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Graphical abstract

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Repurposing calcium-sensing receptor agonist cinacalcet for treatment of CFTR-mediated secretory diarrheas

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Introduction

Diarrhea is a major cause of global mortality, and outbreaks of secretory diarrhea such as cholera remain an important problem in the developing world. Current treatment of secretory diarrhea primarily involves supportive measures, such as fluid replacement. The calcium-sensing receptor (CaSR) regulates multiple biological activities in response to changes in extracellular Ca²⁺. The FDA-approved drug cinacalcet is an allosteric activator of CaSR used for treatment of hyperparathyroidism. Here, we found by short-circuit current measurements in human colonic T84 cells that CaSR activation by cinacalcet reduced forskolin-induced Cl⁻ secretion by greater than 80%. Cinacalcet also reduced Cl⁻ secretion induced by choleragen, heat-stable E. coli enterotoxin, and vasoactive intestinal peptide (VIP). The cinacalcet effect primarily involved indirect inhibition of cystic fibrosis transmembrane conductance regulator–mediated (CFTR-mediated) Cl⁻ secretion following activation of CaSR and downstream phospholipase C and phosphodiesterases. In mice, cinacalcet reduced fluid accumulation by more than 60% in intestinal closed loop models of cholera and traveler’s diarrhea. The cinacalcet effect involved both inhibition of CFTR-mediated secretion and stimulation of sodium-hydrogen exchanger 3–mediated absorption. These findings support the therapeutic utility of the safe and commonly used drug cinacalcet in CFTR-dependent secretory diarrheas, including cholera, traveler’s diarrhea, and VIPoma.
The CaSR can be activated by organic cations (Ca^{2+}, Mg^{2+}, Gd^{3+}, etc.), certain heavy metals, amino acids, polyamines, and small molecules, the latter including calcimimetics (CaSR activators) (9). Targeting CaSR in the parathyroid gland by the FDA-approved drug cinacalcet or other calcimimetics suppresses parathyroid hormone (PTH) secretion, which is commonly used to treat secondary hyperparathyroidism associated with chronic kidney disease or renal failure. Cinacalcet is also used to treat hyperparathyroidism and hypercalcemia associated with parathyroid gland tumors (10).

Here, we provide evidence that activation of CaSR by cinacalcet in a human colonic cell line and in mouse intestine inhibits intestinal fluid secretion and enhances fluid absorption, offering a simple, novel, and rapidly translatable therapeutic strategy for secretory diarrheas, including cholera.

Results

CaSR activation by cinacalcet inhibits CFTR-mediated Cl– secretion in T84 cells. Cl– secretion was measured by short-circuit current (I_{sc}) in T84 cells, a commonly used human intestinal epithelial cell line that expresses CaSR (11). Though cinacalcet did not alter baseline I_{sc}, it largely inhibited the increase in I_{sc} following cAMP elevation by forskolin, which activates CFTR to produce a Cl– secretory current (Figure 1, A and B). The forskolin-induced increase in I_{sc} was inhibited by approximately 50% and 85% by pretreatment of the T84 cells with 10 μM and 30 μM cinacalcet, respectively (Figure 1C), which is consistent with its in vitro EC_{50} of 2.8 μM (12).

The forskolin-induced increase in I_{sc} was partially reversed by the CFTR-selective inhibitor CFTRinh-172 (Figure 1, A and D). Cinacalcet pretreatment strongly inhibited the CFTRinh-172–sensitive I_{sc} in a concentration-dependent manner. These findings support CFTR inhibition as a major mechanism of cinacalcet action. Similarly, pretreatment of T84 cells with the experimental CaSR agonist R-568 reduced the forskolin-induced maximal I_{sc} increase (Supplemental Figure 1, A and B; supplemental material available online with this article; https://doi.org/10.1172/jci.insight.146823DS1). R-568 appeared to be more potent than cinacalcet in T84 cells with approximately 70% reduction in I_{sc} at 10 μM, which is consistent with its greater reported in vitro potency for CaSR activation compared with cinacalcet (12). As found with cinacalcet, R-568 pretreatment inhibited the CFTRinh-172–sensitive I_{sc} (Supplemental Figure 1, A and C). Although R-568 inhibited CFTR-mediated Cl– secretion in T84 cells with modestly greater in vitro potency, subsequent studies were done with cinacalcet because it is an FDA-approved drug with favorable safety profile.

Cinacalcet-induced CFTR inhibition is indirect and dependent on CaSR. To test whether the cinacalcet effect in T84 cells may be due to direct CFTR inhibition, I_{sc} measurements were done in CFTR-transfected Fischer rat thyroid (FRT) cells, which do not express CaSR (13, 14). These cells show a robust cAMP-dependent Cl– current and have been widely used to identify and characterize CFTR modulators (15). In permeabilized FRT cells with a basolateral-to-apical Cl– gradient, cinacalcet pretreatment did not affect CFTR-mediated Cl– conductance as evidenced by the unchanged I_{sc} responses to maximal forskolin and CFTRinh-172 (Figure 2, A and B).

The cinacalcet effect was also studied in well-differentiated human bronchial epithelial (HBE) cells, which like T84 cells express CaSR (16), as well as the ion channels CFTR, ENaC, and CaCC (17). Cinacalcet pretreatment had no significant effect on ENaC or CaCC activities, as evidenced by comparable I_{sc} responses to amiloride and ATP, respectively (Figure 2, C and D). However, as found in T84 cells, CFTR-mediated Cl– secretion was largely inhibited by cinacalcet, as seen by the reduced responses to forskolin and CFTRinh-172.

Type II calcimimetics such as cinacalcet are allosteric modulators of CaSR that increase its sensitivity to extracellular Ca^{2+} and so do not activate CaSR in the absence of extracellular Ca^{2+} (9, 12). To further support the conclusion that the cinacalcet effect in T84 cells is dependent on CaSR activation, forskolin-induced Cl– secretion was measured using Ca^{2+}- and Mg^{2+}-free bathing solution. In this setting, 30 μM cinacalcet had only minimal effect on the forskolin-induced secretory response and its reversal by CFTRinh-172 (Figure 3, A and B), compared with the large responses in cells in the presence of 1 mM Ca^{2+} and Mg^{2+}. Since Ca^{2+} and Mg^{2+} are stored intracellularly, we hypothesized that leakage of intracellular Ca^{2+} or Mg^{2+} into the bathing solution might be responsible for the small residual effect of cinacalcet on the forskolin response in the Ca^{2+}- and Mg^{2+}-free solution. In T84 cells, cinacalcet’s effect on forskolin and CFTRinh-172 responses was completely abolished in Ca^{2+}- and Mg^{2+}-free solution with 1 mM EDTA, a chelator of Ca^{2+} and Mg^{2+}. These results in FRT, HBE, and T84 cells indicate that CFTR inhibition by cinacalcet is indirect and dependent on CaSR activation.

Cinacalcet exerts its antisecretory effect from the apical side in T84 cells. CaSR expression has been reported in both apical and basolateral membranes of intestinal epithelial cells (18). In T84 cells, addition of cinacalcet to the apical chamber inhibited the forskolin-induced increase in I_{sc} to a comparable extent to its addition to both the apical and basolateral chambers (Figure 4, A and B). No inhibition of the forskolin-induced I_{sc} was
seen when cinacalcet was added to the basolateral chamber only. Similarly, cinacalcet pretreatment reduced the CFTRinh-172 effect when added to the apical or both chambers, but not to the basolateral chamber alone.

Cinacalcet inhibits apical membrane Cl− and basolateral membrane K+ conductance in T84 cells. Forskolin-induced Cl− secretion in intestinal cells involves the coordinated action of several transporters, including apical membrane CFTR, and basolateral membrane K+ channels and cotransporters (4). To ascribe the cinacalcet inhibition of ISC in T84 cells to an action on CFTR, ISC was measured in T84 cells following selective permeabilization of the basolateral membrane in the presence of a basolateral-to-apical Cl− gradient, in which ISC provides a direct measure of apical membrane Cl− conductance (19). In this setting cinacalcet pretreatment largely inhibited forskolin-induced Cl− secretion and the CFTRinh-172 effect (Figure 4, C and D).

To investigate possible cinacalcet effect on basolateral membrane K+ channels, ISC was measured in T84 cells following selective permeabilization of the apical membrane in the presence of an apical-to-basolateral K+ gradient, in which ISC provides a direct measure of basolateral membrane K+ conductance (20). In this setting cinacalcet pretreatment substantially reduced basolateral membrane K+ conductance in T84 cells as seen by the reduced forskolin- and BaCl2-induced ISC changes (Figure 4, E and F). These results support a dual cinacalcet effect in T84 cells involving inhibition of apical membrane CFTR Cl− channel as well as basolateral membrane K+ channels.

Cinacalcet inhibits Cl− secretion in T84 cells induced by cholera toxin, heat-stable E. coli enterotoxin, and vasoactive intestinal peptide. The cinacalcet effect on Cl− secretion in T84 cells was also studied in response to 2 biologically relevant enterotoxins that cause secretory diarrhea: cholera toxin and heat-stable E. coli enterotoxin (STa toxin). Cholera toxin produced a slow but large increase in ISC, whereas the STa toxin effect was faster but of smaller magnitude. Cinacalcet pretreatment significantly inhibited the maximal ISC increase induced by cholera toxin (Figure 5, A and B) and STa toxin (Figure 5, C and D) as well as the CFTRinh-172 responses.

Vasoactive intestinal peptide–secreting (VIP-secreting) neuroendocrine tumors (VIPomas) are rare causes of severe secretory diarrhea also known as “pancreatic cholera.” In this condition high circulating VIP levels stimulate intestinal fluid secretion by elevating cyclic nucleotides in enterocytes (21). Treatment
of T84 cells with VIP caused an immediate and large increase in I_{SC}. Cinacalcet pretreatment significantly reduced VIP-induced ISC and the CFTRinh-172 effect (Figure 5, E and F).

Cinacalcet activates PLC and reduces cAMP levels in T84 cells through PDEs. CaSR can be coupled to various G proteins and signaling pathways in different tissues (6). In rat colonocytes, CaSR was shown to be functionally coupled to G_{q}, and PLC, causing IP_{3} generation and Ca^{2+} release from intracellular stores with consequent PDE activation and cAMP breakdown (7, 8). This proposed signaling mechanism was tested in human T84 cells. Cinacalcet produced a rapid and marked increase in intracellular Ca^{2+} as measured by Fluo-4 fluorescence, which was abolished by pretreatment with the PLC inhibitor U73122 (Supplemental Figure 2, A and B).

Because cAMP is the major activator of CFTR, increased PDE activity via CaSR activation might explain the inhibitory effect of cinacalcet on CFTR-mediated Cl^- secretion as well as on cAMP-activated basolateral K+ channels. Consistent with this mechanism, cinacalcet pretreatment significantly reduced forskolin-induced elevations in cAMP (Supplemental Figure 2C). The cinacalcet effect was completely reversed by the nonselective PDE inhibitor IBMX. Together, these results suggest PLC-mediated intracellular Ca^{2+} elevation and PDE activation as the antisecretory mechanism of cinacalcet in T84 cells.

Cinacalcet inhibits fluid accumulation in closed intestinal loop models of cholera and traveler’s diarrhea in mice. Motivated by its antisecretory action in T84 cells, we tested cinacalcet in mouse models of cholera and traveler’s diarrhea in which CFTR activation is the major secretory pathway following exposure to cholera toxin or STa toxin, respectively. Closed loop studies were done in mouse midjejunum in which cinacalcet was administered intraperitoneally prior to loop creation and instillation of toxin (Figure 6A). In cholera toxin–injected loops, cinacalcet at 30 mg/kg inhibited intestinal fluid accumulation over 3 hours (assessed by loop weight/length ratio) by approximately 75%, with significant inhibition also seen at lower doses of 1
and 10 mg/kg (Figure 6B). Cinacalcet was also effective in closed loops containing STa toxin, with approximately 60% inhibition of intestinal fluid accumulation over 3 hours at 30 mg/kg (Figure 6C).

**Cinacalcet stimulates fluid absorption from mouse small intestine.** In secretory diarrheas, intestinal fluid accumulation in response to enterotoxins involves elevation in cyclic nucleotides that results in fluid secretion due to CFTR activation and reduced fluid absorption due to NHE3 inhibition (2, 3). An earlier study showed that CaSR activation can stimulate fluid absorption from rat colonic crypts through increased NHE activity (8). In order to avoid the confounding effect of absorption in the cholera model and directly demonstrate cinacalcet action on fluid secretion, jejunal closed loops were injected with the nonabsorbable NHE3 inhibitor tenapanor together with cholera toxin. Interestingly, cinacalcet was less effective in preventing fluid accumulation in the presence of tenapanor (Figure 6B). To determine the effect of cinacalcet on NHE3 in the cholera model, closed loop experiments were done in cystic fibrosis (CF, ΔF508 homozygous) mice lacking functional CFTR. In CF mice, cholera toxin induced mild fluid accumulation as seen by the modestly increased loop weight/length ratio (Figure 6D) compared with the WT mice (Figure 6B). Cinacalcet prevented fluid accumulation in jejunal closed loops in CF mice, and this effect was abolished in the presence of luminal tenapanor (Figure 6D). These results suggest that the cinacalcet effect in the cholera model is largely due to CFTR inhibition and in part due to increased NHE3 activity.

Experiments were also done in jejunal closed loops in the absence of secretagogues in order to further characterize the cinacalcet effect on intestinal fluid absorption (22). Closed midjejunal loops were injected with phosphate-buffered saline (PBS), and fluid absorption was determined at 15, 60, and 120 minutes (Fig-
Figure 4. Cinacalcet acts from the apical side in T84 cells for inhibition of apical membrane CFTR-mediated Cl\(^-\) secretion and inhibits cAMP-activated basolateral membrane K\(^+\) conductance. (A) \(I_{sc}\) traces showing effects of 10 \(\mu\)M forskolin and 10 \(\mu\)M CFTR\(_{inh-172}\) with 20-minute pretreatment with 30 \(\mu\)M cinacalcet applied from apical, basolateral, or both sides. (B) Summary of data from experiments as in A showing \(\Delta I_{sc}\) following forskolin and CFTR\(_{inh-172}\). (C) \(I_{sc}\) traces with basolateral permeabilization (amphotericin B, 250 \(\mu\)g/mL) and 60 mM basolateral-to-apical Cl\(^-\) gradient showing responses to 10 \(\mu\)M forskolin and 10 \(\mu\)M CFTR\(_{inh-172}\) without and with 20-minute pretreatment with 30 \(\mu\)M cinacalcet. (D) Summary of experiments as in C showing \(\Delta I_{sc}\) following forskolin and CFTR\(_{inh-172}\). (E) \(I_{sc}\) traces with apical permeabilization (amphotericin B, 20 \(\mu\)M) and apical-to-basolateral K\(^+\) gradient showing responses to 10 \(\mu\)M forskolin and 5 mM barium chloride (BaCl\(_2\), cAMP-activated K\(^+\) channel inhibitor, added to basolateral side) without and with 20-minute pretreatment with 30 \(\mu\)M cinacalcet. (F) Summary of experiments as in E showing \(\Delta I_{sc}\) following forskolin and BaCl\(_2\), \(n = 5-6\) experiments per group. Mean ± SEM, 1-way ANOVA with Newman-Keuls multiple comparisons test (B), and Student’s t test (D and F), *\(P < 0.05\), **\(P < 0.001\).
reversed the cinacalcet effect and blocked fluid absorption at all time points (Figure 7B). These data support the conclusion that cinacalcet stimulates in vivo intestinal fluid absorption through NHE3.

**Discussion**

Here we showed that CaSR regulates CFTR-mediated Cl⁻ secretion in human T84 cells, and the FDA-approved CaSR activator cinacalcet prevents intestinal fluid accumulation in mouse models of cholera and...
traveler’s diarrhea. In humans, cinacalcet is administered at doses up to 180 mg per day (approximately 2.5 mg/kg for a 70 kg adult) for secondary hyperparathyroidism and at higher doses up to 360 mg per day for parathyroid carcinoma (24). Cinacalcet treatment in mice had a marked antisecretory action in closed intestinal loop models of cholera and traveler’s diarrhea at 30 mg/kg, a dose previously used in rodent studies (25) and equivalent to 2.5 mg/kg in humans by consideration of the body surface area conversion factor (26). Cinacalcet is thus predicted to have antidiarrheal effect in human secretory diarrheas at usual therapeutic doses, supporting its potential repurposing for major secretory diarrheas.

Consistent with earlier studies in rat colonocytes (7, 8), we found here in human T84 cells that CaSR activation by cinacalcet activated PLC to elevate intracellular Ca\(^{2+}\), resulting in PDE activation and cAMP degradation. Because cAMP is the major activator of CFTR, this mechanism is likely in large part responsible for the cinacalcet-mediated suppression of Cl\(^{-}\) secretion in T84 cells. In mice, cinacalcet also stimulated intestinal fluid absorption in an NHE3-dependent manner. Because cAMP inhibits NHE3 activity (3), the stimulatory effect of cinacalcet on intestinal fluid absorption is likely explained by reduced cAMP levels as well. In secretory diarrheas, elevation of intracellular cyclic nucleotides is the key mechanism of diarrhea. Cinacalcet might thus exert its antidiarrheal effect through multiple mechanisms, including inhibition of fluid secretion and stimulation of fluid absorption.

CaSR is also expressed in enteric neurons. Earlier studies suggested that CaSR modulates the regulatory effect of the enteric nervous system (ENS) on intestinal fluid secretion in secretory diarrhea (27). Although cinacalcet had marked antisecretory effect in T84 cells where enteric nerves are absent, its
antisecretory actions in mouse intestine in vivo might also involve modulation of ENS activity. In addition to cholera or traveler’s diarrhea, cinacalcet may be effective in other forms of secretory diarrheas, such as VIPoma, as suggested by its efficacy in reducing VIP-induced secretory response in T84 cells and by its mechanism involving reducing cAMP levels in enterocytes. Although somatostatin analogs such as octreotide and/or surgical resection are the standard of care for VIPoma (28), cinacalcet may offer an alternative option for patients who do not tolerate or respond to these treatments. Other potential indications for cinacalcet include medullary thyroid cancer–associated diarrhea (29), bile acid diarrhea (30), and tyrosine kinase inhibitor diarrhea (19), in which increased CFTR-mediated Cl− secretion is a major cause of diarrhea. Cinacalcet may also be effective in some congenital diarrheas such as gain-of-function mutations in \textit{GUCY2C}, which is associated with excessive cyclic nucleotide-mediated CFTR activation in enterocytes (31), as well as microvillus inclusion disease, which may involve impaired intestinal absorption and unbalanced CFTR-mediated Cl− secretion (32).

In addition to gut, CaSR is expressed in various organs, including parathyroid gland, kidney, and bone (6). Cinacalcet has good oral bioavailability and is thus expected to produce systemic effects, including hypocalcemia due to PTH suppression. Cinacalcet-induced hypocalcemia is generally asymptomatic, transient, and easily managed by dose adjustment or oral calcium supplementation (33). For short-term use as in cholera, hypocalcemia may be of lesser concern and can be prevented, if needed, by calcium supplementation. However, cinacalcet use in chronic diarrheas might be problematic due to sustained PTH suppression, which can impair bone mineralization even if hypocalcemia is prevented with calcium supplementation. Interestingly, we found here that cinacalcet exerts its effect with apical membrane administration in T84 cells. Therefore, nonabsorbable CaSR activators acting from the gut lumen can potentially have antidiarrheal efficacy without systemic action.

CaSR is expressed in both apical and basolateral membranes of human and rodent intestinal epithelium (7, 18). We previously showed efficacy of intraperitoneally administered modulators of apical membrane CFTR in mouse models of intestinal fluid transport (closed loops) and constipation (22, 34). Thus, the efficacy of intraperitoneally administered cinacalcet in mice here may result from its action on both basolateral and apical CaSR. To our knowledge, there are no reports of the apical versus basolateral membrane distribution of CaSR in T84 cells. The lack of cinacalcet efficacy when added to the basal surface of T84 cells may be due to low basolateral CaSR expression or perhaps limited physical access of cinacalcet through the porous filter because of its high hydrophobicity. Since cinacalcet is thought to bind to the transmembrane domain of CaSR (9), cellular uptake may be required for its action, in which case the apical versus basolateral location of CaSR may not be an important determinant in its action.

In conclusion, we demonstrated that the FDA-approved drug cinacalcet suppressed CFTR-mediated Cl− secretion in human colonic T84 cells and prevented diarrhea in mouse models of cholera and traveler’s diarrhea. Cinacalcet or other calcimimetics can be lifesaving and logistically feasible treatments for cholera and offer an alternative treatment option for many other types of secretory diarrheas.
Methods

Chemicals. All chemicals were purchased from MilliporeSigma unless otherwise specified.

Cell culture. T84 cells (ATCC CCL-248, human colon carcinoma cells) were cultured in a 1:1 mixture of DMEM/Ham’s F12 medium supplemented with 10% fetal bovine serum, 2 mM l-glutamine, 100 U/mL penicillin, and 100 μg/mL streptomycin. T84 cells were grown on inserts (12 mm diameter, 0.4 μm polyester membrane; Corning Life Sciences) at 37°C in 5% CO2/95% air and used for Isc experiments 7 days after plating. FRT cells (obtained from UCSF Cystic Fibrosis Drug Discovery Core Center) stably expressing human WT CFTR (FRT-CFTR cells) were cultured as described (34) on inserts and used for Isc experiments 5 days after plating. Well-differentiated HBE cells (obtained from UCSF Cystic Fibrosis Drug Discovery Core Center) were cultured at an air-liquid interface on inserts as described (22). HBE cells were used for Isc experiments 21 days after plating, when they typically form a tight epithelium (Rte > 1000 Ω cm²).

Isc measurements. Cells were mounted in Ussing chambers with each hemichamber containing bicarbonate-buffered Ringer’s solution (pH 7.4, in mM: 120 NaCl, 5 KCl, 1 MgCl₂, 1 CaCl₂, 10 d-glucose, 5 HEPES, and 25 NaHCO₃). Unless otherwise specified, secretagogues and ion transport modulators were added to both apical and basolateral bathing solutions. Some experiments were done using Ca²⁺ and Mg²⁺-free Ringer’s solution with and without EDTA to chelate Ca²⁺ and Mg²⁺. The solutions were aerated with 95% O₂/5% CO₂ and maintained at 37°C during experiments. Isc was measured using an EVC4000 multichannel voltage clamp (World Precision Instruments) via Ag/AgCl electrodes and 3 M KCl agar bridges.

In some experiments to measure apical Cl⁻ conductance directly, the basolateral membrane was permeabilized with 250 μg/mL amphotericin B, and a basolateral-to-apical Cl⁻ gradient was applied, as described (22, 34), in which Ringer’s was the basolateral bathing solution (120 mM NaCl) with the apical solution containing 60 mM NaCl and 60 mM Na gluconate. To measure basolateral membrane K⁺ conductance, an apical-to-basolateral potassium gradient was applied, and the apical membrane was permeabilized with 20 μM amphotericin B (20). The apical solution (pH 7.4) contained in mM: 142.5 K-gluconate, 1 CaCl₂, 1 MgCl₂, 0.43 KH₂PO₄, 0.35 Na₂HPO₄, 10 HEPES, and 10 d-glucose. In the basolateral solution (pH 7.4) 142.5 mM K-gluconate was replaced by 5.5 mM K-gluconate and 137 mM N-methylglucamine.

Intracellular Ca²⁺ and cAMP measurement. T84 cells were plated in 96-well, black-walled microplates (Corning Life Sciences) and used at 72 hours after plating. Confluent cells were loaded with calcium indicator Fluo-4 NW (Invitrogen, Thermo Fisher Scientific) per manufacturer’s instructions. Fluo-4 fluorescence was measured in each well continuously with a Tecan Infinite M1000 plate reader (Tecan Group) at excitation/emission wavelengths of 495 nm/516 nm after manual addition of 30 μM cinacalcet (or 1% DMSO vehicle control). In some studies cells were pretreated with PLC inhibitor U73122 (10 μM) for 5 minutes prior to addition of cinacalcet. For cAMP assay, T84 cells were grown in clear 24-well plates and pretreated for 20 minutes with 30 μM cinacalcet with or without 500 μM IBMX or vehicle control (0.2% DMSO). Then cells were treated with 10 μM forskolin (for 5 minutes) and lysed by repeated freeze/thaw and centrifuged to remove cell debris. The supernatant was assayed for cAMP using the cAMP Parameter immunoassay kit according to the manufacturer’s instructions (R&D Systems, Bio-Techne).

Intestinal closed loop model of cholera and traveler’s diarrhea. Mice (male and female CD1, 8–12 weeks old, developed and bred in-house) were fasted overnight with access to 5% dextrose in water but no solid food before experiments. In some experiments, mice were treated with specified amounts of intraperitoneal cinacalcet hydrochloride (1, 10, and 30 mg/kg), or vehicle (saline containing 5% DMSO and 10% Kolliphor HS), 30 minutes prior to the start of abdominal surgery. Mice were anesthetized with isoflurane, and body temperature was maintained during surgery at 36°C–38°C using a heating pad. After a small abdominal incision to expose the small intestine, midjejunal loops (2–3 cm in length) were isolated by sutures as described (22, 34). Loops were injected with 100 μL PBS (pH 7.4, in mM: 137 NaCl, 2.7 KCl, 8 Na₂HPO₄, 1.8 KH₂PO₄, 1 CaCl₂, 0.5 MgCl₂) containing 1 μg choler toxin or 0.1 μg heat-stable enterotoxin of E. coli (STα toxin, Bachem Americas Inc.) or PBS alone. In some experiments 10 μM tenapanor was injected into the loops together with choler toxin or PBS to block NHE3-mediated fluid absorption (23). After loop injections the abdominal incision was closed with sutures, and mice were allowed to recover from anesthesia. Intestinal loops were removed at 3 hours, and loop length and weight were measured to quantify fluid secretion.

To quantify intestinal absorption from closed loops, midjejunal loops were isolated in mice treated with cinacalcet (30 mg/kg) or vehicle as described above. Loops were injected with 100 μL PBS and excised immediately (0 minutes), or 15, 60, or 120 minutes after injection, and loop length and weight were
measured to quantify fluid absorption (23). In some experiments 10 μM tenapanor (in 100 μL PBS) was injected into the loops to block NHE3.

**Statistics.** Experiments with 2 groups were analyzed using 2-tailed Student’s t test; for 3 or more groups, analysis was done with 1-way ANOVA and post hoc Newman-Keuls multiple comparisons test. P < 0.05 was considered statistically significant.

**Study approval.** The experimental protocols were approved by the UCSF Institutional Animal Care and Use Committee. Animals were bred in UCSF Laboratory Animal Resource Center. Animal experiments were done in adherence to the NIH Guide for the Care and Use of Laboratory Animals (National Academies Press, 2011).

**Author contributions**
OC conceived the study and designed the experiments. AAO, PDC, and OC conducted the experiments. AAR prepared and maintained the cells. AAO, PDC, and OC analyzed the data. OC wrote the paper. ASV revised the paper. All authors read the paper and approved the submitted form.

**Acknowledgments**
This work was supported by grants from the NIH (DK126070, DK072517), American Heart Association (18POST33990365), and Cystic Fibrosis Foundation.

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24. FDA. Sensipar (cinacalcet hydrochloride) tablets label. https://www.accessdata.fda.gov/drugsatfda_docs/