

FUNCTIONAL TISSUE SELECTIVITY OF SVT-40776 A NEW SELECTIVE M3 ANTAGONIST FOR THE TREATMENT OF OVERACTIVEBLADDER

Hypothesis/Aims Of The Study

Antimuscarinic agents are the first line of treatment for overactive bladder (OAB). However side effects such as dry mouth or cardiovascular adverse events safety due to M2 or M3 blocking may be a limiting factor in their clinical use. SVT-40776 is a novel substituted quinuclidine derivative highly selective (203-fold) for human M3 over M2 receptors. SVT-40776 exhibited the highest selectivity for bladder over salivary gland (23-fold in mice tissues and 14-fold in human samples) when compared with other antimuscarinic agents. Surprisingly there was not a correlation between the binding affinities and the values for the functional assays in both tissues, bladder and salivary gland. The aim of this study was to investigate whether the differences found in salivary gland and bladder were due to a different behaviour of the M3 receptor in these tissues.

Study Design, Materials And Methods

Mice salivary gland and bladder membrane preparation. Adult mice CD-1 were killed by cervical dislocation and exanguined. Salivary glands (SG) and bladder (B) were removed, cleaned and minced with scissors. Afterwards, animal tissues were homogenized with an Ultraturrax homogeniser. The homogenate was centrifuged and the final pellet was suspended in Tris-HCl (pH 7.4), and aliquots were frozen at -80°C. The same procedure was performed for human samples.

Competition studies. Competition curves performed with mice salivary gland and bladder membrane preparations were determined incubating [³H]-N-methyl scopolamine 0.5 nM, 200 µg of the membrane preparation and different concentrations of antagonist in a total volume of 2 mL at 30°C for 3 hours. Non-specific binding was defined in the presence of 5µM atropine.

Measurement of Ins-4-monophosphate accumulation in submandibular glands and bladder tissue. The measurement of Ins-4-monofosfate was adapted from the method of (Berridge, 1983). Mice SG and B suspension was labelled with [³H]-myo-inositol for 2 h at 37°C. When indicated, Pertussis Toxin treatment was added 24 hours before performing the experiment at a concentration of 1mg/ml. Tissues were preincubated for 20 min with antagonists, before the addition of carbachol (Cch, 10 µM), and then further incubated for 1 h at 37°C in the presence of Lithium chloride (10 mM). The reaction was stopped and [³H]Ins4P eluted as previously described.

Results

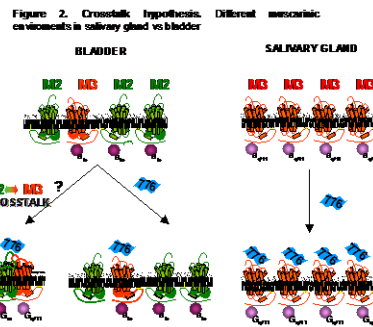
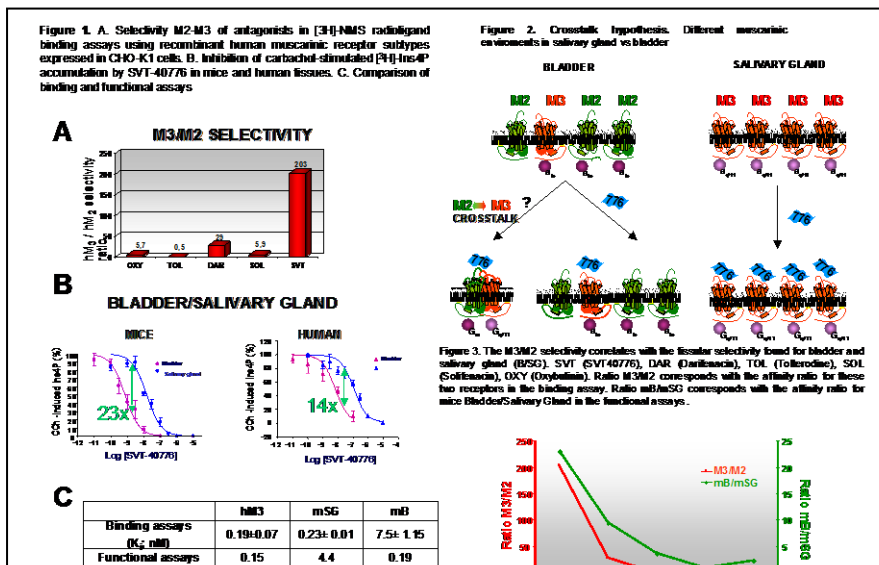


Figure 3. The M3/M2 selectivity correlates with the tissue selectivity found for bladder and salivary gland (BSG). SVT (SVT4076), DAR (Darifenacin), TOL (Tolterodine), SOL (Solifenacin), OXY (Oxybutinin). Ratio M3/M2 corresponds with the affinity ratio for these two receptors in the binding assay. Ratio mB/mSG corresponds with the affinity ratio for mice Bladder/Salivary Gland in the functional assays.

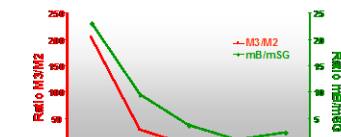
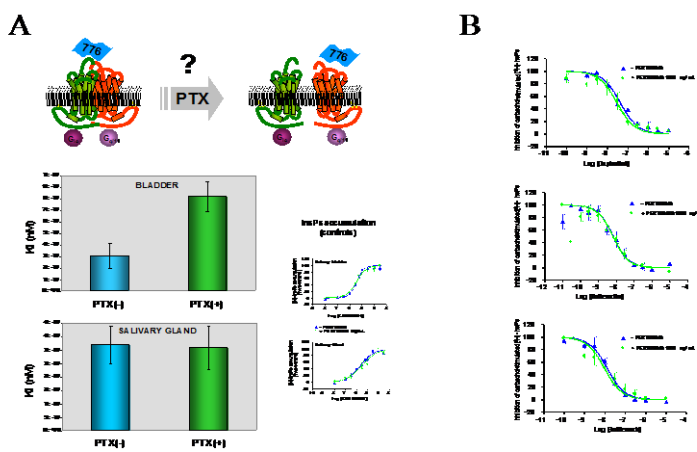


Figure 4. A. Inhibition of carbachol-stimulated [³H]-Ins4P accumulation by SVT-40776 in mice tissues in the presence (+PTX) or not (-PTX) of Pertussis Toxin. B. Inhibition of carbachol-stimulated [³H]-Ins4P accumulation by Oxybutinin, Tolterodine and Solifenacin in mice bladder in the presence) or not of Pertussis Toxin



Interpretation of Results

- SVT-40776 has the highest selectivity for M3 over M2 for all the Muscarinic antagonists tested
- SVT-40776 displays the highest selectivity for bladder over salivary gland in inhibition of Cch-induced Ins4P accumulation assays.
- SVT-40776 in bladder exhibited an excellent correlation between Ins4P inhibition and the *in vitro* human M3 receptor binding but not with the tissue binding assays.
- We hypothesized the existence of a crosstalk between M3 and M2 receptors that could account for this difference.
- Despite the SVT-40776 lack of affinity for the M2 receptor, PTX pretreatment of tissues resulted in changes in the affinity of in functional assays only in bladder but not in salivary gland receptor. This change correlates with the crosstalk between M3 and M2 receptors. SVT-40776 seems to be unique among the anti-muscarinic compounds in the ability to interact with the heterodimer.

Concluding Message

These data demonstrate that SVT-40776 is highly selective for human M3 over M2 receptors and it exhibited the highest selectivity for bladder over salivary gland when compared with other antimuscarinic agents. These data can foresee a better tolerability in terms of dry mouth in clinical trials.

<i>Specify source of funding or grant</i>	No grant
<i>Is this a clinical trial?</i>	No
<i>What were the subjects in the study?</i>	NONE