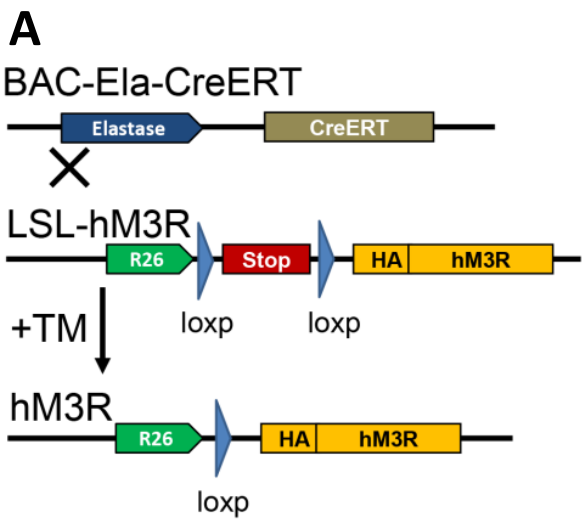
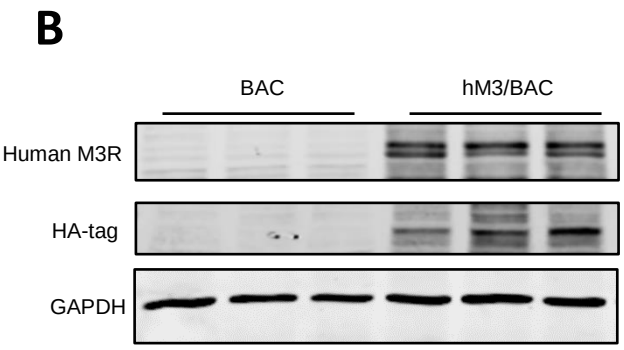
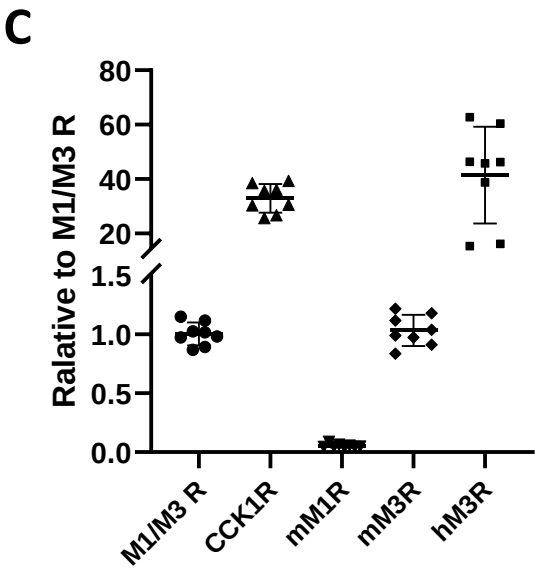
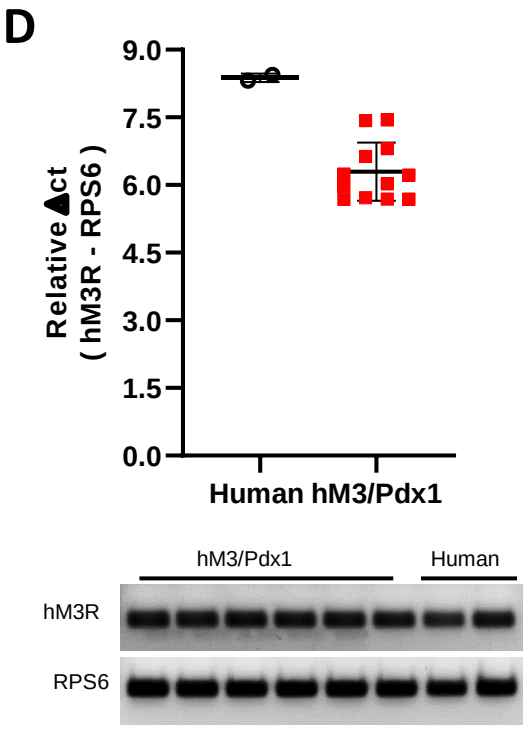
Suppl Fig 1


A
BAC-Ela-CreERT
Elastase
CreERT
LSL-hM3R
R26
Stop
HA
hM3R
loxp
loxp
+TM
hM3R
R26
HA
hM3R
loxp


B
BAC
hM3/BAC
Human M3R
HA-tag
GAPDH


C
Ralative to M1/M3 R
80
60
40
20
1.5
1.0
0.5
0.0
M1/M3 R
CCK1R
mM1R
mM3R
hM3R

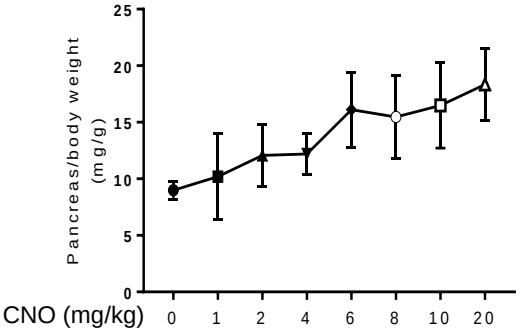

D
Relative Δct
(hM3R - RPS6)
9.0
7.5
6.0
4.5
3.0
1.5
0.0
Human hM3/Pdx1
hM3/Pdx1
Human
hM3R
RPS6

Supplementary figure 1. A mutant hM3R was conditionally expressed in the pancreata of transgenic mice.

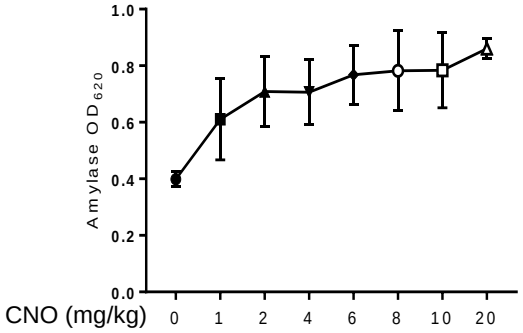
A. Mice were crossed to generate LSL-hM3R/BAC-Ela-CreERT (hM3/BAC) double transgenic mice for conditional pancreatic acinar specific expression of mutant human M3R. Upon tamoxifen (TM) induction to activate CreERT, Cre recombinase activity will remove the transcription stop between hM3R cDNA and the R26 promoter to allow the expression of the HA-tagged hM3R. **B.** Western blot using antibodies against hM3R or HA tag detected hM3R in double transgenic mice. **C.** Realtime RT-PCR was used to quantify the expression levels of mM1R, mM3R, CCK1R, and transgenic hM3R. The expression levels were normalized to total mM1/M3R expression. **D.** Expression levels of M3R mRNA in transgenic mice and human pancreas were compared by realtime RT-PCR.

Suppl Fig 2

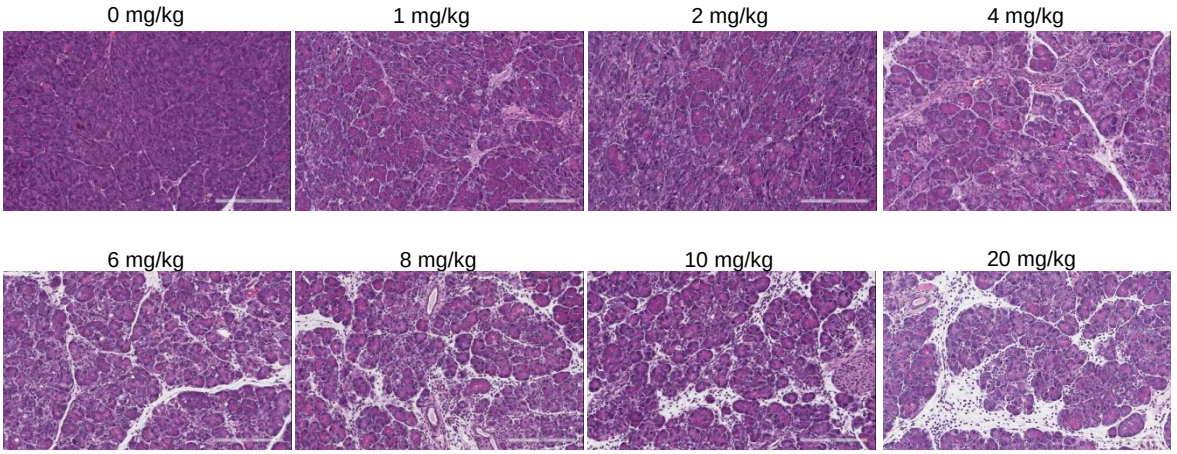
A



B



C

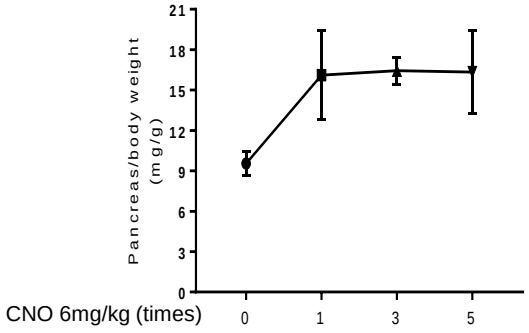


Supplementary figure 2. Dose dependent effects of CNO on the severity of acute pancreatitis in hM3/BAC mice.

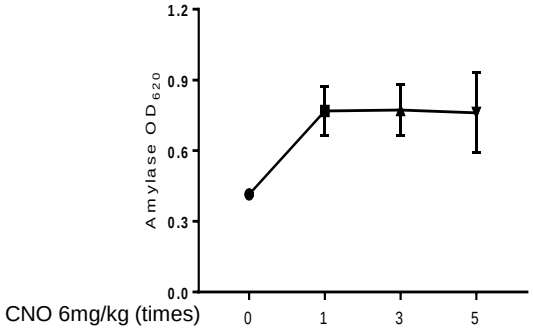
The mice were injected (ip) with the indicated dose of CNO and pancreatitis was evaluated at 24 hours by pancreas edema (A), serum amylase (B), and histology (C). (scale bar: 200 μm, n=3/group).

Suppl Fig 3

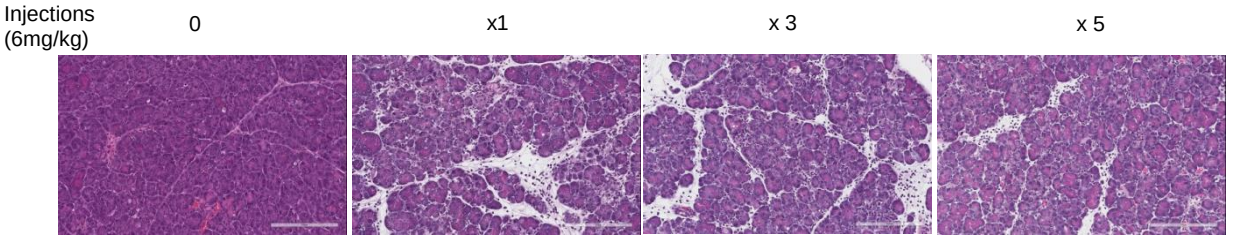
A



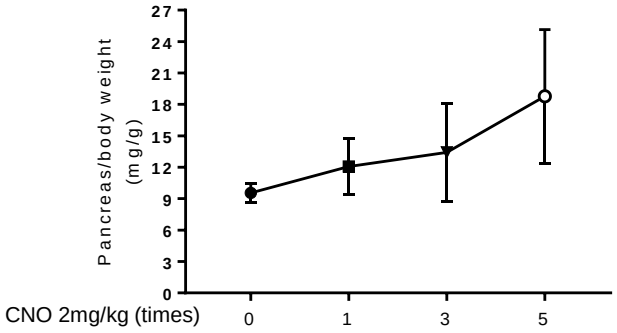
B



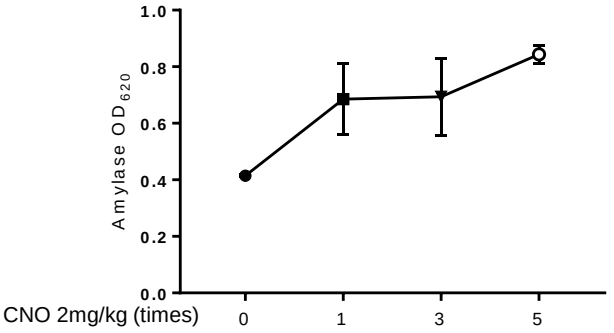
C



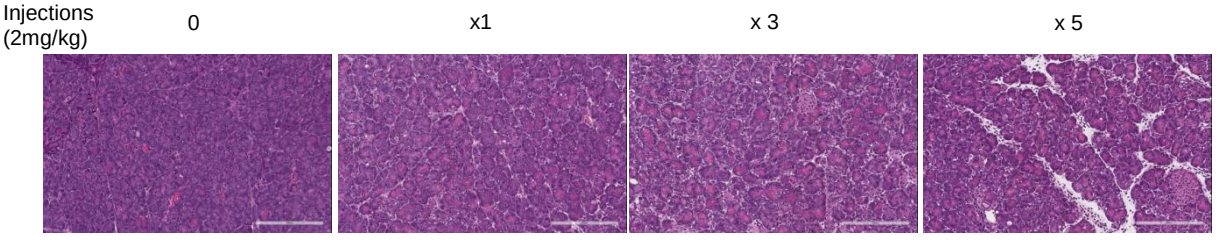
D



E



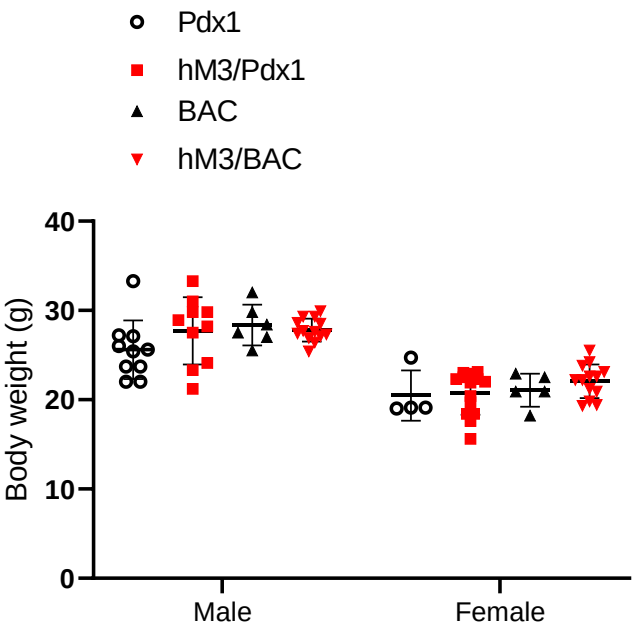
F



Supplementary figure 3. The effects of the number of repeated injections of CNO on the severity of acute pancreatitis in hM3/BAC mice.

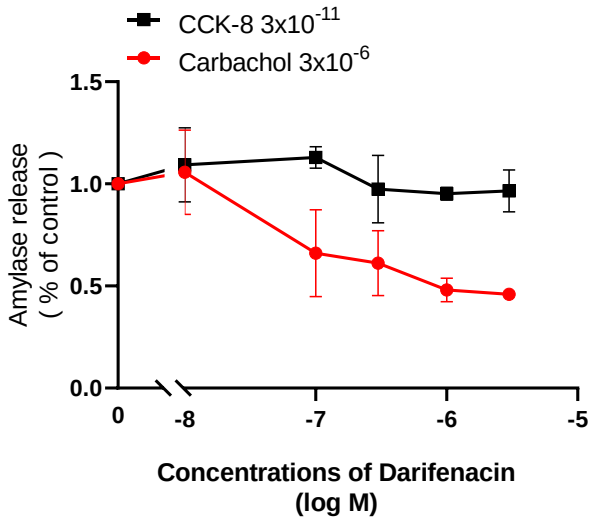
The hM3/BAC mice were injected with CNO (6mg/kg/h, ip) at the indicted repeats. Pancreatitis was evaluated at 24 hours by pancreas edema (A), serum amylase (B), and histology (C). (scale bar: 200 μm, n=3/group). Similar experiments were performed with CNO at 2mg/kg/h (D-F).

Suppl Fig 4.


Pdx1
hM3/Pdx1
BAC
hM3/BAC
Body weight (g)
40
30
20
10
0
Male
Female

Supplementary figure 4. There were no differences in overall body weight among these groups at 4 weeks after CNO treatment.

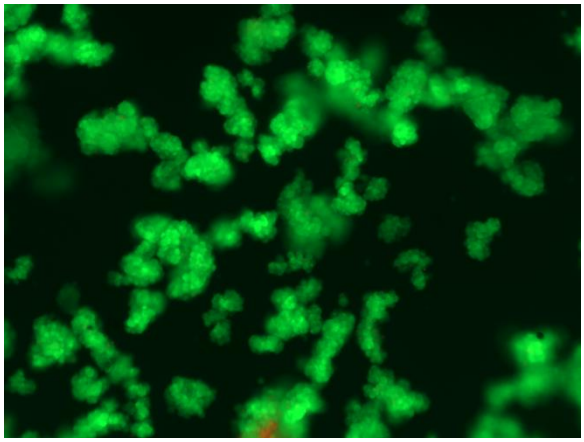
Suppl Fig 5.


CCK-8 3x10-11
Carbachol 3x10-6
Amylase release
(% of control)
1.5
1.0
0.5
0.0
0
-8
-7
-6
-5
Concentrations of Darifenacin
(log M)

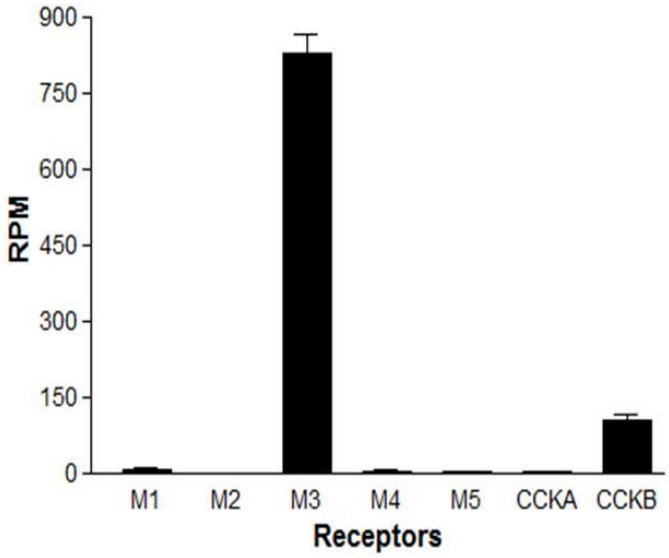
Supplementary figure 5. Darifenacin had no direct effect on CCK1R on pancreatic acinar cells as measured by amylase secretion.

Suppl Fig 6.

A



B



Supplementary figure 6. Human pancreatic acinar cells express the M3 acetylcholine receptor.

A. Pancreatic acinar cells were prepared from organ donors free of exocrine pancreatic diseases at Prodo Laboratories with a proprietary protocol. Cell viability was examined by staining with Fluorescein Diacetate (Green) and Ethidium Bromide (Red). **B.** Expression profile of muscarinic and CCK receptors in normal human pancreatic acinar cells by RNAseq. Pancreatic acinar RNA was prepared from 3 different human organ donors free of exocrine pancreatic diseases. Unbiased RNAseq showed high expression of M3 receptors but no CCK1R expression (RPM = reads per million mapped reads).

Supplementary Methods

Reagents: Clozapine N-oxide (CNO) (#QV-1707) and darifenacin hydrobromide (#QA-1979-001) were purchased from Combi-blocks Inc. Cerulein (#170412-15A) was synthesized by CPC Scientific. Soybean trypsin inhibitor (T6522), tamoxifen (#H7904) and carbachol (#C4382) were obtained from Sigma Aldrich. Cholecystokinin octapeptide CCK-8 (#H2080.0001) was purchased from Bachem Americas. Collagenase (#LS005273) was from Worthington Biochemicals. The following primary antibodies were used for Western blotting or immunohistochemistry (IHC) studies: HA-tag (#3724), LC3I/II (#12741P), p62 (#5114S), p-p65 (#3033s), IκBα (#2859S), and cleaved-caspase-3 (#9661S) were from Cell Signaling Technology. HMGB1 (#AB-18256), p65 (#AB-16502), CD11b (#AB-133357), α-SMA (#AB-5694), Ki-67 (#AB-15580) were from Abcam. Human M3 receptor (#324719) was from US Biological. GAPDH (#G8795) was from Sigma-Aldrich. F4-80 (#MCA497GA) was from Bio-Rad. The second antibodies IRDye 800CW Goat anti-Rabbit (#926-32211) and IRDye 680RD Goat anti-Mouse (#926-68070) were from LI-COR Biosciences.

Genotyping: Mouse genomic DNA was isolated from 1mm tail biopsies with 10min digestion at 100°C in 250ul 0.05M NaOH followed by cooling and neutralization with 25ul 1M Tris-HCl containing 10mM EDTA (pH 8.0). PCR was performed using the primer pairs hM3R F, 5'-TCGCCTAGGGATGCATGA-3' and hM3R R, 5'-GAA ACAAAGGCGAGGTTGTACT-3'; hM4R F, 5'-TCGCCTAGGGATGCATGA-3' and hM4R R, 5'-GACTGATTGCCCGAGCTG-3'; CreER F, 5'-ATACCGGAGATCATGCAAGC-3' and CreER R, 5'-ATAGATCATGGGCGGTTCAG-3'; Pdx1 F, 5'-GAACTGGGGAGGAAAAGGAG-3' and Pdx1 R, 5'-AGGCAAATTTTGGTGTACGG-3').

Western blot analysis: Total protein from pancreatic tissues was extracted by RIPA buffer (#RB4477, Bio Basic Canada Inc) containing 50 mM Tris-HCL (pH 7.4), 150 mM NaCl, 1 % NP-40, 0.5% Deoxycholate, 0.1 % SDS, 1mM EGTA, 5mM EDTA and phosphatase inhibitors (Roche Applied Science, Basel, Switzerland). The concentration of the total protein was detected by BCA protein assay kit (Thermo Scientific) according to the

manufacturer's recommendations. SDS-PAGE was used to separate proteins of different molecular weights. Nonspecific binding was blocked with 5% Bovine serum albumin (BSA). Immuno-reactive banding was visualized using Odyssey CLx (LI-COR Biosciences).

Total RNA extraction and Quantitative Reverse Transcription Polymerase Chain Reaction (RT-PCR) analysis: Total RNA from pancreatic tissues was extracted using the TRIzol reagent (Life Technologies) and RNeasy Plus Mini Kit (#74136; Qiagen). Reverse Transcription System (#A3500, Promega) was used to transcribe RNA into cDNA using 2 μg of total RNA. Quantitative PCR was performed with SYBR Green qPCR master mix (Fisher Scientific) on the Quant Studio 7 Flex Real-Time PCR System (Thermo Fisher Scientific). GAPDH was used as RNA loading control. The primers used for qPCR are listed in Supplementary Table 1.

Supplementary Table 1. Primers for qPCR

Gene	Forward primer	Reverse primer
mM1 receptor	5'-TGGGAGCCAGGTGTTTGGGT-3'	5'-GGCTGAAGTGTTCATGGTGGCT-3'
mM2 receptor	5'-GAGGTACCCGAATAGGTGTTGCC-3'	5'-AGCTGCGCACCGCTTAGAGT-3'
mM3 receptor	5'-GAGGAGACGAAGGGAAGGTGGA-3'	5'-AGGCAGCAATGGACTGGGTAG-3'
mM4 receptor	5'-GCCTGGTCACGTCATCATCCCA-3'	5'-AGCACACGCCAGGCTGAAGA-3'
mM5 receptor	5'-TTACCTGCTCAGCTTAGCCTGTG-3'	5'-AGTAACGGTCAAAACTGATCACCAG-3'
mCCK1R	5'-ACAATAACCAGACGGCGAAC-3'	5'-CAGGGATAAGGAAGAGGATGAG-3'
mIL-1β	5'-AAATACCTGTGGCCTTGGGC-3'	5'-TTTGGGATCTACACTCTCCAGCT-3'
mIL-6	5'-GTAGCCGCCCCACACAGA-3'	5'-CATGTCTCCTTTCTCAGGGCTG-3'
mTNF-α	5'-CCCAGGGACCTCTCTCTAATCA-3'	5'-GCTTGAGGGTTTGCTACAACATG-3'
mRPS6	5'-CGCCAGTATGTTGTCAGGAA-3'	5'-GTTGCAGGACACGAGGAGT-3'
hM3R	5'-AGGAGGAGCTGGGGATGG-3'	5'-TGGAAAACTGCCTCCATCG-3'
hRPS6	5'-AAGGAGAGAAGGATATTCCTG-3'	5'-AGAGAGATTGAAAAGTTTGCG-3'

Isolation of Mouse Pancreatic Acini and amylase release

Mouse pancreatic acinar cells were isolated as previously described(1). Briefly, the pancreas was dissected and digested by 100U/ml collagenase (Worthington) in DMEM in the presence of soybean trypsin inhibitor (Sigma) for 50 min at 37°C. The digested pancreatic cells were filtered through a mesh. The acini were allowed to settle by gravity for 5-10 min to remove damaged cells in DMEM containing 4% BSA. Isolated acini were aliquoted and stimulated with different concentrations of CCK-8, carbachol and CNO for 30 min at 37°C. Supernatant and the pancreatic acini were collected for amylase and ethidium bromide staining.

Intracellular Ca2+ Measurements

Pancreatic acinar cells were simultaneously loaded with 5 μM of the Ca2+ sensitive dye fura-2 (AM) (Life Technologies) and allowed to adhere to a Cell-Tak (Corning) coated glass coverslip at room temperature for 30 min. Cells were perfused in a rapid exchange perfusion chamber with HEPES-buffered saline solution (HBSS) containing 137 mM NaCl, 0.56 mM MgCl2, 4.7 mM KCl, 1 mM Na2HPO4, 10 mM HEPES, 5.5 mM glucose, and 1.26 mM CaCl2 (pH 7.4). Clozapine N-oxide (CNO), obtained from Cayman Chemical, was dissolved in DMSO before diluting in HEPES buffer. Cholecystokinin Octapeptide (CCK8) was obtained from Tocris and diluted to 100 μM in PBS. Carbachol (CCh) was obtained from Sigma and diluted prior to use. Imaging was performed using an inverted Olympus IX71 microscope through a 40X oil immersion objective lens (numerical aperture, 1.35). Fura-2 loaded cells were excited alternately with light at 340 and 380 nm using a monochromator-based illumination system (TILL Photonics), and the emission at 510 nm was captured using a digital CCD camera. Images were captured every second. Fluorescence was measured as the ratio of (F340 / F380). (F/ F0) was calculated by dividing (F340 / F380) by the average of the first 20 control values of (F340 / F380). Origin 6 software was used to analyze the frequency and peak height of Ca2+ oscillations and plot all statistical graphs.

Trypsinogen Activation Assay

Rhodamine 110 (BZiPAR) was obtained from Biotium, Inc. and resuspended in DMSO prior to use. Pancreatic acinar cells were simultaneously incubated in HBSS with 100 μM BZiPAR and either 10 μM CNO, 10 nM CCK8 or plain HBSS for 40 minutes in a glass bottom 35 mm culture dish at 37C. BZiPAR fluorescence was measured using an inverted Olympus microscope through a 40X oil immersion objective lens (numerical aperture, 1.35) and a monochromator based illumination system (TILL Photonics). Cells were excited at 488 nm and emission was measured at 520 nm. Regions of interest (ROI) were placed around each individual acinar cell within the TILLvision software and the average levels of gray were calculated. Origin 6 software was used to plot the statistical graphs of the grouped data.

1. Gaiser S, Daniluk J, Liu Y, Tsou L, Chu J, Lee W, et al. Intracellular activation of trypsinogen in transgenic mice induces acute but not chronic pancreatitis. *Gut.* 2011;60(10):1379-88.