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SVT-40776: A SELECTIVE, COMPETITIVE AND REVERSIBLE ANTAGONIST OF THE HUMAN RECOMBINANT MUSCARINIC M3 RECEPTOR

Hypothesis / Aims of Study

The muscarinic M3 receptor subtype is one of the primary receptors involved in the pathophysiology of overactive bladder (OAB). SVT-40776 is a novel substituted quinuclidine derivative that is highly selective (200-fold, aprox) for human M3 over M2 receptors [1,2], that is currently ongoing Phase II clinical testing for the treatment of OAB. The aim of the study was to characterize the binding properties of SVT-40776 to the human M3 receptor expressed in CHO-K1 cells.

Study Design, Materials and Methods

Binding Studies: Membranes were prepared from CHO cells stably transfected with the M3 subtype of human muscarinic receptor. Saturation studies: Saturation curves were performed incubating different concentrations of [^{3}H]-NMS and the membrane preparations in the presence of increasing concentrations of antagonist, in a total volume of 200 µL at 25ºC for 1 hr. In alternative assays, membranes were pre-incubated for 90 min with the antagonist before adding the radioligand. Non-specific binding was determined in the presence of 10 µM of atropine. The reaction was terminated by vacuum filtration over 96-well glass fiber filter (type C) plates using the Millipore vacuum manifold; filters were then washed and dried. Scintillation liquid was added to the filters, and the retained radioactivity was determined in a scintillation counter (Microbeta® TriLux; PerkinElmer). Dissociation experiments: Human M3 membrane preparations were allowed to equilibrate with the antagonist for 3 h at 25ºC. Specific binding was determined with [3H]-NMS following initiation of dissociation with 50-fold dilution. Data Analysis: The data points derived from the specific binding were analysed using a non-linear curve-fitting programme (GraphPad Prism®, GraphPad Software Inc.). Binding parameters were obtained as the best-fit values for the data using the least-squares method.
Acetylcholine-Induced Ca^{2+} Mobilization in CHO Cells Expressing the Human M3 Receptor: CHO cells expressing the human M3 receptor were loaded with Fluo-3 (4 µM) for 45 min at 37ºC in the dark in HEPES buffer, containing probenecid (2.5 mM) and BSA (0.1%). Fluorescence due to changes in intracellular free calcium induced by the addition of acetylcholine (0.3 µM) was measured in a 96-well multilabel reader (Victor, Perkin Elmer). Antagonists were incubated at 37ºC, 15 min before the addition of the agonist.
Reagents: SVT-40776, darifenacin, solifenacin and tolterodine were dissolved in DMSO to prepare a concentrated stock solution of 10 mM. 4-DAMP mustard was dissolved in 50% ethanol solution and let to stand at RT for 1 h to form the aziridinium ion before subsequent dilution.

Results

Table 1: Effect of Muscarinic Antagonists on [^{3}H]-NMS Binding Parameters to the Human M3 Receptor after 90 min Preincubation

[Antagonist] nM	**0**	**0.1**	**0.3**	**1**	**3**	**10**	**30**	**60**	**100**	**200**	**300**	**600**
SVT-40776												
K_D	0.69	0.78	0.91	1.7**	3.9*	9.5**	27.5					
B_{max}	1364	1291	1251	1447	1458	1420	1493					
Darifenacin												
K_D	0.71			2.1	1.2	1.5	2.9**	7.7*	11.9*			
B_{max}	1463			1526	1411	1263	1128	1171	1488			
Solifenacin												
K_D	1.1	0.6	0.49	0.67	0.79	1.1			8.4**			
B_{max}	1571	1321	1293	1328	1278	1240			1499			
[Antagonist] nM	**0**	**0.1**	**0.3**	**1**	**3**	**10**	**30**	**60**	**100**	**200**	**300**	**600**

Tolterodine										
K_D	1.99			0.64		0.9	3.3	8.4	12*	27**	52**
B_{max}	1639			1247		1491	1525	1358	1328	1530	1278
4-DAMP must.										
K_D	1.4	1.5	1.3	1.3	0.99					
B_{max}	1527	1365	1212	773	330*					

Kd is expressed in nM, Bmax in fmols/mg. *p<0.05 (t-student) .**p<0.01 (t-student)

Figure 1: Dissociation of SVT-40776 from the Muscarinic hM3 Receptor

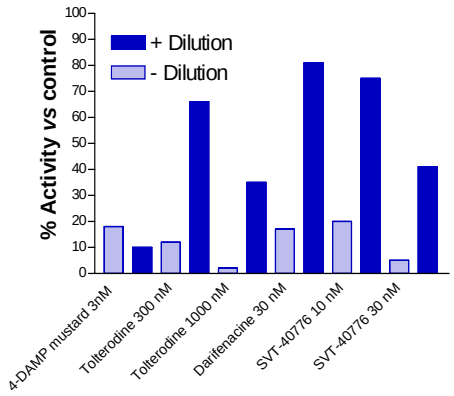
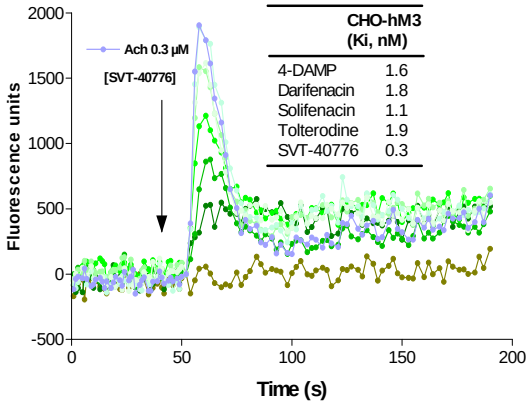


Figure 2: Representative Effect of SVT-40776 on Ca^{2+} Mobilization Induced by Acetylcholine in hM3-CHO Cells



Interpretation of Results

- SVT-40776, like darifenacin, solifenacin and tolterodine, produced a dose-dependent righward shifts of [^{3}H]-NMS saturation curves without significantly changing the Bmax value and accompanied by an increased in the Kd value, consistent with a competitive binding. This behaviour is a time-independent process. 4-DAMP mustard produced a significant dose-dependent reduction in Bmax for radioligand binding, consistent with a non-competitive binding (Table 1).
- Preincubation of CHO membranes containing hM3 receptors with the ligands and further diluted in the presence of [^{3}H]-NMS, showed a reversible binding to the muscarinic receptor of SVT-40776, as well as darifenacin, solifenacin and tolterodine. As expected, 4-DAMP mustard binding to the M3 receptor was, however, irreversible (Figure 1).
- SVT-40776 showed to be the most potent product inhibiting the acetylcholine-induced Ca^{2+} mobilization through human M3 receptors expressed in CHO cells, thus demonstrating its muscarinic antagonistic ability (Figure 3). The potency of SVT-40776 in inhibiting this response correlates well with its binding affinity [see 1].

Concluding Message

These data indicate that SVT-40776 is the most potent ligand of the human recombinant M3 muscarinic receptor compared to the other compounds assayed. SVT-40776 exhibits a competitive and reversible antagonistic profile.

References

[1] Functional characterization of SVT-40776, a novel and potent M3 receptor antagonist, on mice detrusor and atria isolated preparations using *in vitro* and *ex vivo* protocols. *Br J. Pharmacol* (2002) **136**(supp): 45P.

[2] SVT-40776, a new selective M3 muscarinic antagonist: human receptor binding profile and bladder effects in the guinea-pig. *Neurourol Urodyn* (2003) **22**(5):382-384.