HCO$_3^-$ transport through anoctamin/transmembrane protein ANO1/TMEM16A, in pancreatic acinar cells, regulates luminal pH

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Short Title: TMEM16A regulates pancreatic luminal pH

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2Abbreviations used are: ACh, acetylcholine; CCK, cholecystokinin; CFTR, cystic fibrosis transmembrane regulator; HPTS, hydroxypyrene-3,6,8-trisulfonate; SRB, sulforhodamine B.

Abstract

The identification of ANO1/TMEM16A as the likely calcium-dependent chloride channel of exocrine glands has led to a more detailed understanding of its biophysical properties. This includes a calcium-dependent change in the channel selectivity and evidence that HCO$_3^-$ permeability can be significant. Here, we use freshly isolated pancreatic acini that preserve the luminal structure to measure intraluminal pH and test the idea that ANO1/TMEM16A contributes to luminal pH balance. Our data show that under physiologically-relevant stimulation, with 10 pM CCK, the luminal acid load that results from the exocytic fusion of zymogen granules is significantly blunted by HCO$_3^-$ buffer, in comparison to HEPES, and that this is blocked by the specific TMEM16A inhibitor, T16inh-A01. Furthermore, in a model of acute pancreatitis we observed substantive luminal acidification and provide evidence that ANO1/TMEM16A acts to attenuate this pH shift. We conclude that ANO1/TMEM16A is a significant pathway in pancreatic acinar cells for HCO$_3^-$ secretion in to the lumen.

Introduction

Luminal pH dysregulation in exocrine organs is a key factor in diseases like cystic fibrosis and acute pancreatitis (1,2). This luminal pH is controlled by the epithelial cells that line the lumen. In exocrine glands a major pathway for HCO$_3^-$ secretion is Slc26a6, a Cl$^-$/HCO$_3^-$ exchanger that is found in the apical membrane of ductal epithelium and works, in these cells, in consort with the cystic fibrosis transmembrane regulator CFTR (2) to produce a HCO$_3^-$ rich fluid exudate from the gland (3,4). At least part of the role of this secreted HCO$_3^-$ is to neutralise a luminal acid load that results from the exocytosis of the highly acidic zymogen granule content of the pancreatic acinar cells (5,6). Whether the acinar cells play a more active role in luminal pH regulation is not clear. The identification of ANO1/TMEM16A as the likely candidate for
the apical calcium-dependent chloride channel in exocrine acinar cells (7-11) and some recent work on its control (12) now highlights the possibility that it may act as an apical HCO$_3^-$ exit pathway and thus be an additional component in pancreatic luminal pH regulation (13).

A common finding from work on exocrine tissues is that the calcium-dependent chloride channel has an anion permeability consistent with the Eisenman series I which shows an order of permeability of I$^->$Cl$^->$HCO$_3^->$F$^-$(14,15). In exocrine cells, Cl$^-$ and HCO$_3^-$ are arguably the most physiologically relevant ions. While Cl$^-$ exit in to the lumen is known to be the major ion participating in fluid movement, HCO$_3^-$ movement through the Cl$^-$ channel is not well understood. There is one study that suggests HCO$_3^-$ might exit through the Cl$^-$ channel and if this was true then HCO$_3^-$ would contribute to luminal pH control (16). Now, using cloned and expressed ANO1/TMEM16A, the Eisenman permeability series I for anions for this channel has been confirmed (7,17). Interestingly, studies show that there are dynamic changes in the anion selectivity that are dependent on the concentration of intracellular calcium (18-20). In one paper these selectivity changes in ANO1/TMEM16A permeability include a shift, as the calcium concentration is increased (from 400 nM to 3 µM), from the normal $P_{HCO_3^-} / P_{Cl^-}$ ratio of 0.3 to a ratio of 1.1 (12). It has been suggested that calmodulin mediates this permeability change (12), however, this mechanism has been contested by others (21). In distinction to its precise regulation, an open question is whether the HCO$_3^-$ permeability of ANO1/TMEM16A in acinar cells is large enough to play a significant role in luminal pH balance.

Here, we have used isolated mouse pancreatic fragments and lobules, which preserve the structure of the intact acini, to measure pH changes in the lumen in response to cell stimulation (5). We used 10 pM choleystokinin (CCK) (22) to study the physiological response and 10 nM CCK to model the cell responses in acute pancreatitis. In both cases we observe that the stimulated exocytosis of zymogen granules release their acid content into the lumen. In HCO$_3^-$ buffered solutions the recovery from this acidification is faster than with HEPES buffer and, importantly, is slowed by the ANO1/TMEM16A blocker, T16Ainh-A01. Our data show that at both normal and high levels of stimulation ANO1/TMEM16A provides a HCO$_3^-$ exit pathway in acinar cells that regulates luminal pH.

**Results**

We prepared acini from freshly isolated, collagenase digested, mouse pancreas which then remain viable for 3-4 hours, as reported (24). The tissue preparation is composed of multicellular fragments of acini that retain their structural integrity, cell organisation and polarity, and lumens. When stimulated, exocytic fusion of zymogen granules occurs exclusively in to the lumen. Our method for visualising exocytosis bathes the tissue fragments in sulforhodamine B (SRB) (24). This dye diffuses in to the lumens and enters each zymogen granule as it fuses with the cell membrane. Imaging with two-photon microscopy, granule fusion is observed as the sudden appearance of bright spots of fluorescence about 1 µm in diameter (24). In addition to SRB we also include hydroxyprobe-3,6,8-trisulfonate (HPTS) in the extracellular solution. This dye is pH sensitive (fluorescence decreases with acidification) with a $pK_a$ of 7.3, and, with the assumption of a starting pH of 7.4, is calibrated and mapped on to a pseudocolour scale, to indicate luminal pH changes after cell stimulation (5).
Fig. 1 shows the typical response of a cluster of acinar cells to stimulation with cholecystokinin (CCK) at a physiologically relevant concentration of 10 pM. In these images, two exocytic events are shown (Fig. 1A arrows) with the bright flash of SRB fluorescence occurring as the dye enters the granules (Fig. 1A, B time point, ii). HPTS also enters the granule but shows an apparently slower rate of rise which is consistent with the suppression of fluorescence that is expected within the acid environment of the granule lumen (Fig. 1B). The simultaneous record of luminal fluorescence changes (Fig. 1A circles) shows a small increase in the SRB fluorescence which reflects the dye accumulated on the exiting granule content (25). In contrast, the HPTS fluorescence decreases and, as we have observed before (5), this occurs before the rise in SRB fluorescence and suggests an initial release of protons into the lumen through a narrow fusion pore. The luminal acidification, associated with each granule fusion event, is transient and reaches a nadir within a few seconds, followed by a pH recovery (Fig. 1A, B). The calibrated HPTS ratio changes, mapped on to a pseudocolour scale, show the spatial extent of the luminal pH changes (Fig. 1A).

Experiments performed in the presence of 2 mM HEPES (Fig. 1A, D, E; n=36 exocytic events from 6 mice) show a relatively bigger luminal acidification and a slower rate of pH recovery when compared to experiments performed in the presence of 2 mM HCO$_3^-$ (Fig. 1B, D, E; n=33 exocytic events from 6 mice). These data demonstrate that the different buffers have a different effect on the dynamics of luminal pH.

Since recent work has implicated TMEM16A as a significant HCO$_3^-$ transporter (7,12), and confirmed it is the luminally-located calcium-dependent chloride channel in acinar cells (8,9,26), we set out to test if TMEM16A was playing a role in the different luminal pH responses we observed when using HCO$_3^-$ as a buffer.

The TMEM16A inhibitor, T16Ainh-A01 (26), provides an ideal tool to test for a role of this chloride channel in HCO$_3^-$ transport. Acutely applied to the preparation we observed that T16Ainh-A01 did not affect the CCK-stimulated exocytic events which showed very similar SRB fluorescence profiles to controls (Fig. 2). However, the luminal pH changes were affected by T16Ainh-A01 and show a significantly more acidic nadir (Fig. 2D, n=32 exocytic events in control, n=11 with T16Ainh-A01, p<0.0001) and a slowing of luminal pH recovery (n=32 exocytic events, 4 mice in control, n=11, 3 mice with T16Ainh-A01, P<0.01). This data provides direct evidence that TMEM16A is a significant HCO$_3^-$ pathway and contributes to the dynamic pH regulation of the lumen.

To further strengthen this finding we conducted control, whole-cell patch clamp experiments to directly measure the agonist-evoked calcium-dependent chloride currents in isolated acinar cells. In mouse acinar cells CCK stimulation principally activates calcium-dependent chloride channels, as shown by the significant reduction in currents in the TMEM16A knockout mouse (8). In mouse pancreatic acinar cells there is no calcium-dependent potassium conductance (27) and only a minor contribution from a non-selective cation conductance (28). Under conditions we have previously used to characterise the calcium-dependent chloride currents (14) we show that CCK activates a voltage-insensitive current that is significantly blocked in the presence of T16Ainh-A01 (Fig. 3A, B).

In addition, we were concerned that the inhibitor might be acting directly on the zymogen granules, prior to exocytosis, to affect the intra-granular pH and consequently change the proton load in to the lumen. To test this possibility, we incubated the cells in lysosensor Yellow/Blue DND-160. The dye enters acidic
compartments within the cell (Fig. 3C) which in acinar cells are dominated by the zymogen granules although it would include lysosomes (5). After calibration, the lyosensor dye showed that the intragranular pH was not different in control compared to cells treated with T16Ainh-A01 (Fig. 3C, D). Our results therefore demonstrate that T16Ainh-A01 is acting on the calcium-dependent chloride channel in pancreatic acinar cells and, although we cannot rule out that it might act elsewhere (29), this action alone is sufficient to explain the actions on luminal pH.

In another control experiment we tested whether T16Ainh-A01 might affect the agonist-evoked calcium response. This might be the case in other systems (29) and any change in intracellular calcium could lead to have downstream consequences that might alter luminal pH independently of an action on the chloride channel. Our experiments, using Fura-2 loaded cells, show that the acinar cells have a robust response to stimulation in the control (Fig. 4, DMSO) that is not affected by the application of 100 µM T16Ainh-A01.

Our results show that the likely major action of HC0$_3^-$ buffer on luminal pH is via TMEM16A leading us to conclude that TMEM16A is a transport pathway for apical HC0$_3^-$ exit that can significantly alter luminal pH, under physiologically relevant stimulation.

Luminal pH control is of particular importance in cystic fibrosis and acute pancreatitis where in diseased tissue the luminal pH can become significantly acidic (1). This is likely to be due either to the imbalance of luminal proton loading from secretion of acidic zymogen granule content (5) which is in excess in acute pancreatitis (6), or because of the lack of normal HC0$_3^-$ buffering because of the reduced activity of the CFTR in the ductal cells (1). Our findings that TMEM16A contributes to luminal pH buffering could therefore be of significance to understanding the mechanistic basis of disease. To test this idea we turned to a commonly used in vitro model of acute pancreatitis, supramaximal stimulation, which recapitulates the early stages of acinar cell damage (6). Fig. 5 shows the responses to 10 nM CCK, a supramaximal stimulation that triggers the fusion of a large number of zymogen granules, as observed by the influx of SRB in to granules. These granules rapidly coalesce to form the intracellular vacuoles that characterise the initial stages of acute pancreatitis (Fig. 5 A, B ii). Bathing the acini in 2 mM HEPES led to a large and relatively sustained drop in luminal pH. In contrast, bathing the cells in 2 mM HC0$_3^-$ led to a significantly smaller acidic nadir (n=11 from 4 mice in HEPES, n=9 from 6 mice in HC0$_3^-$, p<0.01), and over the time period of recordings less sustained acidification (Fig. 5C, D).

As before, to test for a role of TMEM16A in acute pancreatitis, we pretreated the acini with T16Ainh-A01 and then stimulated with supramaximal CCK (Fig. 6). The induced fusion of zymogen granules was similar under the two conditions (Fig. 6E). However, the luminal acidification was significantly increased in the presence of the inhibitor (Fig. 6C, D; n=8 from 5 mice in DMSO; n=8 from 3 mice with T16Ainh-A01; p<0.05).

We then tested for consistency of the action of T16Ainh-A01 with the use of another inhibitor of the calcium-dependent chloride channel, niflumic acid. Cells were bathed in 2 Mm HC0$_3^-$ buffer and stimulated with supramaximal CCK (10 nM) to evoke a response and tested for the action of 100 µM niflumic acid on the luminal acid changes (Fig. 7). As with T16Ainh-A01 we observed a significant increase in luminal pH that was not accompanied by any change in the exocytic response (Fig 7E). Our data reveal that TMEM16A plays a role in the control of luminal pH that is important in these pathological responses.

Discussion
The major finding of our study is that under physiologically-relevant stimulation, HCO₃⁻ exit through the apical ANO1/TMEM16A channel of pancreatic acinar cells is a significant regulator of intraluminal pH. This observation builds on the biophysical characterisation of ANO1/TMEM16A and provides evidence that acinar cells play an active role in regulating the pH of the pancreatic lumen. Furthermore, we show this channel also plays a role in normalising the extreme luminal acidification observed in a model of acute pancreatitis.

There is now extensive evidence that supports the idea that ANO1/TMEM16A is the apical agonist-induced calcium-dependent chloride channel in acinar cells. Immunofluorescence of ANO1/TMEM16A show it is present in the apical membrane (7,9), it has the same ion selectivity sequence (7,18) and current rectification (11), and calcium dependence (17,30) when compared to the native calcium-dependent chloride channel (14). Furthermore, chloride flux is significantly reduced in the ANO1/TMEM16A knock-out mouse (8) and finally, T16Ainh-A01 selectively blocks the salivary gland chloride conductance (26). We extend these latter studies, to show a block of the agonist-activated Cl⁻ current in the pancreatic acinar cells (Fig. 3).

Protein expression of ANO1/TMEM16A is now enabling a much better characterisation of the channel properties in terms of the control of ion permeability and the potential role for calcium regulation. In this context, the cell physiology we describe in this paper is consistent with past biophysical characterisation of the calcium control of permeability. Our experiments stimulate the cells with 10 pM CCK which triggers global calcium oscillations, that as we have previously shown, reach ~ 2 μM in the subplasmalemmal domain, and activate both zymogen granule exocytosis and the opening of the calcium-dependent chloride channel (22). This relatively high concentration of cytosolic calcium is known to elicit changes in the ANO1/TMEM16A ion selectivity that can lead to much greater permeability to HCO₃⁻ even up to a P_{HCO₃⁻}/P_{Cl⁻} of 1.1 (12).

Our data show HCO₃⁻ efflux through the ANO1/TMEM16A channel must be moving down its electrochemical gradient and is likely to be due to intracellular accumulation of HCO₃⁻ through basolateral transporters. For the pancreatic acinar cells there is evidence for basolateral Na⁺-HCO₃⁻ cotransporter (31) and a Cl⁻/HCO₃⁻ exchanger (32), possibly the Slc4a9 anion exchanger, as found in salivary glands (33). It should be noted that in the tissue fragments we have to conduct our experiments using 2 mM buffer concentrations; any higher than this and luminal pH changes cannot be recorded with HPTS (5). Thus, at the higher HCO₃⁻ concentrations that normally would be bathing the acinar cells, we would expect a greater driving force for HCO₃⁻ entry across the basal pole.

We conclude that our work provides evidence to support the physiological relevance of HCO₃⁻ exit through TMEM16A in mouse pancreatic acinar cells.

**Experimental Procedures**

**Cell preparation**

Mice were humanely killed according to local, University of Queensland, animal ethics procedures (approved by the University of Queensland, Anatomical Biosciences Ethics Committee). Isolated mouse pancreatic tissue was prepared by a collagenase digestion method in normal NaCl rich extracellular solution (Solution A), modified to reduce the time in collagenase and limit mechanical trituration. The resulting preparation was composed mainly of pancreatic lobules and fragments (50-100 cells), which were plated onto poly-l-lysine-coated glass coverslips.
Solutions

Two types of extracellular solutions were used to bathe acini. Solution A, in mM: 135 NaCl, 5 KCl, 10 glucose, 8 sucrose, 2 MgCl$_2$, 2 CaCl$_2$ and 2 HEPES, pH 7.4. Solution B, in mM: 135 NaCl, 5 KCl, 10 glucose, 8 sucrose, 2 MgCl$_2$, 2 CaCl$_2$ and 2 NaHCO$_3$, pH 7.4. The HCO$_3^-$ buffered solution was bubbled with CO$_2$ prior to and during the experiments.

Live-cell Two-photon Imaging

We used a custom-made, video-rate, 2-photon microscope with a 60x oil immersion objective (NA 1.42, Olympus), providing an axial resolution (full width, half maximum) of ~1 µm. We imaged exocytic events using Sulforhodamine B (SRB, 400 µM) as a membrane-impermeant fluorescent extracellular marker excited by femtosecond laser pulses at 950 nm, with fluorescence emission detected at 550-650 nm. To image pH changes we used extracellular HPTS (400 µM, ThermoFisher, Scoresby VIC, Australia) excited at 950 nm and fluorescence detected at 420-520 nm. As a negative control we also employed 8-methoxypyrene-1,3,6-trisulfonate, MPTS (400 µM, ThermoFisher, Scoresby VIC, Australia) which is a pH-insensitive analogue of HPTS. Images (resolution of 10 pixels/µm, average of 15 video frames) were analysed with the Metamorph program (Molecular Devices Corporation, Sunnyvale, CA, USA). Exocytotic event kinetics were measured from regions of interest (0.78 µm$^2$, 78 pixels) centered over individual granules. Traces were rejected if extensive movement was observed. All data are shown as mean ± SEM.

Estimation of granule pH

An in situ calibration solution was, in mM: 130 KCl, 1 MgCl$_2$, 15 MES, 15 HEPES, 10 µg/mL nigericin, 10 µM monensin, pH 4.2-7). Calibration was carried out on Lysosensor Yellow/blue DND 160 (ThermoFisher, Scoresby VIC, Australia) loaded cells after incubating in high potassium/nigericin solutions at different pH. After equilibration in these solutions for 10 minutes, Lysosensor Yellow/blue DND 160 was excited at 750 nm, and fluorescence below 490 nm or above 520 nm was recorded. Calibration curves were constructed from these treatments for comparison with experimental measurements (Fig. 3).

Electrophysiology

Cells were whole-cell patched using pipettes with a resistance of 2–4 MΩ when filled with KCl-rich pipette solution. Seal resistance was 10–50 GΩ and series resistance was <10 MΩ. The pipette solution was (in mM): 140 KCl, 10 NaCl, 1.13 MgCl$_2$, 10 HEPES-KOH, 2 Na$_2$ATP, EDTA 0.1, pH 7.2. The bath solution was (in mM): 135 NaCl, 5 KCl, 10 glucose, 2 MgCl$_2$, 2 CaCl$_2$ and 10 HEPES, pH 7.4.

A voltage-pulse protocol stepped the cells from -100 mV to +40 mV (400 ms pulse width) in 10 mV increments. Current/voltage (I/V) relationships were constructed from the average steady-state current measured 350 ms after the onset of the pulses. All currents were normalized against cell capacitance and expressed as mean ± SEM (pA/pF).

Statistics

Unpaired, two-tailed Student’s t tests were used for all experiments.

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Conflict of Interest

The authors declare no conflicts of interest.
Author Contributions

YH, AS and PT designed the experiments, YH performed experiments, PT, YH and AS wrote paper.

References

Figure Legends

FIGURE 1. Luminal acidification after zymogen granule exocytosis is decreased in a HCO$_3^-$ buffer. A, two-photon live-cell imaging of pancreatic fragments, bathed in HEPES, SRB and HTPS, shows the dyes outline the cells and enter the lumen between the cells. Zymogen granule exocytosis, triggered by stimulation with CCK (10-20 pM), is observed as the sudden appearance of fluorescent spots (two events are shown, indicated by arrows) as the dyes enter the granule shown here at the time points (i, ii, iii – also see panel B and Supplemental Movie 1). The pseudocoloured pH changes were obtained from calibrated ratios of the HPTS fluorescence and show spatial pH changes in the lumen synchronous with the granule fusion events (see also Supplemental Movie 2. B, plots of fluorescence over time (baseline subtracted, n>33 events, n=3+ animals) within regions of interest (indicated by arrows in A) over the region of the fusing granules, show both SRB and HPTS enters the granules. In the lumen (A, white circles) SRB shows a small fluorescence increase but the HPTS shows a decrease, consistent with luminal acidification and quenching of HPTS fluorescence. C, pancreatic fragments bathed in HCO$_3^-$ buffer show similar fluorescent changes in the granule and the lumen to those in HEPES buffer (see also Supplemental Movies 3 and 4). D, E, comparison of the responses in HEPES and HCO$_3^-$ show the changes of fluorescence within the granule are similar but, in the lumen, the pH nadir and recovery, were significantly (Student’s t test p<0.05 * or p<0.01 **) less acidic in HCO$_3^-$.

FIGURE 2. TMEM16A is a significant transporter of HCO$_3^-$ in to the acinar lumen. A, B, two-photon live-cell imaging of pancreatic fragments, bathed in HCO$_3^-$ stimulated with CCK (10-20pM) in the absence (DMSO control, A, see also Supplemental Movies 5 and 6) or presence of 10 µM T16Ainh-A01, (B, see also Supplemental Movies 7 and 8). The SRB fluorescence shows an example granule fusion event (arrow) and also shown are pseudocoloured pH changes as assessed with the HPTS fluorescence changes. C, D, show that the inhibitor leads to a significant (Student’s t test p<0.001 *** or p<0.01 **) increase in the stimulus-induced luminal acidification (n>10 events, n>3 mice). Scale bars, 5 µm.

FIGURE 3. T16Ainh-A01 blocks the agonist-evoked calcium-dependent chloride current in pancreatic acinar cells but has no effect on zymogen granule acidification. A, whole-cell patch clamp with voltage clamp steps shows activation of a large current after stimulation with 10 pM CCK, that is blocked when cells were stimulated in the presence of 10 µM T16AinhAO1 as shown in the current-voltage relationships (B). C, Cells, incubated in lysosensor were two-photon imaged with excitation at 750 nm and emission collected at wavelength >520 nm and <490 nm. Images were ratioed (C, D) and calibrated across a range of pH values (D). The cellular pH was not different (Student’s t test) after treatment with 10 µM T16Ainh-A01. Scale bar, 5 µm.

FIGURE 4. T16Ainh-A01 does not affect the agonist-evoked calcium response. A, B application of 200 nM acetylcholine induced a rapid rise in intracellular calcium in control (DMSO treated, n=10 acini, n=3 mice) and T16Ainh-A01 (10 µM, n=6 acini, n=3 mice) treated pancreatic acini as measured with calibrated, Fura-2 loaded cells. A shows example images taken at the time points (i, ii, iii, iv indicated in B). B, are the time traces from the examples shown in A. C, D show that the average calcium responses were not different (Student’s t test) in the peak (C) or temporal changes (D) between control and T16Ainh-A01 treatment.
FIGURE 5. Supramaximal CCK stimulation (10 nM) induces a large exocytic response; the consequent acid load in the lumen with HEPES buffer is significantly smaller with HCO₃⁻ buffer. A, two photon live-cell imaging in HEPES buffer, before (C, i) and after (C, ii) supramaximal CCK stimulation shows a large exocytic response (SRB) and significant luminal acidification (A, C, D see also Supplemental Movies 9 and 10). In the presence of HCO₃⁻ buffer (B) the exocytic response is similar but the luminal acidification is much less (B, C, D, see also Supplemental Movies 11 and 12). Scale bar, 5 µm. (n>=9 lumens, n>=3 mice)

FIGURE 6. TMEM16A transports HCO₃⁻ into the lumen during supramaximal agonist stimulation. Addition of 10 µM T16Ainh-AO1 to HCO₃⁻ buffered solution significantly increases the luminal acidification (B, C, D, see also Supplemental Movies 15 and 16) when compared to the HCO₃⁻ buffered control responses (A, C, D; DMSO see also Supplemental Movies 13 and 14). Counts of the numbers of exocytic events per luminal length show that this is not altered in the presence of T16Ainh-AO1 (E). Scale bar, 5 µm.

FIGURE 7. Niflumic acid also enhances luminal acidification in response to supramaximal agonist stimulation. Addition of 100 µM niflumic acid (NFA) to HCO₃⁻ buffered solution significantly increases the luminal acidification (B, C, D see also Supplemental Movies 19 and 20) when compared to the HCO₃⁻ buffered control responses (A, C, D; DMSO, see also Supplemental Movies 17 and 18). Counts of the numbers of exocytic events per luminal length show that this was not altered in the presence of niflumic acid (E). Scale bar, 5 µm.
Fig. 1
A

B

C

D

E

Fig. 3

Control
100 pA
100 mS

CCK

T16Ainh-A01 + CCK

pA/pF

pA/pF

50 mV

50 mV

> 520 nm

< 490 nm

Ratio

<490/100 mS

<490/100 pA

<490/100 mS

<490/100 pA

pH

DMSO

T16Ainh-A01

ns
Fig. 5
Fig. 6

A

SRB

pH

B

SRB

C

Luminal pH

D

Luminal pH

E

No. of events/μm Lumen

DMSO

T16Ainh-A01

DMSO

T16Ainh-A01

ns
Fig. 7

A

SRB

pH

ii i

DMSO NFA

B

SRB

pH

ii i

DMSO NFA

C

Luminal pH

Time (s)

0 20 40 60 80 100

D

luminal pH

DMSO NFA

E

No. of events/μm lumen

DMSO NFA

* ns
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