Pharmacological characterisation of TMEM16A regulators

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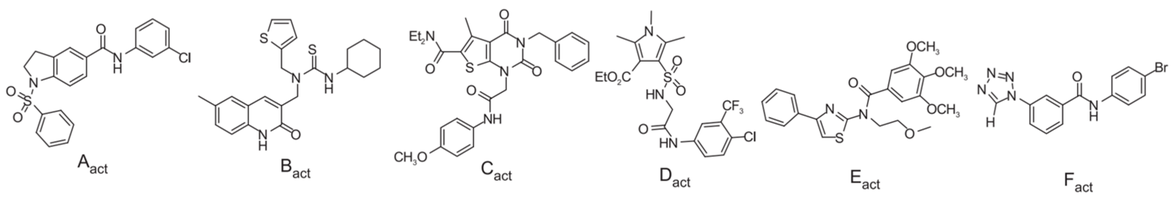
Introduction

TMEM16A was recently identified as a calcium-activated chloride conductance and a key orchestrator of anion secretion in the human airway epithelium (Caputo et al 2008; Schroeder et al 2008; Yang et al 2008). It is now clinically established that promoting anion secretion in the airway leads to enhanced mucus clearance and reduced exacerbation frequency in CF patients and as such TMEM16A represents an important target for the next generation of mucokinetics. Importantly, positive regulators of TMEM16A function will be expected to be of benefit in all CF patients, irrespective of their CFTR mutational status.

To date a small number of tool compounds have been described in the literature as TMEM16A activators (Namkung et al. 2011). Our aim is to identify novel 'potentiators' of TMEM16A that will promote airway epithelial anion secretion in the CF lung. 'Potentiator' compounds will act by enhancing TMEM16A-mediated secretion whilst maintaining normal physiological regulation of the channel. To this end we have established a series of biological assays of TMEM16A function to both identify novel regulators of channel function and to understand their mechanisms of action.

The aim of the present study was to establish the pharmacological profile of literature 'activators' in a series of key functional assays.



FRT-TMEM16A assay formats

An FRT-hTMEM16Aabc cell line, generously provided by Prof. Luis Galietta, was used in a high throughput membrane potential assay format. In addition, the same cell line was cultured on Snapwell inserts and used for ion transport studies.

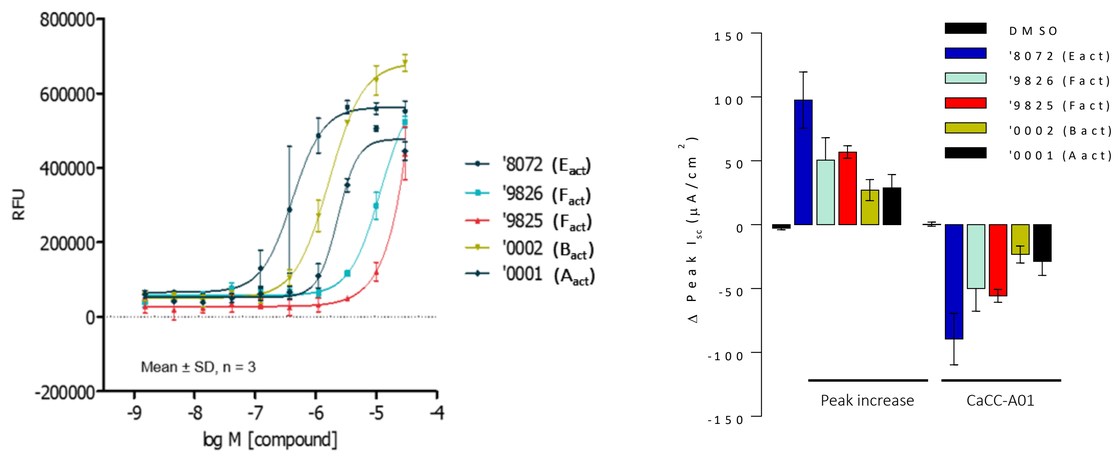
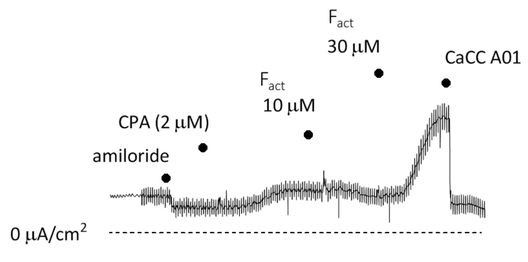


Figure 1. Concentration-response data (A) illustrating the positive effects of literature TMEM16A activators in the membrane potential assay. Each of these compounds (30 μM) likewise increased the short-circuit current (ISC) under an imposed basolateral to apical chloride gradient in the same FRT-TMEM16Aabc line (B).

CF HBEC ion transport

CF HBEC were cultured on Snapwell inserts with the final 14-21 days at air-liquid interface. Cells were treated with IL-13 (10 ng/mL) for 48-96h before use in the assay. HBEC were placed into Ussing chambers bathed in isometric Ringers solution and voltage clamped to 0 mV. The ENaC mediated short-circuit current (ISC) was inhibited with amiloride and then an EC_{20} concentration of the SERCA pump inhibitor CPA (2 μM) was added to induce a small increase in ISC. Under these conditions, the effects of literature TMEM16A activators were examined.



Compound	CaCC-A01 dependent ISC
DMSO	4.5 ± 1.4 μA/cm²
'8072 (E_{act})	29.9 ± 7.1 μA/cm²
'9826 (F_{act})	15.3 ± 5.1 μA/cm²
'9825 (F_{act})	14.7 ± 4.4 μA/cm²
'0002 (B_{act})	7.1 ± 2.9 μA/cm²
'0001 (A_{act})	11.5 ± 2.3 μA/cm²

Figure 2. Raw data trace illustrating a typical CF HBEC ISC response to a literature compound. The table shows the mean ± SEM ISC response to 100 μM CaCC-A01 added at the end of each study to quantify the magnitude of the TMEM16A-mediated current.

Qpatch electrophysiology

The effects of literature activators were evaluated on FRT-TMEM16Aabc cells under patch-clamp conditions. Intracellular Ca^{2+} was clamped to 260 nM to activate the channel to approximately 20% of its maximum current. Under these conditions, the CaCC-A01 sensitive current was unaffected by any of the literature compounds.

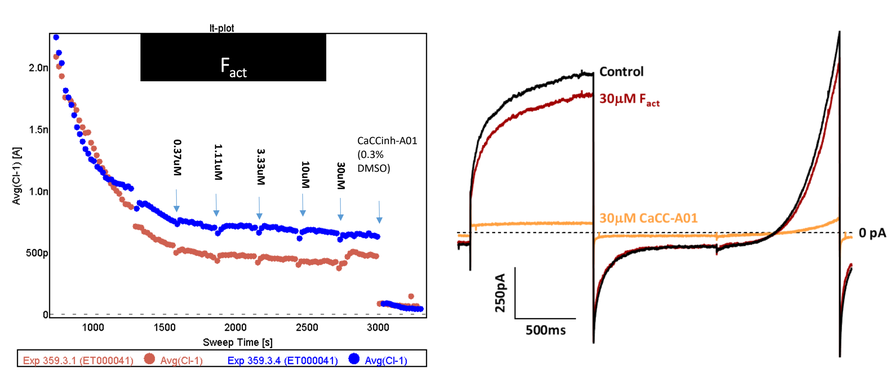


Figure 3. Raw data trace illustrating a typical Qpatch current response, highlighting the lack of activity of compound Fact that was representative of all of the literature compounds.

Intracellular Ca^{2+} assay

In view of the disconnect between the membrane potential and ion transport assays, where intracellular Ca^{2+} levels were unclamped, versus the patch-clamp studies where Ca^{2+} levels were clamped, the FRT-TMEM16Aabc cell line was used to assess the potential of the literature compounds to influence intracellular Ca^{2+} levels.

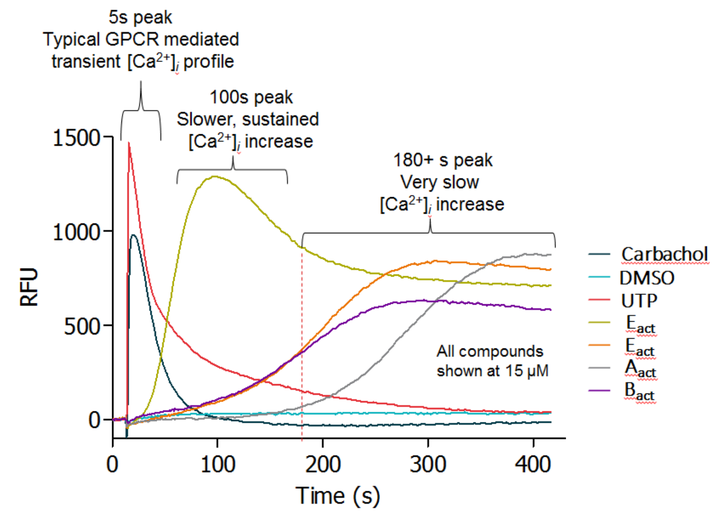


Figure 4. Raw data traces illustrating the effects of literature compounds on the levels of intra-cellular Ca^{2+}. All of the compounds induced an elevation of Ca^{2+} levels.

Conclusions

Together these data suggest that the TMEM16A activators described in the literature act through a non-specific effect on intracellular Ca^{2+} rather than through a direct effect on TMEM16A. As such, care should be taken when using these compounds in cell-based assays and ascribing pharmacological activity to a TMEM16A-specific mechanism.

Using these assays, drug-discovery efforts are ongoing to develop novel potentiators of TMEM16A as drug candidates that will lead to enhanced channel function without elevating intracellular Ca^{2+} levels – novel compounds with potentiator activity have already been identified by employing the Qpatch automated electrophysiology described here in a medium throughput screening format (figure 5).

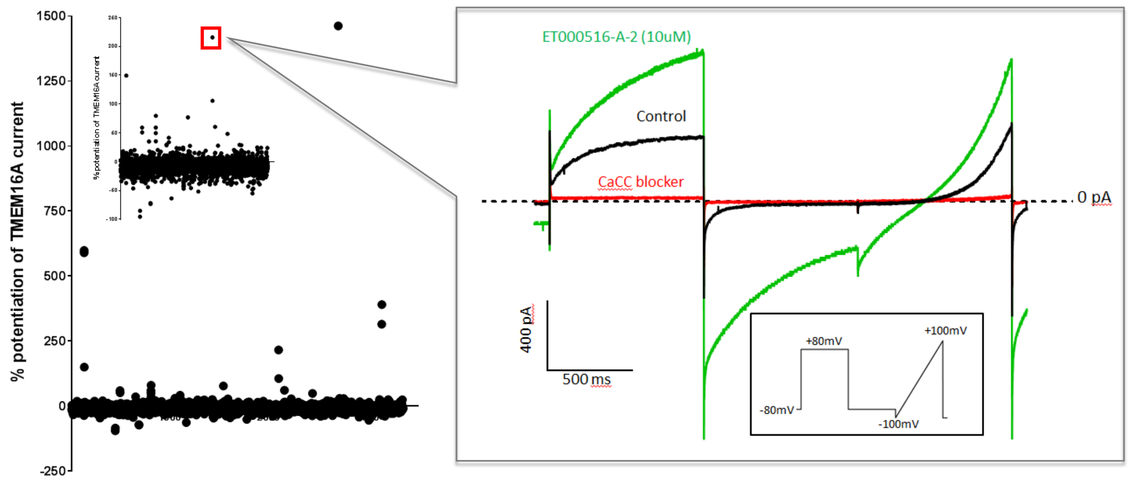


Figure 5. Medium throughput library screening using the Qpatch FRT hTMEM16A assay has identified novel compounds which enhance channel activity when intracellular Ca^{2+} is clamped.

References:

Caputo et al (2008) Science 322(5901); 590 – 4

Schroeder et al (2008) Cell 134(6); 1019 – 29

Yang et al (2008) Nature 455(7217); 1210 – 15

Namkung et al (2011) FASEB J 25; 4048 - 62