Bitter Taste Receptor (TAS2R) Function in Airway Smooth Muscle of Cystic Fibrosis

Nicholas M Dalesio MD[1,2]; Jessie Huang[3]; Charles Emala, MD[4]; Pamela L. Zeitlin, MD, Ph.D[5]; Dan E. Berkowitz, MBBS[1]; Steven An, Ph.D[2]

Johns Hopkins School of Medicine, Department of Anesthesiology;[2] Johns Hopkins School of Medicine, Department of Otolaryngology-Head and Neck Surgery; [3]Johns Hopkins School of Public Health, Department of Environmental Health and Engineering; [4] Columbia University, Department of Anesthesiology; [5] National Jewish Health, Department of Pediatrics

INTRODUCTION

Cystic Fibrosis (CF), a disease caused by the dysfunctional cystic fibrosis transmembrane conductance regulator (CFTR) alters chloride movement, is the most common fatal genetic disease in the USA. For CF of the lung, this leads to hyper-secretion of mucus, decreased mucociliary clearance (MCC), and respiratory infections. Approximately 50% of patients with CF also have concomitant diagnosis of reversible hyper-reactive airway disease caused by constriction of airway smooth muscle (ASM). Unfortunately, these individuals are refractory to traditional bronchodilator therapy with β_2-agonists. Most recently, bitter taste receptors (TAS2R) have been detected in ASM, altering intracellular calcium signaling and causing ASM relaxation and bronchodilation. The physiologic function of TAS2R in CF is entirely unknown.

HYPOTHESIS

We hypothesized that TAS2Rs on ASM cells are functional in CF and that activation of TAS2R signaling pathways can mitigate airway hyper-reactivity in CF patients.

METHODS

Cell Models:

- Murine Models
  - CFTR -/- (S489X mut) and CFTR +/+ (WT) mice
- Human Airway Smooth Muscle (ASM)
  - Primary ASM cells isolated from normal lung
  - Human telomerase reverse transcriptase immortalized ASM cells
- Human CF Models
  - Human ASM cells treated with the CFTR inhibitor (CFTRinh-172) 5 minutes prior to study
  - Human ASM cells from CF patients with ΔF508 mutation (Lonza; CF-DBSMC, #196980)

Bitter Agonist: Chloroquine, a known activator of the TAS2R-10 & -14 receptors expressed on ASM cells.

Magnetic Twisting Cytometry (MTC; Figure 1)

- Cells serum deprived 2 days prior to study
- Cells were treated with increasing doses of chloroquine
- Dynamic changes in cell stiffness measured with MTC

FIGURES

Figure 1: Magnetic Twisting Cytometry

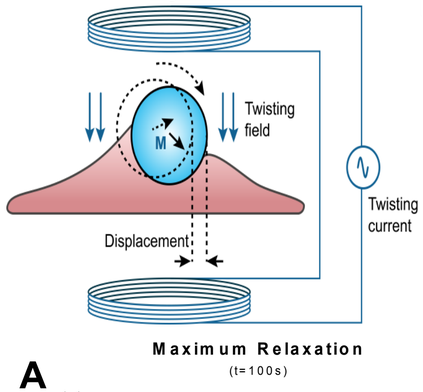


Figure 2: ASM cells from 3 CFTR -/- compared to 3 CFTR +/+ WT mice. For each individual ASM cell, baseline stiffness was measured for 60 s and the changes in stiffness in response to chloroquine (250, 500, 1000μM) measured continuously for 240 seconds.

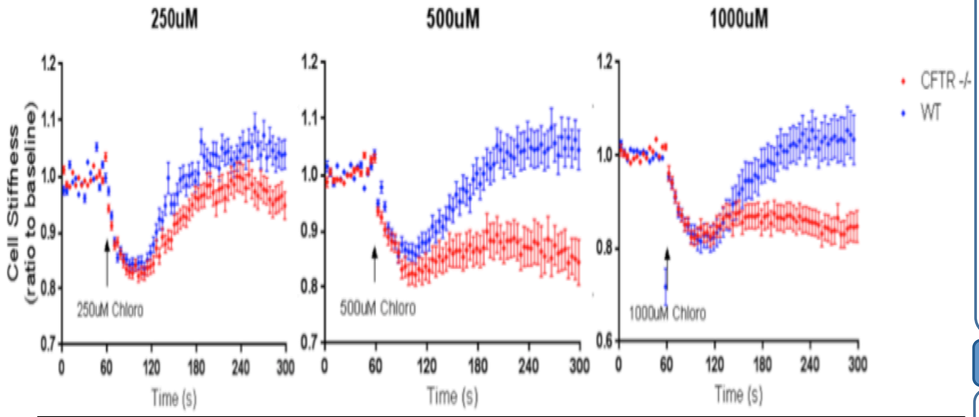
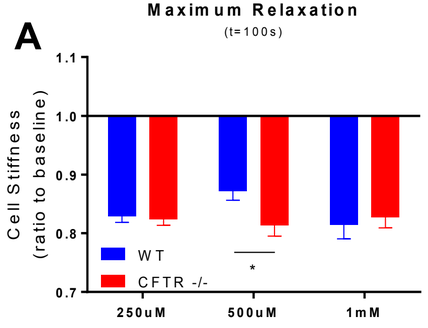
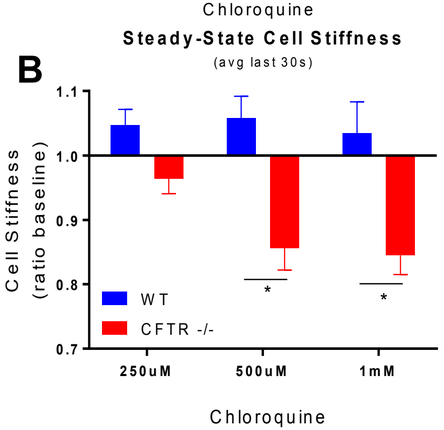
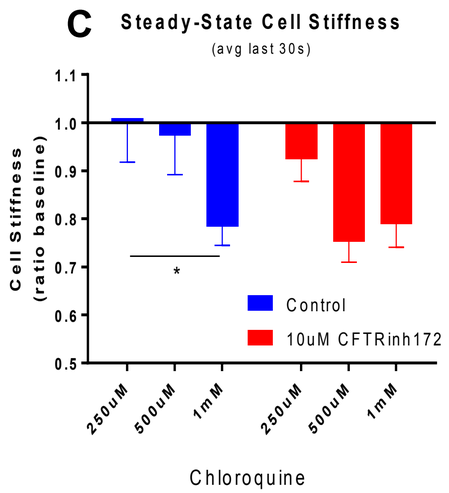
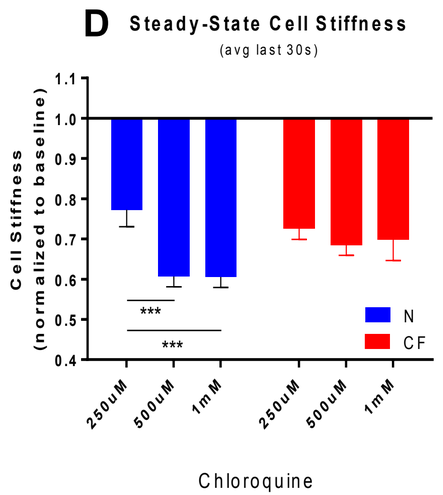


Figure 3: Effects of chloroquine on mouse and human ASM cells. (A) Max cellular relaxation at t=100s and (B) cell stiffness of CFTR -/- compared to CFTR +/+ WT mice; (C) Cell stiffness of human ASM cells treated with and without 10 μM CFTRinh-172; (D) Cell stiffness of primary human ASM cells compared to ASM cells isolated from CF patients.









RESULTS

Murine CFTR -/- versus CFTR +/+

- Enhanced and sustained relaxation response to chloroquine in the CFTR -/- cells compared to the WT cells (Figure 2, 3A)

Human Normal versus CFTRinh-172 treated

- CFTRinh-172 treated cells had enhanced relaxation ($P < 0.02$), however, effects peaked at 500 μM dose (Figure 3B)

Human Normal versus CF

- CF cells had enhanced relaxation trend when compared to the 2 normal cell lines (Figure 3C)

DISCUSSION

These results suggest that dysfunctional CFTR leads to alterations in cell signaling, possibly calcium signaling, enhancing airway smooth muscle cell relaxation in response to bitter agonists.

Possible mechanisms include:

1. Dysfunctional Ca^{2+} - mediated Cl^- channels
2. CFTR alterations affecting G-proteins/Ca^{2+} oscillations
3. Bitter receptor – CFTR ion channel interaction

CONCLUSION

Results suggest dysfunctional CFTR alters Ca^{2+} signaling ***enhancing*** the effects of bitter taste receptor agonists. Future studies to evaluate the linkage between these observations are necessary. TA2SR may be an excellent target for therapy in reactive airways disease in CF

REFERENCES

1) K. S. Robinett. *Am J Respir Cell Mol Biol*, 2011; 2) Deshpande, D. A., *Nat Med*, 2010; 3) An, S. S. *Am J Physiol*. 2012; 6) Keen, C. *Pediatric Pulmonology*, 2010.