo* protocol, carbachol concentration-effect curves were fitted as described above. CR was determined from pEC_{50} values, considering curves obtained from vehicle-treated groups as 'control pEC_{50}' and curves from antagonist-treated animals as 'treated animals pEC_{50}'. A pA_2-equivalent dose (pA_2-ED) value was obtained from the Schild plot, using the oral doses instead of bath concentrations for each compound and given as means with 95% confidence interval. All other data are shown as mean ± SEM.

In the *in vivo* study, ED_{25} values were obtained fitting the percentage of variation to a cubic spline curve (GraphPad Prism software). These values represent the doses needed to inhibit 25% bladder contractions. For the results of *in vitro* assays, a non-paired Student's *t*-test was used for statistical analysis.

Materials

SVT-40776, darifenacin, solifenacin and tolterodine were synthesized by the Medicinal Chemistry Department (SALVAT). Carbachol, indomethacin and hexamethonium were purchased from Sigma Chemical Co. (St. Louis, MO, USA). For the *in vitro* protocol, compounds tested were prepared at 1 $mmol \cdot L^{-1}$ in dimethylsulfoxide (DMSO) and dilutions made in deionized water. For the *ex vivo* protocol, compounds tested were freshly suspended in vehicle (methylcellulose 0.5% and Tween-80 0.1%) 1 h before oral administration (10 $mL \cdot kg^{-1}$). For the anaesthetized animal study, stock solutions of SVT-40776 and solifenacin were dissolved in saline. Tolterodine was dissolved in deionized water. Darifenacin stock solution was prepared in 10% DMSO in deionized water. Subsequent dilutions for all compounds were made in saline.

Results

In vitro *functional characterization of SVT-40776 on mouse isolated bladder and atria preparations*

Carbachol induced concentration-dependent contractions of mouse urinary bladder smooth muscle. The first curve yielded a mean $pEC_{50} = 5.66 \pm 0.04$ with a maximum response (E_{max}) of 11.7 ± 2.2 mN. A consecutive additional carbachol concentration-response curve within this preparation in the absence of antagonist yielded a mean $pEC_{50} = 5.66 \pm 0.08$ with an E_{max} of 11.9 ± 1.9 mN (n = 4). Thus, two consecutive concentration-effect curves to carbachol could be constructed in the same tissue with no significant change in the agonist potency and maximum response (Figure 1A). All the compounds tested antagonized cumulative agonist concentration-response curves, in a concentration-dependent fashion, with parallel right-ward shifts. While tolterodine and solifenacin did not significantly alter maximum carbachol response (Figure 1B,C), darifenacin exposure clearly reduced E_{max} (from 66% at 3 $nmol \cdot L^{-1}$ to 58% at 100 $nmol \cdot L^{-1}$) (Figure 1D). SVT-40776 slightly reduced E_{max} at 10 $nmol \cdot L^{-1}$ and 100 $nmol \cdot L^{-1}$ concentrations (to 71% and 80% respectively) (Figure 1E). All four antagonists yielded Schild regression lines, with slopes close to unity.

In mouse atrial preparations, carbachol induced concentration-dependent negative chronotropism of spontaneous beating right atria. Two consecutive concentration-effect curves to carbachol could be constructed in the same tissue with no significant change in the agonist potency and maximum response. First and second curves yielded pEC_{50} of 6.23 ± 0.05 and 6.89 ± 0.08 (n = 10) respectively. Maximum effect, which was the complete inhibition of beating, was also maintained in the second curve (Figure 2A). The four antagonists tested shifted the carbachol curve dose-dependently to the right (Figure 2B–E).

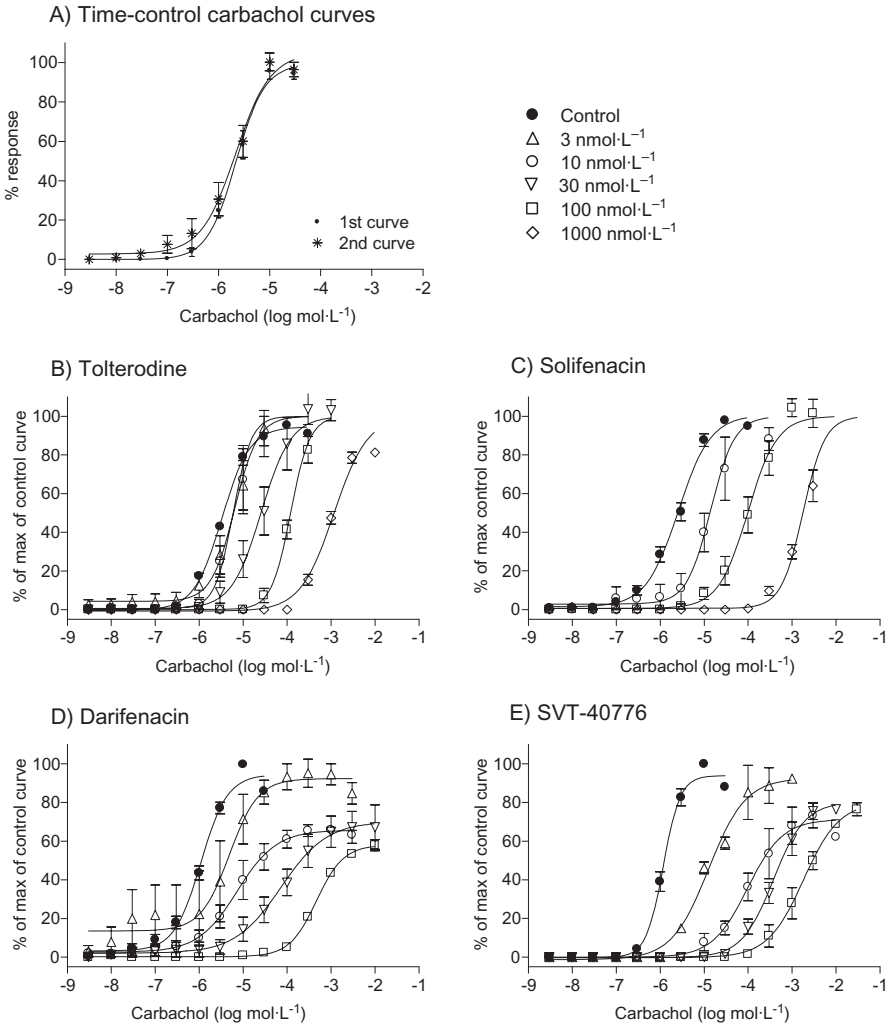

Figure 1 Effects of muscarinic receptor antagonists on the cumulative consecutive concentration-response curves to carbachol on mouse urinary bladder. In (A), two repeated control concentration-response curves show the reproducibility and stability of the preparation. In (B)–(E), the effects of anatagonists (3–1000 nmol·L^{-1}) on the carbachol concentration-response curves are shown. Direct contractile effects were expressed as percentages of the maximum response of the control curve. Data are expressed as mean ± SEM, $n = 4$–8 animals per concentration.

Antagonist affinities (pA_2) on carbachol-induced responses on bladder and heart isolated tissues *in vitro* are summarized in Table 1. SVT-40776 was clearly the most potent antagonist in the bladder, lacking any relevant effect in atria at the same range of concentrations. Furthermore, as shown in Table 1, SVT-40776 exhibited the highest urinary versus cardiac selectivity (199-fold).

Ex vivo *functional characterization of SVT-40776 on mouse isolated bladder and atria preparations*

Detrusor smooth muscle from control animals killed 3 h after receiving an oral dose of vehicle did not behave differently from that of non-treated animals. Carbachol induced concentration-response curves, yielding a $pEC_{50} = 5.16 \pm 0.06$ ($n = 71$) (Figure 3A). This value was assigned as control, in order to compare it with pEC_{50} from antagonist-treated animals. KCl produced a maximum effect similar to carbachol (11.4 ± 1.8 mN). Right-ward shifts of the carbachol response curves for tolterodine, solifenacin, darifenacin and SVT-40776 were obtained (Figure 3B–E). While tolterodine, solifenacin and SVT-40776 did not significantly affect the maximum response to carbachol, darifenacin at 50 mg·kg^{-1} significantly reduced the E_{max}. All four antagonists yielded Schild regression lines with slopes close to unity.

Atria from control animals killed 3 h after receiving an oral dose of vehicle showed the same behaviour as those from non-treated animals. Carbachol induced concentration-response curves, yielding $pEC_{50} = 6.55 \pm 0.09$ ($n = 27$) (Figure 4A). This value was assigned as control, in order to compare it with the pEC_{50} from antagonist-treated animals. In this protocol, as in the *in vitro* atria, maximum effect was seen when complete inhibition of beating was obtained. Tolterodine and solifenacin dose-dependently shifted carbachol curves to the right. Darifenacin exhibited less potency than tolterodine and solifenacin. SVT-40776 did not induce any relevant displacement of carbachol curves to the right, up to a dose of 30 mg·kg^{-1} (Figure 4B–E).

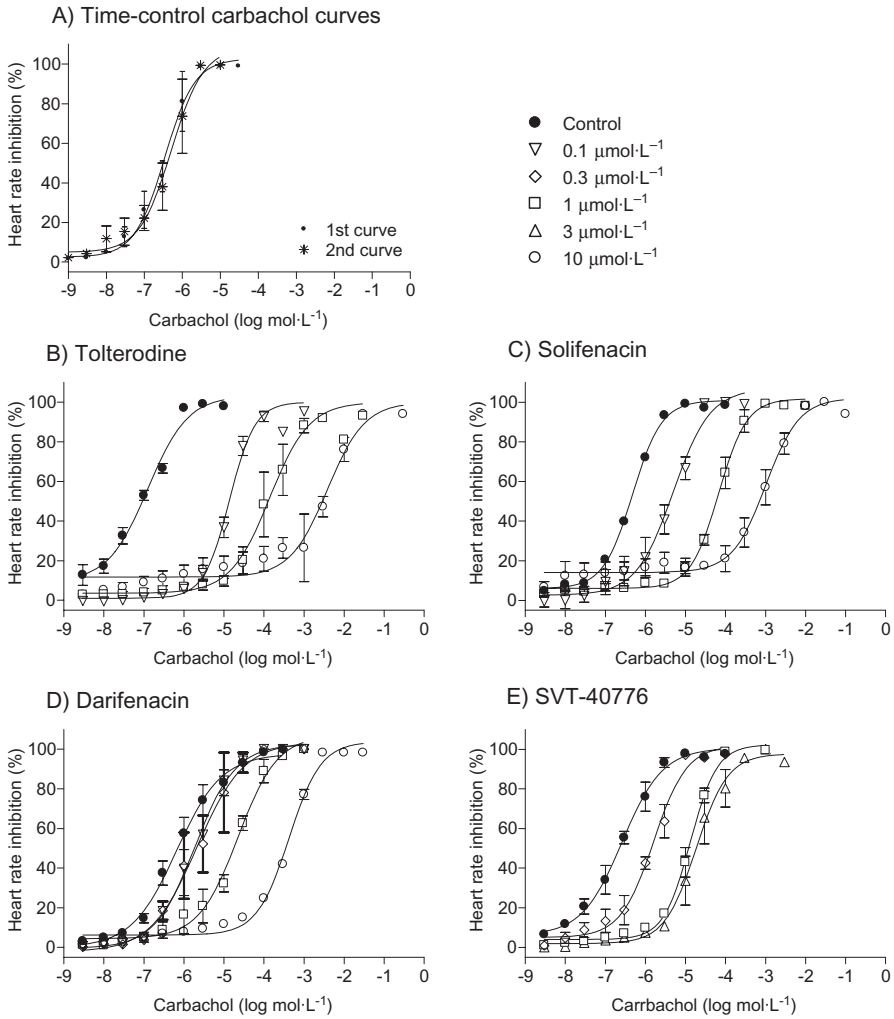

Figure 2 Effect of tolterodine (B), solifenacin (C), darifenacin (D) and SVT-40776 (E) treatment on the cumulative consecutive concentration-response curves to carbachol (A) on mouse atria. As in Figure 1, the reproducibility of the control curves is shown in (A) and the effects of the antagonists (0.1–10 µmol·L^{-1}) in (B)–(E). Direct contractile effects were expressed as percentages of the maximum response of the control curve. Data are expressed as mean ± SEM, $n = 4$–8 animals per concentration.

Table 1 *In vitro* affinities (pA_2) of muscarinic M_3 receptor antagonists on carbachol-induced responses in isolated bladder and atrial tissues

Compound	$pA_{2\ (CI)}$				*Selectivity ratio*
	Bladder	*Slope*	*Atria*	*Slope*	
Tolterodine	8.4 (8.2–8.6)	1.09 ± 0.10	8.5 (7.8–9.2)	1.24 ± 0.17	0.79
Darifenacin	8.8 (8.2–9.4)	1.27 ± 0.52	7.3 (7.2–7.4)	1.26 ± 0.02	31.6
Solifenacin	8.6 (8.3–8.9)	1.11 ± 0.06	7.8 (7.7–7.8)	1.20 ± 0.02	6.3
SVT-40776	9.5 (9.2–9.8)	1.36 ± 0.12	7.2 (7.0–8.1)	1.15 ± 0.45	199

pA_2 values are expressed as mean (95% CI); the slope shown is from Schild plot analysis, mean ± SEM, $n = 4$–8 animals for each antagonist concentration.

Only at the very high dose of 50 mg·kg^{-1}, it was able to shift carbachol curves to the right, to yield pED_{50} values twice that of control.

Antagonist activities (expressed as pA_2-ED) at carbachol-induced responses on bladder and heart isolated tissues *ex vivo* are reported in Table 2. In accordance with results obtained in the *in vitro* study, SVT-40776 exhibited the highest urinary versus cardiac selectivity (58-fold), whereas darifenacin, solifenacin and tolterodine showed much lesser selectivity (2.4-, 1.5- and 0.21-fold respectively).

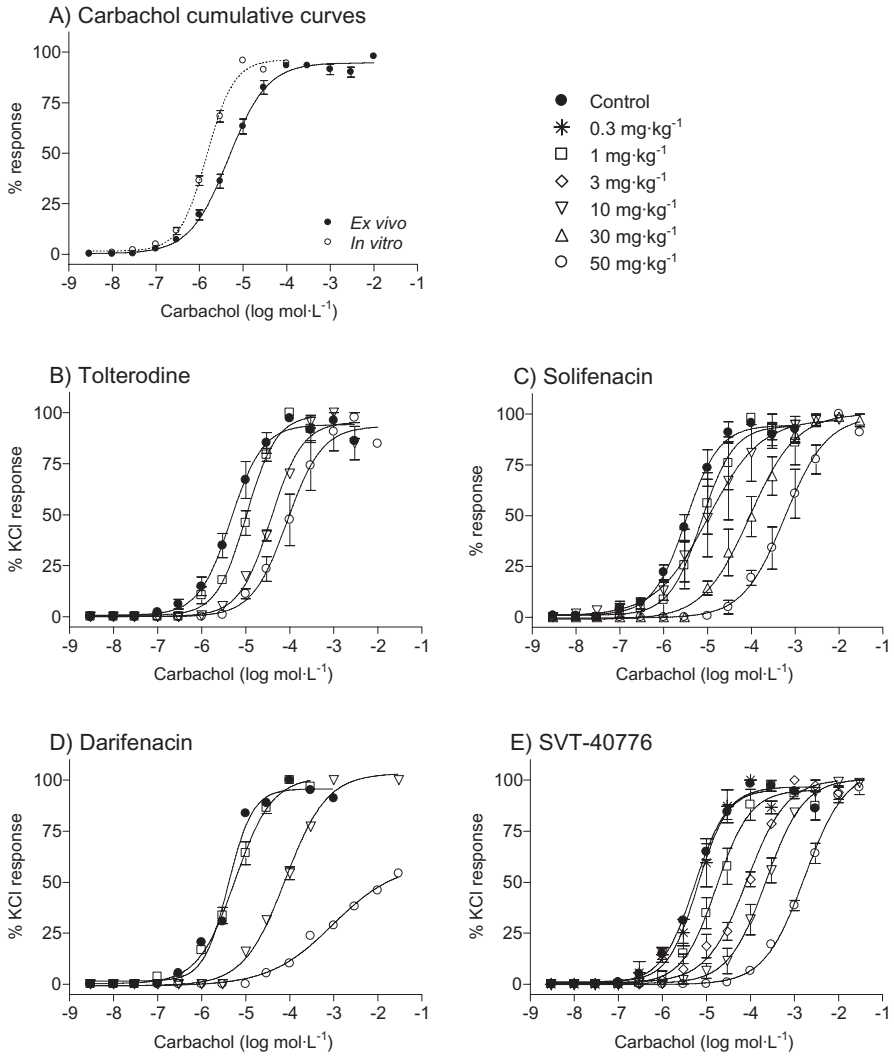

Figure 3 Effect of oral administration of the M_3 receptor antagonists on the contractile response to carbachol in mouse urinary bladder *ex vivo*. Data are expressed as mean ± SEM, $n = 4–6$ animals per dose.

Table 3 shows carbachol EC50 values from detrusor and atria preparations, comparing *in vitro* and *ex vivo* experiments. These values are very similar, which demonstrates the reliability of the *ex vivo* technique.

In vivo *functional characterization of SVT-40776 on guinea pig bladder*

Figure 5 shows a representative trace of spontaneous contractions before and after consecutive saline (A) or antagonist (B) bolus. Amplitudes of bladder contraction were measured during the 15 min period between doses and the per cent change in amplitude was calculated, relative to baseline values. Intravenous administration of SVT-40776, tolterodine, darifenacin and solifenacin changed bladder contraction amplitude in a dose-dependent manner (Figure 6). SVT-40776 was the most potent compared with the antagonists tested in the guinea pig *in vivo* model, inhibiting 25% of spontaneous bladder contractions at a very low dose of 17.1 nmol·kg^{-1} i.v. (6.97 μg·kg^{-1}). Calculated effective doses of darifenacin, solifenacin and tolterodine were 3-, 12- and 17-fold higher than that of SVT-40776 respectively (Table 4). In addition, the bladder versus vascular selectivity of SVT-40776 was more than 175-fold. SVT-40776, darifenacin and solifenacin showed higher urinary selectivity than tolterodine in this assay (Table 4).

Discussion and conclusions

Antimuscarinic agents (muscarinic receptor antagonists) are commonly the first line of treatment for OAB. However, their side effects, stemming from a lack of selectivity, compromise their clinical use (Andersson, 2002b; 2004; Andersson and Olshansky, 2007). Because of this, there are clear potential benefits in terms of efficacy and tolerability to be provided by selective antagonists of muscarinic M_3 receptors (Andersson, 2002b).

Efficacy of antimuscarinic drugs for the treatment of OAB has been evaluated in several clinical trials. A systematic review of 32 randomized controlled trials conducted by Herbison *et al.* (2003) concluded that antimuscarinic agents pro-

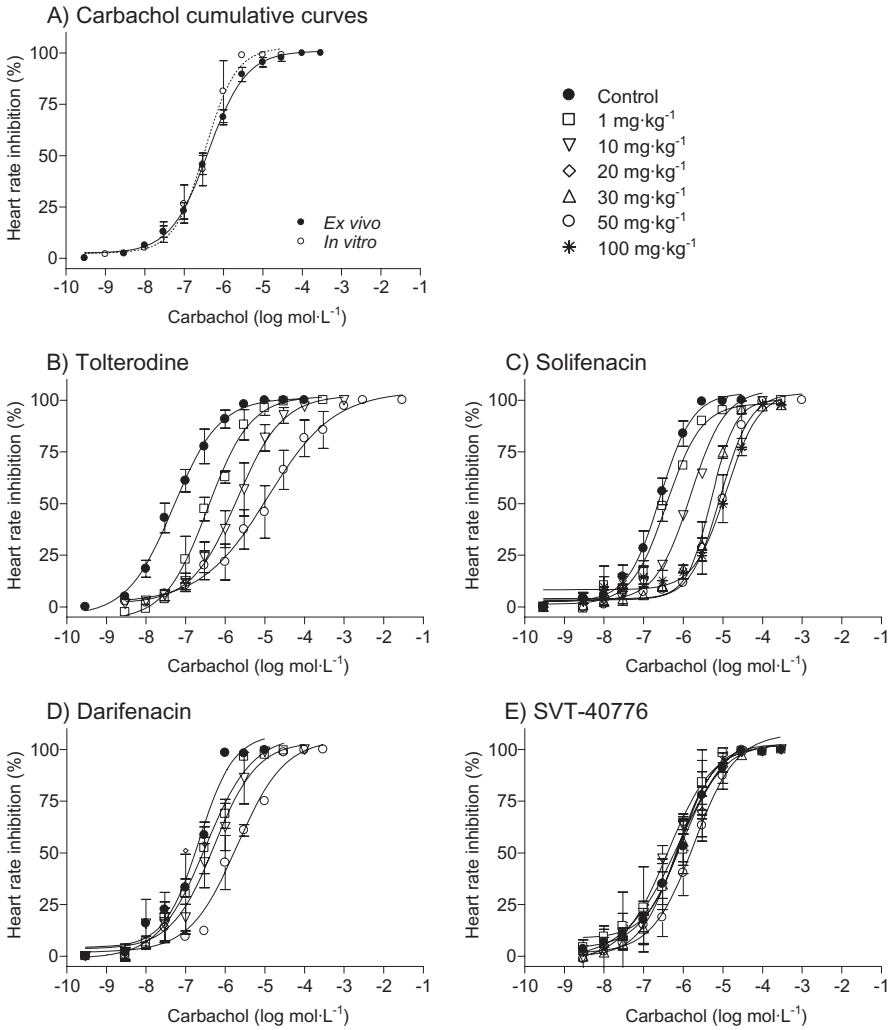

Figure 4 Effect of oral administration of the M_3 receptor antagonists on the contractile response to carbachol in mouse atria *ex vivo*. Data are expressed as mean ± SEM, $n = 4–6$ animals per dose.

Table 2 *Ex vivo* affinities, shown as pA_2-equivalent dose (pA_2-ED) of muscarinic receptor antagonists on carbachol-induced responses in isolated bladder and atrial tissues

Compound	*pA_2-ED ($mg·kg^{-1}$ oral)*		*Selectivity ratio*
	Bladder	*Atria*	
Tolterodine	0.7 (0.4–1.0)	0.14 (0.07–0.3)	0.21
Darifenacin	1.6 (0.35–6.7)	3.9 (2.1–7.5)	2.4
Solifenacin	1.3 (0.46–4.2)	2.0 (1.6–2.6)	1.5
SVT-40776	0.7 (0.4–1.1)	40.3 (32.6–51.6)	58

Mice were killed 3 h after receiving a single oral dose of antagonists or vehicle. Values are expressed as mean (95% CI) n = 4–6 animals for each antagonist dose, three to five doses for each compound.

Table 3 Comparative *in vitro* and *ex vivo* carbachol pEC_{50} obtained in isolated detrusor and in atria from mice

	In vitro *pEC_{50}* (n)	Ex vivo *pEC_{50}* (n)
Detrusor	5.66 ± 0.04 (4)	5.16 ± 0.06 (71)
Atria	6.23 ± 0.05 (10)	6.55 ± 0.09 (27)

pEC_{50} values are expressed as mean ± SEM, n = number of animals used.

duced significant improvements in OAB symptoms compared with placebo, even though the clinical relevance of these differences was uncertain. A recent update of a Cochrane systematic review has corroborated the efficacy of anticholinergic medication, suggesting also that improvements in symptoms may be associated with modest improvement in quality of life (Nabi *et al.*, 2006). The overall concept of improvement in quality of life was introduced to support a reported efficacy that did not reflect the real limited effectiveness, a handicap that no recently launched treatment has been able to overcome.

From a rational point of view, two logical questions are: is efficacy compromised because the dose level is limited and is the dose limited because of the probability of adverse events?

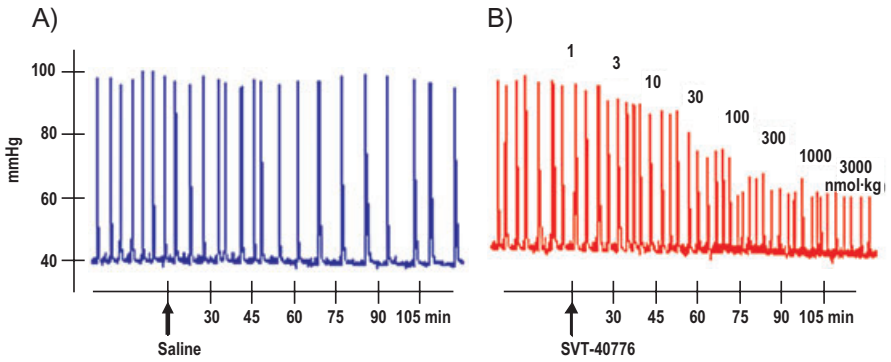

Figure 5 Representative trace of spontaneous contractions of guinea pig bladder *in vivo* before and after consecutive saline (a) or antagonist (b) bolus. Amplitude of bladder contraction was measured during the 15 min period between doses and the per cent change in bladder contraction amplitude was calculated, relative to baseline values.

Table 4 *In vivo* effects of treatment with M_3 receptor antagonists on bladder intravesical pressure and arterial blood pressure

Compound	*ED_{25} (nmol·kg^{-1}, i.v.)*	
	IVP inhibition	*MAP increase*
Tolterodine	299.2	820
Darifenacin	53.2	>1000
Solifenacin	200.9	>3000
SVT-40776	17.1	>3000

Values are calculated from $n = 4–6$ animals per dose. Darifenacin was not given at higher doses because of its solubility problems.
IVP, intravesical pressure; MAP, mean arterial pressure.

The incidence of typical muscarinic adverse events such as constipation or dry mouth has been shown to increase with dose (Chapple *et al*., 2005; Hay-Smith *et al*., 2005). However, cardiac effects due to blockade of M_2, receptors which would be unacceptable for a non-life-threatening condition, have been clearly under-reported. Increase in heart rate is an adverse effect exhibited by non-selective anticholinergic agents, which may become prominent when used at high doses (Howell and Kovalsky, 1995; Andersson and Olshansky, 2007). The last two antimuscarinic agents launched for OAB, solifenacin and darifenacin, were developed with the intention of obtaining a safer cardiovascular profile. It should be noted that cardiovascular disorders, including hypertension, ischaemic heart disease and arrhythmias, have a prevalence of 47% in OAB patients treated with anitmuscarinics (Andersson and Olshansky, 2007). The effect of darifenacin and tolterodine treatment on heart rate have been recently evaluated in patients with OAB (Romanzi *et al*., 2005; Olshansky *et al*., 2006; 2008). Tolterodine significantly increased heart rate in comparison with darifenacin and placebo. The percentage of patients with an increase in heart rate of ≥5 bpm from baseline to last observation was significantly greater with immediate-release tolterodine (2 mg twice daily) (39.3%) than placebo (23.2%) or darifenacin (15 mg·kg^{-1} once daily) (23%) (Olshansky *et al*., 2006). Very similar results were obtained in a second study where extended release-tolterodine (4 mg once daily) and darifenacin (15 mg·kg^{-1} once daily) were compared with placebo (Olshansky *et al*, 2008). Tiotropium, a well-known non-selective muscarinic antagonist developed for the treatment of chronic obstructive pulmonary disease (COPD), showed in a pooled clinical trial analysis, a slightly elevated risk of tachycardia when compared with placebo (Kesten *et al*., 2005). Moreover, Barr *et al*. (2006) published a meta-analysis of available randomized trials, in which, among the adverse events reported, the authors pointed out that the frequency of arrhythmias was significantly higher with tiotropium than with placebo.

In order to increase both the efficacy and tolerability of antimuscarinics in the treatment of OAB, new antagonists with greater selectivity for M_3 receptors are being developed. The present study reports the functional activity of a novel antimuscarinic, SVT-40776, with M_3 receptor antagonist properties. Previous binding studies performed in our laboratory have characterized the binding properties of SVT-40776 (data not shown). These studies demonstrated the high affinity and selectivity for binding to M_3 receptors over M_2 of SVT-40776 (Ki of 0.19 nmol·L^{-1} for human M_3 receptor affinity and 203-fold for M_3 vs. M_2 affinity) (Farrerons *et al*., 2002 SALVAT S.A., PCT Patent application WO02/00652; Salcedo *et al*., 2003; Balsa *et al*., 2004; Fernández *et al*., 2005). In the present study, we have characterized the functional activity of SVT-40776 on bladder contraction and compared it with its activity on atrial contractions in order to assess the functional selectivity of the compound.

Functional *in vitro* studies in mouse urinary bladder smooth muscle have shown that SVT-40776 was more potent in inhibiting carbachol-induced bladder contractions than the marketed antimuscarinic agents tolterodine, solifenacin and darifenacin. SVT-40776 was able to produce a right-ward parallel shift of the cumulative agonist concentration-response curves, obtaining a pA_2 of 9.5, while pA_2 values for tolterodine, solifenacin and darifenacin were 8.4, 8.6 and 8.8 respectively. Tolterodine and solifenacin did not significantly alter maximum carbachol response. However, darifenacin reduced E_{max} (from 66% at 3 nmol·L^{-1} to 58% at 100 nmol·L^{-1}), which means that darifenacin behaves insurmountably in the mouse bladder. SVT-40776 slightly reduced the E_{max} to 71% at 10 nmol·L^{-1} and to 80% at 100 nmol·L^{-1}, concentrations that are 25 and 250-fold higher than its intrinsic activity. Although the pharmacological meaning of these results has not been elucidated so far and would require further investi-

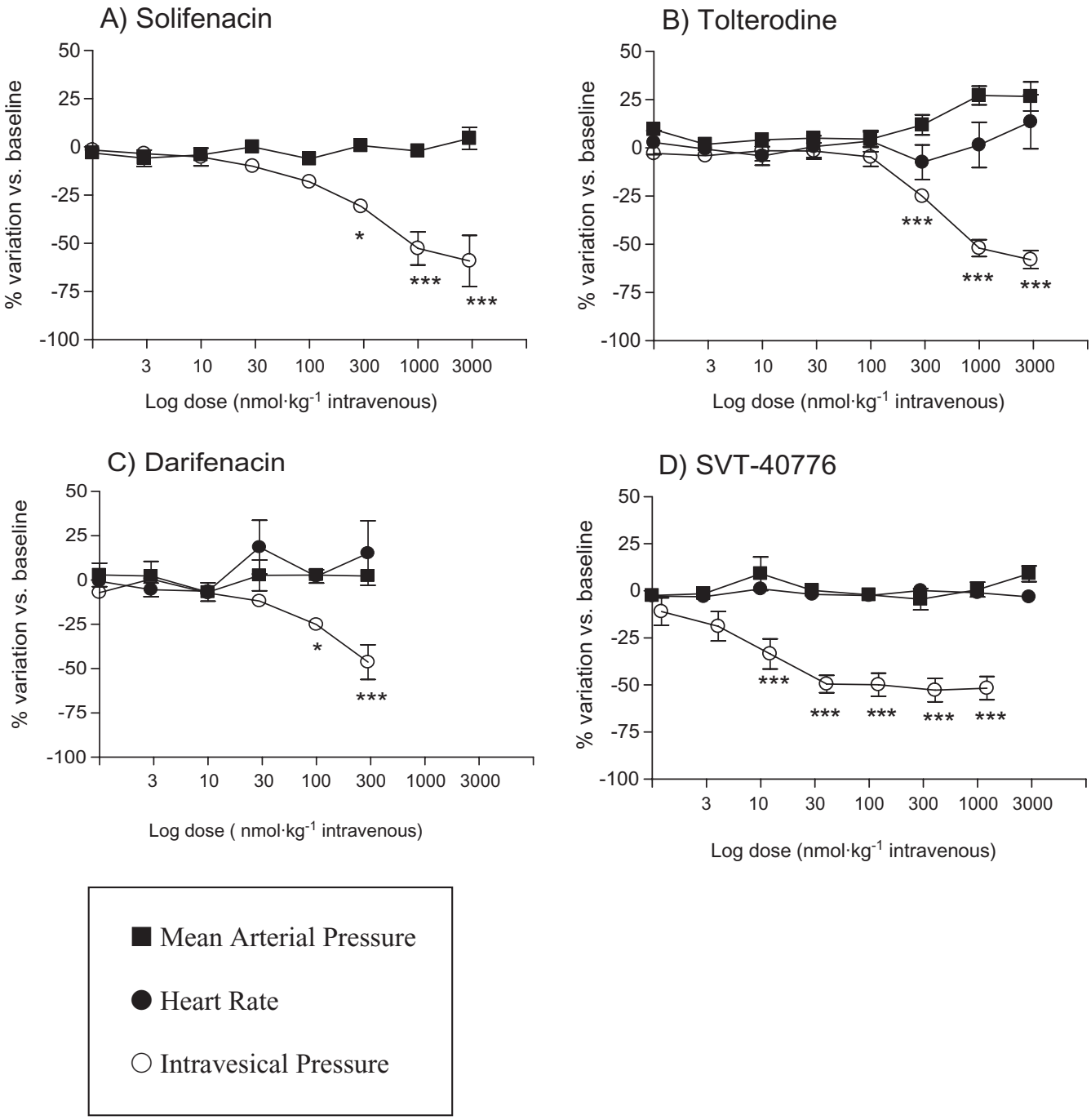

Figure 6 Effect of intravenous administration of four muscarinic receptor antagonists on bladder contraction, blood pressure and heart rate in guinea pigs. Amplitude of bladder contraction, mean arterial blood pressure and heart rate were measured at the same times (see *Methods*). The per cent change in each variable was calculated relative to baseline values. *$P < 0.05$, **$P < 0.01$ and ***$P < 0.001$ versus control; Student's *t*-test: $n = 4$–6 animals per compound.

gation, one explanation can be that at these high concentrations a depletion of the antagonist from the medium can occur as a consequence of binding to other receptors or other structures, which would lead to a slope higher than 1. However, it should be noted that at concentrations in the order of its affinity for M_3 receptors, SVT-40776 behaves as a competitive antagonist, as do tolterodine and solifenacin, but not darifenacin. The results from darifenacin were compatible with an insurmountable blockade and are consistent with previous findings in rat (Hegde *et al.*, 1997), dog (Choppin and Eglen, 2001), mouse (Yamada, 2006) and human bladder (Fetscher *et al.*, 2002).

In mouse atrial preparations, carbachol curves were antagonized by all compounds tested, in a concentration-dependent fashion, with parallel right-ward displacements at lower potencies than in bladder tissue. The rank order of antagonist

activities (pA_2) was tolterodine (8.5), solifenacin (7.8), darifenacin (7.3) and SVT-40776 (7.2).

Having in mind that the M_3 receptors represent the main receptor system controlling bladder contractility and that the M_2 receptor is mainly responsible for muscarinic cardiac effects, the high urinary versus cardiac functional selectivity (199-fold) exhibited by SVT-40776 is in agreement with the results previously found in binding studies where SVT-40776 showed a high affinity for the M_3 receptor ($K_i = 0.19$ nmol·L^{-1}) and a clear selectivity (203-fold) for M_3 versus M_2 receptor subtypes (Salcedo *et al*. 2003, Balsa *et al*. 2004, Fernández *et al*., 2005). As a result of the pharmacological profile exhibited by SVT-40776 in binding as well as in functional studies, it is therefore not expected for the compound to cause any M_2 receptor-related unwanted cardiac effects.

The *ex vivo* protocol was intended to get a closer approach to the *in vivo* situation, as it integrates the pharmacokinetic and distribution pattern of the compounds. SVT-40776 inhibited carbachol-induced bladder contractions in a concentration-dependent manner 3 h after oral administration. Neither tolterodine, solifenacin nor SVT-40776 significantly altered the maximum response to carbachol. On the contrary, darifenacin significantly reduced the E_{max} at 50 mg·kg^{-1}, verifying the antagonist profile shown in the *in vitro* protocol. Likewise, darifenacin exhibited less potency than tolterodine and solifenacin in the atria, which accounts for the M_3 versus M_2 receptor selectivity attributed to darifenacin (Gillberg *et al*., 1998). Interestingly, SVT-40776 did not induce any significant right-ward shifts of the carbachol curves at doses up to 30 mg·kg^{-1}. As the *ex vivo* model reflects more accurately the physiological conditions, these data support the fact that SVT-40776 is a M_3 receptor antagonist clearly devoid of any relevant M_2 receptor affinity.

In line with the above observations, in the anaesthetized guinea pig model, SVT-40776 inhibited isovolumetric-induced contractions in a dose-dependent fashion, without changing cardiovascular parameters. Tolterodine, darifenacin and solifenacin also changed bladder contraction amplitude in a dose-dependent manner. Nonetheless, SVT-40776 revealed itself as the most potent muscarinic antagonist of the compounds tested in this *in vivo* model, with an ED_{25} of 17.1 nmol·kg^{-1} (6.97 μg·kg^{-1}). These experiments provide *in vivo* functional data that may indicate potential advantages for an M_3 selective drug, in terms of cardiovascular safety.

The present study has shown that SVT-40776, a novel substituted quinuclidine derivative, is a potent inhibitor of M_3 receptor-related detrusor contractile activity. Its functional selectivity for urinary bladder over cardiac tissues is in the order of 200-fold, a value not reached with any of the current agents used in the treatment of OAB. Studies performed in our laboratory have shown that SVT-40776 exhibits also a relevant selectivity for mouse bladder tissue over salivary glands, a tissue known to contain M_1 and M_3 receptors and whose activation is necessary for salivary secretion (Balsa *et al*. 2005). This is an important characteristic of the compound as it may predict a lack of dry mouth, a side effect exhibited by most of the antimuscarinic agents used for OAB. In summary, the wide experimental selectivity ratio for bladder over cardiac function (and salivary glands) exhibited by SVT-40776 may predict a good tolerability profile regarding to antimuscarinic adverse effects. The compound has successfully completed Phase I clinical trials and is currently undergoing Phase II clinical trials for the treatment of OAB.

Conflict of interest

All the authors were employees of Laboratorios Salvat at the time of these experiments.

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