The K^+ channel KCNQ1 modulates tubulovesicle Ca^{2+} release during gastric acid secretion

Nirakar Sahoo[1, 2], Tripti Saini[1], Mingxue Gu[2], Ce Wang[2], Xiaoli Zhang[2], Raul Calvo[3], Samarjit Patnaik[3], and Haoxing Xu[1]

[1]Department of Biology, University of Texas Rio Grande Valley, Edinburg, TX, 78539, USA

[2]The Department of Molecular, Cellular, and Developmental Biology, the University of Michigan, 3083 Natural Science Building (Kraus), 830 North University, Ann Arbor, MI 48109, USA

[3]National Center for Advancing Translational Sciences, National Institute of Health, 9800 Medical Center Drive, Rockville, MD 20850, USA

Abstract

Gastric acid secretion is an active secretory process where histamine induces trafficking and exocytosis of proton pump H^+/K^+ ATPase carrying tubulovesicle to the apical membrane. While it is crucial for digestion of food and killing of pathogens, any imbalance in the homeostasis contributes to many pathophysiological conditions which include atrophic gastritis, peptic, and duodenal ulcers, gastroesophageal reflux disease (GERD) and eventually leads to stomach cancer. In recent years, there has been growing recognition of tubulovesicle ion channels as a modulator of gastric acid secretion. Here, we show that the K^+ channel KCNQ1 controls TRPML1 mediated Ca^{2+} release from the tubulovesicle. We used an integrative approach that combines tubulovesicle electrophysiology, Ca^{2+}- imaging, cell biology, and biochemical techniques to dissect the impact of K^+ channel KCNQ1 on tubulovesicle trafficking and exocytosis. Our study showed that TRPMl1 is a tubulovesicular Ca^{2+} channel and responsible for tubulovesicles trafficking, exocytosis, and acid secretion. Tubulovesicle-targeted Ca^{2+} imaging revealed that inhibition of tubulovesicle K^+ channel KCNQ1 significantly abolished histamine-TRPML1 induced Ca^{2+} release from the tubulovesicles. Histamine-mediated tubulovesicle trafficking to the apical membrane was also blocked by KCNQ1 inhibitors. Conclusions: Overall, this study provided a novel regulatory mechanism that could be targeted to treat acid-related gastric diseases.

Introduction

Acid secretion in the stomach requires an active proton pump (H+/K+ ATPase) where H^+ is secreted to the apical side in exchange with K^+ ion. Several studies have shown that K^+ channel KCNQ1 in the tubulovesicle controls gastric acid secretion. However, the role of KCNQ1 in the trafficking and exocytosis of tubulovesicle remains unknown. Our previous study showed that Transient Receptor Potential Mucolipin-1 (ML1, aka TRPML1 or MCOLN1) is the principal Ca^{2+} release channel in the tubulovesicle; it regulates vesicle trafficking and exocytosis and modulates gastric acid secretion. The aim of the present study was to investigate whether KCNQ1 has a direct role in modulating ML1 channels in the tubulovesicle and ultimately controls gastric acid secretion.

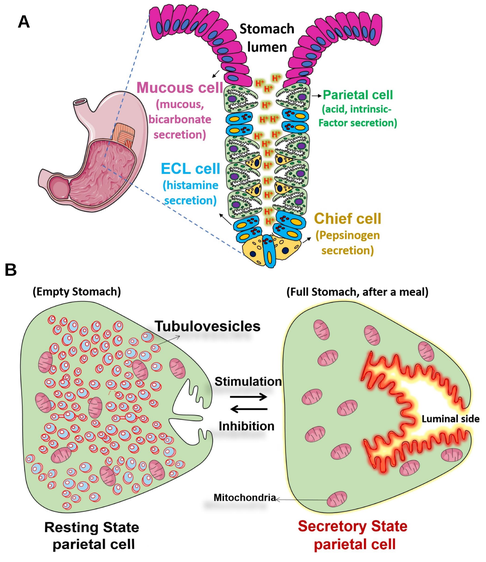

Figure 1. Tubulovesicle in the parietal cell is responsible for gastric acid secretion. (A) Schematic representation of a single gastric gland. Acid is secreted by the gastric gland, and parietal cells in the gland take an active role in this process. **(B)** Parietal cells are packed with small organelles called tubulovesicles. At resting conditions (empty stomach), tubulovesicle carrying proton pump remains in the cytosol and. Upon stimulation (after a meal), tubulovesicles moves to the stomach lumen and resulting in secretion of gastric acids.

Tubulovesicle ion channels modulate gastric acid secretion

Does KCNQ1 play a role in trafficking and exocytosis of tubulovesicles?

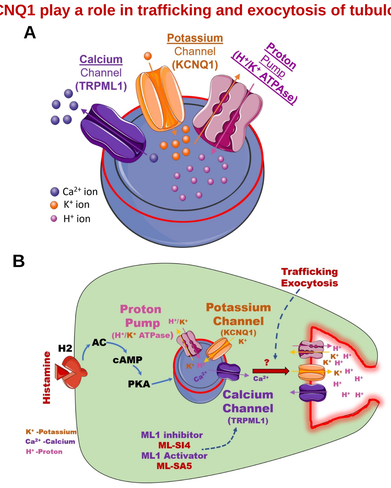

Figure 2. ML1 ion channels regulate trafficking and exocytosis of tubulovesicles. (A, B) The cAMP-PKA signaling pathway controls ML1-mediated Ca^{2+} from the tubulovesicle. Activation of ML1 increases acid secretion and inhibition of ML1 decreases acid secretion.

1. KCNQ1 localizes in the tubulovesicle of the parietal cells

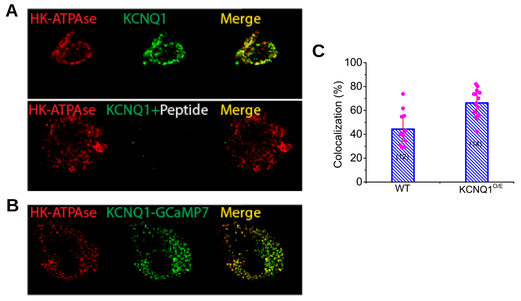

Figure 3. KCNQ1 resides in the tubulovesicles. (A) Dual-STED images of wild-type parietal cells immuno-labeled with anti-HK-α and anti-KCNQ1. A pre-incubation of anti-KCNQ1 with ML1 epitope peptide confirmed the specificity of the KCNQ1 antibody. **(B)** Dual-STED images of a parietal cell transfected with KCNQ1-GCaMP7 and were immuno-labeled with anti-HK-α. **(C)** Analysis of colocalization of HK-α with KCNQ1 in the parietal cells.

2. KCNQ1 traffics to the apical membrane in the stimulated parietal cells

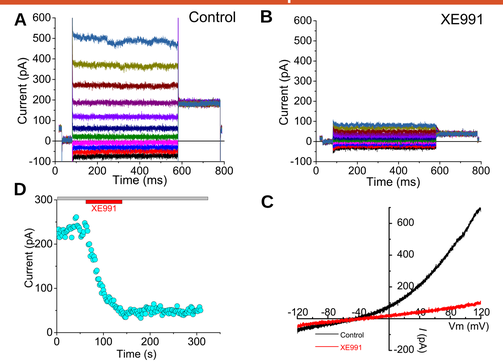

Figure 4. Tubulovesicle K^+ channels KCNQ1 traffics to the apical membrane in the stimulated parietal cells. Patch-clamp recording from apical/ tubulovesicle membranes. Representative whole-VAC recordings of KCNQ1-like currents in histamine-stimulated wild-type parietal cells before and after application of KCNQ1 inhibitor XE991. **(A, B)** current-voltage relation trace measured from -100 mv to + 80 mV, (C) ramp trace, and (D) on rate of induction of XE991 inhibition.

3. Synthetic inhibitors of KCNQ1 disrupts tubulovesicle exocytosis

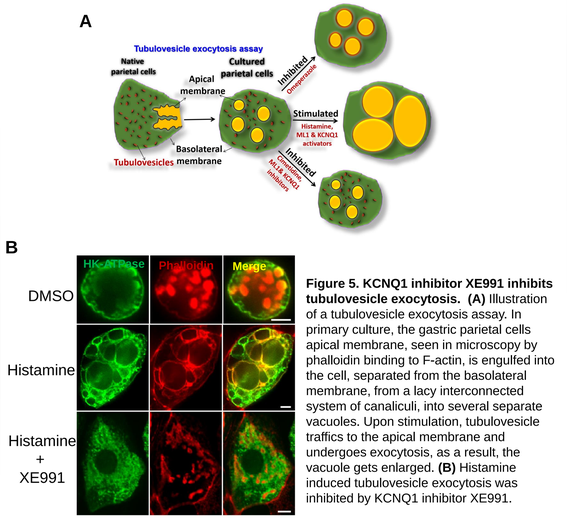

Figure 5. KCNQ1 inhibitor XE991 inhibits tubulovesicle exocytosis. (A) Illustration of a tubulovesicle exocytosis assay. In primary culture, the gastric parietal cells apical membrane, seen in microscopy by phalloidin binding to F-actin, is engulfed into the cell, separated from the basolateral membrane, from a lacy interconnected system of canaliculi, into several separate vacuoles. Upon stimulation, tubulovesicle traffics to the apical membrane and undergoes exocytosis, as a result, the vacuole gets enlarged. **(B)** Histamine induced tubulovesicle exocytosis was inhibited by KCNQ1 inhibitor XE991.

4. KCNQ 1 inhibitor blocks histamine-mediated tubulovesicle Ca^{2+} release

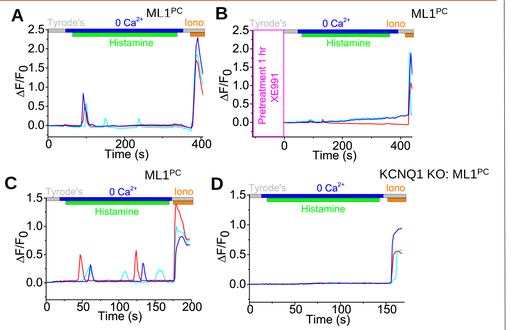

Figure 6. Inhibition of KCNQ1 blocks histamine-mediated Ca^{2+} release from the tubulovesicle. (A, B) Histamine induced tubulovesicle Ca^{2+} release in GCaMP3-ML1-expressing $ML1^{PC}$ parietal cells and was blocked by KCNQ1 inhibitor XE991. **(C, D)** Histamine mediated Ca^{2+} release was absent in GCaMP3-ML1-expressing $ML1^{PC}$:KCNQ1 knock-out parietal cells.

5. Activator of KCNQ1 induces tubulovesicle exocytosis in ML1-dependent manner

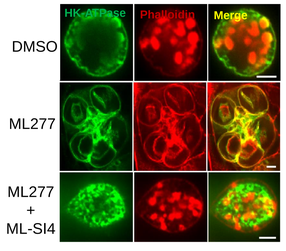

Figure 7. KCNQ1 activator induces trafficking and exocytosis of tubulovesicle in ML1 dependent manner. KCNQ1 opener ML277 facilitates tubulovesicle exocytosis and was inhibited by ML1 inhibitor ML-SI4.

6. KCNQ1 is necessary for ML1-mediated Ca^{2+} release from the tubulovesicle

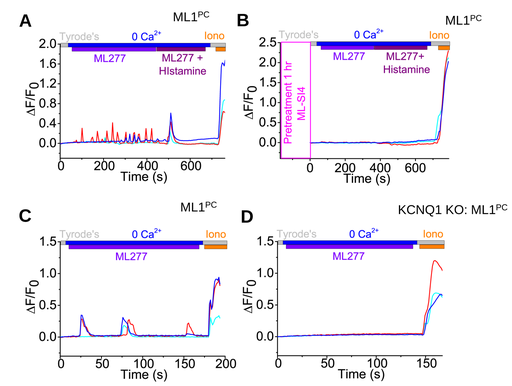

Figure 8. Synthetic opener of KCNQ1 channel induces ML1-mediated Ca^{2+} release from the tubulovesicle. (A, B) KCNQ1 opener ML277 induced tubulovesicle Ca^{2+} release in GCaMP3-ML1-expressing $ML1^{PC}$ parietal cells and was blocked by ML1 inhibitor ML-SI4. **(C, D)** ML277 mediated Ca2+ release was absent in GCaMP3-ML1-expressing $ML1^{PC}$: KCNQ1 knock-out parietal cells.

7. Hypothetical model of KCNQ1 role in tubulovesicle trafficking and exocytosis

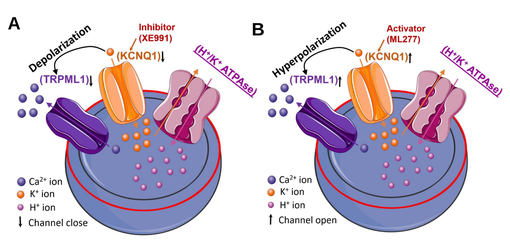

Figure 9. Proposed model shows how KCNQ1 plays a role in non-apical pre-fusion function. KCNQ1 regulates ML1-mediated tubulovesicle Ca^{2+} release by modulating tubulovesicle membrane potential **(A)** KCNQ1 inhibitors block histamine-induced tubulovesicle Ca^{2+} release by depolarization of tubulovesicle membrane potential **(B)** KCNQ1 activator induced tubulovesicle Ca^{2+} release by hyperpolarizing tubulovesicle membrane potential. Because ML1 is strongly voltage-dependent, opening or, inhibiting a K^+ channel in the tubulovesicles may dramatically increase or decrease ML1 current and driving force for Ca^{2+} flux.

Summary

1. **Tubulovesicle K^+ channels KCNQ1 traffics to the apical membrane in ML1-dependent manner during gastric acid secretion.**
2. **KCNQ1 inhibitor blocks tubulovesicle exocytosis and histamine-mediated tubulovesicle Ca^{2+} release.**
3. **KCNQ1 activator potentiates tubulovesicle trafficking and exocytosis by activating ML1-mediated Ca^{2+} release from the tubulovesicles.**

Conclusion

Our study identified that tubulovesicle K^+ channels KCNQ1 is a modulator of tubulovesicle Ca^{2+} channel. KCNQ1 plays a dual role during gastric acid secretion 1) it facilitates the opening of ML1 Ca^{2+} channels in the tubulovesicle, resulting trafficking and exocytosis to the apical membrane, 2) it mediates luminal K^+ efflux in the apical membrane required for K^+-dependence of the H^+/K^+-ATPase activity. Synthetic activator and inhibitor of KCNQ1 may be developed to control acid secretion and treat acid-related gastric diseases.

Acknowledgements

This work was supported by NIH project grants (R01-NS062792 and R01-AR060837 to H.X; University of Rio Grande Valley startup fund, and University of Texas System Rising STARs Award to N.S.