The Calcium-Sensing Receptor (CaSR) acts as a modulator of gastric acid secretion in freshly isolated human gastric glands

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ABSTRACT

Gastric acid secretion is activated by two distinct pathways, a neuronal pathway via the vagus nerve and release of acetylcholine, and an endocrine pathway involving gastrin and histamine. Recently, we have demonstrated that activation of H⁺/K⁺-ATPase activity in parietal cells in freshly isolated rat gastric glands is modulated by the calcium sensing receptor (CaSR). Here we investigated if the CaSR is functionally expressed in freshly isolated gastric glands from human patients undergoing surgery and if the CaSR is influencing the histamine induced activation of H⁺/K⁺-ATPase activity. In tissue samples obtained from patients, immunohistochemistry demonstrated the expression in parietal cells of both subunits of the gastric H⁺/K⁺-ATPase and the CaSR. Functional experiments using the pH-sensitive dye BCECF and measuring intracellular pH changes allowed estimation of the activity of the H⁺/K⁺-ATPase in single freshly isolated human gastric glands. Under control conditions, H⁺/K⁺-ATPase activity was stimulated by histamine (100 µM) and inhibited by omeprazole (100 µM). Reduction of the extracellular divalent cation concentration (0 Mg²⁺, 100 µM Ca²⁺) inactivated CaSR and reduced the histamine induced activation of H⁺/K⁺-ATPase activity. In contrast, activation of the CaSR with the trivalent cation Gd³⁺ caused activation of omeprazole-sensitive H⁺/K⁺-ATPase activity even in the absence of histamine and under low extracellular divalent cations. This stimulation was not due to release of histamine from neighbouring ECL cells as the stimulation persisted in the presence of the H₂-receptor antagonist cimetidine.
(100 µM). Furthermore, intracellular calcium measurements with FURA-2 and Fluo-4 showed that activation of CaSR by Gd^{3+} led to a sustained increase in intracellular Ca^{2+} even under low extracellular divalent cations.

These experiments demonstrate the presence of a functional calcium-sensing receptor in the human stomach and show that this receptor may modulate the activity of the acid-secreting H^{+}/K^{+}-ATPase in parietal cells. Furthermore, our results show the viability of freshly isolated human gastric glands and may allow the use of this preparation for experiments investigating the physiological regulation and properties of human gastric glands in vitro.
INTRODUCTION

Gastric acid secretion by parietal cells is under the control of both neuronal regulation via the vagus nerve involving release of acetylcholine and under the control of endocrine and paracrine factors including gastrin and histamine. Histamine is released from neighbouring enterochromaffin-like cells (ECL) and triggers an intracellular signalling cascade in parietal cells leading to the insertion of H⁺/K⁺-ATPases from tubulo-vesicular structures into the luminal membrane where acid secretion takes place (17). The exposure to histamine also causes a simultaneous rise in intracellular Ca²⁺ which has served as an additional marker for activated acid secretion (2, 4).

In addition to these classic pathways regulating gastric acid secretion, the calcium-sensing receptor, CaSR, has been identified in rat gastric parietal cells (2, 10). The CaSR is activated by divalent cations, Ca²⁺ and Mg²⁺, the trivalent cation Gd³⁺, and by substrates like spermine. Its sensitivity to these ligands is modulated by L-amino acids and pH (5, 6, 18). Activation of CaSR in rat parietal cells induced an increase in intracellular Ca²⁺ concentrations (2, 8) suggesting that CaSR could be involved in regulation of gastric acid secretion. Indeed, further experiments using freshly isolated rat gastric glands demonstrated that activation of CaSR leads to a stimulation of histamine-induced H⁺/K⁺-ATPase activity (8). On the other hand, inactivation of CaSR by a reduction of extracellular divalent cations prevented the histamine mediated stimulation of H⁺/K⁺-ATPase activity. Taken together, these results suggested that the CaSR
represents a novel receptor in the stomach that may modulate the histamine induced stimulation of gastric acid secretion (8, 10).

The investigation of human parietal cells and the regulation of acid-secretion has been hampered in the past by the lack of appropriate human cell models as most cell lines lose their responsiveness to physiological stimuli for acid-secretion, alter morphology or the expression of key proteins involved in ion transport and acid-secretion (17). The use of freshly isolated human gastric glands may therefore be useful to investigate some aspects of short-term regulation and basic properties of ion transport and acid-secretion. Some attempts have been made in the past to use gastric glands obtained from biopsy samples and measure several parameters linked to parietal cell activity (14).

To this end we tested if fresh tissue samples obtained from stomach surgery could be used to isolate intact human gastric glands and if these glands were viable for physiological experiments. Furthermore, we examined if a CaSR-dependent pathway modulating acid secretion via the H⁺/K⁺-ATPase is present in human parietal cells. Our results demonstrate that freshly isolated gastric glands are viable and express functional H⁺/K⁺-ATPases stimulated by histamine. Expression of CaSR was shown by immunohistochemistry and this receptor modulates H⁺/K⁺-ATPase activity in human parietal cells.
MATERIAL AND METHODS

Patients

Over a period of 8 months samples of gastric tissue were obtained from a total of 29 patients. Patients underwent Roux-en-Y gastric bypass operation for morbid obesity (7) and small samples of gastric tissue (including mucosal and muscle layers) from the gastrojejunal anastomosis were collected. The gastric tissue originated from the fundus of the stomach. Samples were collected from 8 male and 21 female patients. Male patients had an average age of 48.6 ± 2.6 years with an average body mass index (BMI) of 44.4 ± 2.6. Female patients were 39.9 ± 2.2 yrs. old and had a BMI of 44.3 ± 1.2. Due to obesity many of the patients suffered from metabolic syndrome (31.0 %). Drugs altering gastric acid secretion (proton pump inhibitors, H₂-receptor antagonists) were discontinued one week prior to surgery.

Informed consent was obtained from all patients and the study was approved by the local Ethics committee.

Isolation of gastric glands, digital imaging for intracellular pH and Ca²⁺

Gastric tissue was stored for transport in ice-cold MEM solution (Gibco, Langley, OK, USA). The tissue was then transferred to the stage of a dissecting microscope and sliced into 0.5 cm square sections. Individual glands were isolated using a hand dissection technique as described previously (8, 13) at a temperature of about 10°C. After isolation, the glands were transferred to
coverslips precoated with adhesive Cell-Tak (BD Cell-Tak Cell and Tissue Adhesion, BD Biosciences) and mounted in a thermostatically controlled chamber maintained at 37 °C on an inverted microscope (Zeiss Axiovert 200) equipped with an video imaging system for the duration of the experiment. Isolated gastric glands were loaded with 10 µM of the pH-sensitive dye 2′,7′-bis (2-carboxyethyl)-5-(and 6)-carboxyfluorescein aceto-methyl ester (BCECF-AM) (Molecular Probes, Eugene, OR) for 10 min in a HEPES-buffered Ringer solution (125 mM NaCl, 5 mM KCl, 1 mM CaCl₂, 1.2 mM MgCl₂, 32.2 mM HEPES, and 5 mM glucose, pH 7.4) at 37 °C. After loading, the chamber was flushed with HEPES-buffered Ringer solution to remove non de-esterfied dye. Measurements were performed in the epifluorescence mode with 40x / 1.30 oil objective on an inverted microscope. BCECF was successively excited at 440 nm and 495 nm from a monochromator light source and the resultant fluorescence signal was monitored at 535 nm using an intensified charge-coupled device camera. Data points were acquired every 7 s. The resulting 495/440 intensity ratio data were converted to intracellular pH (pHᵢ) values using the high K⁺/Nigericin calibration technique (19). Over the pH range of 6.3 - 7.8, fluorescence varied in a linear fashion with extracellular pH. Data were expressed as ΔpH/min. Acid extrusion was monitored in the absence of bicarbonate as intracellular alkalinization after the removal of Na⁺ from the bath and using the NH₄Cl-prepulse technique, which caused a reproducible and sustained intracellular acidification. Alkalinization rates (ΔpHᵢ/minute) for the calculation of Na⁺-independent pHᵢ recovery (H⁺,K⁺-
ATPase activity) and Na⁺-dependent pHᵢ recovery (Na⁺/H⁺ exchanger activity) rates were measured in the range of pH 6.50-6.70 and 6.75 – 6.90, respectively.

To measure intracellular Ca²⁺, gastric glands were loaded with 10 µM of the Ca²⁺-sensing dye Fura-2 AM, Molecular Probes (Eugene, OR, USA) in the chamber for 20 min at room temperature. To eliminate residual de-esterified dye from the bath the glands were superfused with standard HEPES-buffered Ringer solution for 2 min. Fura-2 was excited with light of 340/380 nM wavelengths. Intracellular calcium concentrations [Ca²⁺]ᵢ were calculated from the ratio of fluorescence at excitations of 340/380 and using the equation described previously: 

\[ \text{Ca}^{2+}_i = \left[ \frac{(R - R_{\text{min}})}{(R_{\text{max}} - R)} \right] \times \left( \frac{F_{\text{min}}}{F_{\text{max}}} \right) \times K_d, \]

where \( R \) is the measured ratio of emitted light, \( F_{\text{max}} \) is the fluorescence at 380 nM with 2 mM Ca²⁺ bath solution, \( F_{\text{min}} \) is the fluorescence at 380 nM with 0 mM Ca²⁺ bath solution, and \( K_d = 225 \text{nM} \) for the Fura-2-calcium binding (9).

All chemicals used were all obtained from Sigma and Molecular Probes. Omeprazole was a kind gift from Astra Hässle AB, Mölndal, Sweden.

Activation of acid secretion via histamine and inhibition by omeprazole was induced by a preincubation of the glands for 10 min before the experiment combined with BCECF. All data are summarized as mean ± S.E. and were analyzed by grouping measurements at baseline values and during experimental periods. Significance was determined using an unpaired Student’s t-test with \( p < 0.05 \) considered to be statistically significant.
Immunohistochemistry

Human stomach samples were washed several times with PBS and fixed by immersion with paraformaldehyde-lysine-periodate (PLP) fixative (16) overnight at 4°. Stomachs were washed three times with PBS and thin sections were cut at a thickness of 5 µm after cryoprotection with 2.3 M sucrose in PBS for at least 12 h. Immunostaining was carried out as described previously (13). Sections were incubated with 1 % SDS for 5 min., washed 3 times with PBS and incubated with PBS containing 1 % bovine serum albumin for 15 min prior to the primary antibody. The primary antibodies (mouse monoclonal anti pig β gastric H⁺/K⁺-ATPase (Affinity Bioreagents, CA, USA), rabbit polyclonal anti pig α gastric H⁺/K⁺-ATPase (Chemicon, CA, USA), a rabbit polyclonal affinity-purified anti-CaSR against amino acids 12-27 of rat CaSR (Affinity Bioreagents, CA, USA) were diluted 1:2000, 1:1000, and 1:50, respectively, in PBS and applied overnight at 4 °C. In addition, a rabbit polyclonal antibody was generated to a maltose-binding fusion protein of the entire extracellular domain of the rat CaSR (residues 1-642). The rabbit anti-rat CaSR 1-642 polyclonal antibody was affinity purified using the MBP-CaSR protein (AminoLink Plus Immobilization Trial Kit, Pierce) and used at a dilution of 1:50. Peptide protection experiments were performed by incubating the affinity-purified antibody at 1:50 with the immunizing peptide (0.8 µg/ml) in PBS for 1 h at room temperature and directly applying the dilution after centrifugation to remove precipitates. Stomach sections were then washed twice for 5 min with high NaCl PBS (PBS + 2.7 % NaCl), once with PBS, and incubated with the secondary antibodies (donkey anti-rabbit Alexa 546 and
donkey anti-mouse Alexa 488, Molecular Probes, Oregon, USA) at a dilution of 1:1000 and 1:200, respectively, for 1 h at room temperature. Sections were washed twice with high NaCl PBS and once with PBS before mounting with VectaMount (Vector Laboratories, Burlingame, CA, USA). The specimens were viewed with a Leica SP1 UV CLSM confocal microscope and pictures processed using Adobe Photoshop.
RESULTS

_Freshly isolated human gastric glands are suitable for functional experiments_

In a first series of experiments we tested if the freshly isolated human gastric glands were suitable for functional experiments investigating regulation of H⁺/H⁺-ATPase activity. To this end, immunohistochemistry was performed on the tissue samples obtained to examine expression of both subunits of the gastric H⁺/K⁺-ATPase. Immunohistochemistry demonstrated that both the alpha and beta subunits could be detected and that thus the acid-secretory parietal cells were present in the samples obtained from the antral part of the human stomach (Figure 1). Intracellular pH measurements of single parietal cells within freshly isolated gastric glands were used to measure H⁺/K⁺-ATPase activity. The activity of the pump was calculated from the rate of alkalinization of intracellular pH ($\Delta$pHi/min) after acidification using the NH₄Cl prepulse technique in the absence of sodium and bicarbonate. H⁺-extrusion under these conditions depends mainly on the activity of the H⁺/K⁺-ATPase as previously shown (8). In the absence of any stimulation (i.e. histamine or acetylcholine) only a low rate of intracellular pH recovery was observed (0.010 ± 0.004 pH units/min, n = 60 parietal cells, 4 glands, 4 patients) (Figure 2A,D). After exposure of the glands to histamine (100 µM) the rate of Na⁺-independent pHᵢ alkalinization increased to 0.024 ± 0.003 pH units/min (n = 50 parietal cells, 5 glands, 2 patients) (Figure 2B,D). To confirm that this stimulation of the Na⁺-independent pHᵢ recovery represented H⁺/K⁺-ATPase activity, glands were preincubated with 100 µM of the specific inhibitor of
the gastric H⁺/K⁺-ATPase omeprazole for 10 min prior to the experiments in the presence of histamine (100 µM). Omeprazole prevented the stimulatory effect of histamine on the Na⁺-independent pHᵢ-recovery rate (0.0094 ± 0.002 pH units/min) and reduced it to the same level as seen in glands not exposed to histamine (n = 65 parietal cells, 3 glands, 4 patients) (Figure 2C,D). Thus, the freshly isolated human gastric glands contained functional parietal cells that showed H⁺/K⁺-ATPase activity which could be stimulated with the physiological agonist histamine and inhibited by the specific blocker omeprazole.

Expression of the Ca²⁺-sensing receptor in human parietal cells

Immunohistochemistry using two different antibodies directed against different epitopes of the CaSR demonstrated staining of the basolateral side of a subset of cells along the gastric gland (Figure 3A). No specific signal for CaSR was observed with pre-immune serum (Figure 3F), after peptide protection with the immunizing peptide (Figure 3G) or applying only the secondary antibody (Figure 3H). In order to test if the CaSR was expressed in parietal cells, double-labelling for CaSR and the beta subunit of the gastric H⁺/K⁺-ATPase was performed in samples obtained from patients fasting for at least 12 hours prior to the operation. Co-localization of both the CaSR and the beta subunit of the gastric H⁺/K⁺-ATPase was observed demonstrating expression of the CaSR in human gastric parietal cells (Figure 3). Higher magnification pictures show that CaSR and the βH⁺/K⁺-ATPase subunit do not localize to the same compartment.
of resting parietal cells with the $\beta$H+/K+-ATPase subunit residing in intracellular structures consistent with its localization in tubulo-vesicular structures (Figure 3 D,E).

Modulation of the histamine induced stimulation of H+/K+-ATPase activity by CaSR in human gastric glands

To examine the effect of the CaSR on the activity of the gastric H+/K+-ATPase and its stimulation by histamine we reduced the concentration of total divalent cations from 1 mM Mg$^{2+}$ and 1.2 mM Ca$^{2+}$ in the control solution to only 0.1 mM Ca$^{2+}$ and 0 mM Mg$^{2+}$, a concentration of divalent cations leaving the CaSR inactive. Glands were preincubated in this low divalent cation solution for 10 min prior to the experiment and were stimulated with 100 $\mu$M histamine as described above. Low extracellular divalent cations abolished the histamine-induced alkalinization (0.015 ± 0.003 pH unit/min, n= 61 parietal cells, 9 glands, 9 patients) (Figure 4C). In contrast, stimulation of the CaSR with the trivalent cation Gd$^{3+}$ (100 $\mu$M) in the low divalent cation solution (100 $\mu$M Ca$^{2+}$, 0 mM Mg$^{2+}$) stimulated H$^+$,K$^+$-ATPase activity even in the absence of histamine (Na$^+$-independent pH-recovery: 0.075 ± 0.004 pH units/min n= 68 parietal cells, 8 glands, 6 patients) (Figure 4B). This stimulatory effect was also seen when the gastric glands were preincubated with histamine in a low divalent cation solution (100 $\mu$M Ca$^{2+}$, 0 mM Mg$^{2+}$) and Gd$^{3+}$ applied directly during the phase of Na$^+$-independent alkalinization. Gd$^{3+}$ induced an immediate increase in alkalinization rate (data not shown) suggesting a rapid activation of H$^+$-extrusion. Similarly,
increasing extracellular Ca\textsuperscript{2+} to 5 mM in the presence of histamine stimulated H\textsuperscript{+}/K\textsuperscript{+}-ATPase activity above levels seen at 1 mM extracellular Ca\textsuperscript{2+} (n = 101 parietal cells, 6 glands, 4 patients). These data suggest that also the physiological ligand, Ca\textsuperscript{2+} can stimulate the CaSR and increase H\textsuperscript{+}/K\textsuperscript{+}-ATPase activity.

To rule out that histamine released from neighbouring ECL cells mediated the effect of Gd\textsuperscript{3+} on parietal cells, gastric glands were preincubated with the H\textsubscript{2} receptor inhibitor cimetidine (100 µM) and stimulated with Gd\textsuperscript{3+}. The Na\textsuperscript{+}-independent pH\textsubscript{i}-recovery rate was not altered by incubation with cimetidine and Gd\textsuperscript{3+} was still effective in stimulating realalkalinization (0.095 ± 0.01 pH units/min, n= 34 parietal cells, 4 glands, 2 patients) (Figure 5).

Furthermore, to examine if the Gd\textsuperscript{3+} induced stimulation of the Na\textsuperscript{+}-independent pH\textsubscript{i} recovery was due H\textsuperscript{+}/K\textsuperscript{+}-ATPase activity, gastric glands were preincubated for 10 min with the H\textsuperscript{+}/K\textsuperscript{+}-ATPase inhibitor omeprazole (100 µM) which abolished almost completely intracellular alkalinization (0.011 ± 0.001 pH units/min, n= 58 parietal cells, 6 glands, 4 patients) (Figure 5) demonstrating that CaSR stimulated H\textsuperscript{+}/K\textsuperscript{+}-ATPase activity.

*CaSR activation leads to increases in intracellular Ca\textsuperscript{2+}*

CaSR activation has been shown to induce increases in intracellular Ca\textsuperscript{2+} concentrations in rat gastric parietal cells and in a number of other tissue
preparations and cell culture lines (2, 3, 8). Therefore, we tested if activation of CaSR by Gd\textsuperscript{3+} increased intracellular Ca\textsuperscript{2+}. Activation of the CaSR with Gd\textsuperscript{3+} (100 µM) increased intracellular Ca\textsuperscript{2+} even under conditions of low extracellular divalent cations (Figure 6).
**DISCUSSION**

Gastric acid secretion involves a complex process of either neuronal or paracrine stimulatory pathways converging in the insertion of H⁺/K⁺-ATPases into the luminal membrane of the acid-secretory parietal cells (for review: (20)). Besides the classic routes of activation through acetyl choline, gastrin, and histamine, a number of metabolic factors including serum calcium and protein- or amino acid rich diets, influence gastric acid secretion via only partly characterized pathways. The recent identification of the Calcium-sensing receptor, CaSR, in gastric tissue and its localization to parietal cells has raised the question as to its function in these specialized cells and its potential role as a metabolic sensor (10). We have previously shown that the CaSR is functionally active in rat parietal cells and is able to modulate gastric acid secretion via stimulation of H⁺/K⁺-ATPase activity (2, 8). In the present study we have tested if freshly isolated human gastric glands could be used for functional studies using a modification of the techniques that we have developed for rat and mouse isolated gastric glands. Our results demonstrate that freshly isolated human glands expressed both subunits of the gastric H⁺/K⁺-ATPase, secrete acid in response to histamine, and that acid-secretion is sensitive to the specific inhibitor omeprazole.

We also demonstrate that the CaSR is expressed in human gastric parietal cells and functionally active. Stimulation of CaSR by increased concentrations of divalent or trivalent ions led to enhanced proton extrusion via the omeprazole sensitive H⁺/K⁺-ATPase. A reduction of extracellular divalent
cations resulted in a reduction, or in the case of histamine an inactivation of the histamine induced H⁺/K⁺-ATPase activity. Thus, enhanced CaSR activity can modulate H⁺/K⁺-ATPase activity both in the absence and presence of the potent secretagogue histamine. However, it remains to be established, whether the CaSR provides a pathway for stimulation or regulation of gastric acid secretion independent from the classic route via histamine or ACh in vivo. Activation of CaSR was associated with a rise in intracellular Ca²⁺, an event that has been linked to activation of H⁺/K⁺-ATPases. A direct correlation in intracellular Ca²⁺ levels and CaSR mediated regulation of H⁺/K⁺-ATPase activity requires further investigation.

The CaSR has also been shown to be sensitive to changes in extracellular pH and to be allosterically sensitized by L-amino acids shifting the activation curve for divalent cations to the left (5, 6, 18). Both high extracellular Ca²⁺ and L-amino acids have been shown to stimulate gastric acid secretion through only poorly understood mechanisms. We have recently shown that L-amino acids can stimulate gastric H⁺/K⁺-ATPase activity in isolated rat gastric glands by a dual mechanism. At low concentrations this appears to involve the uptake of the amino acids by amino acid transporters whereas at higher concentrations the CaSR appears to be involved (1, 12). Thus under these conditions CaSR could be acting as a metabolic sensor through which several metabolic pathways could modulate gastric acid secretion (11).
In conclusion our data show both the viability of freshly isolated human gastric glands for investigating human gastric acid secretion, and the identification of the CaSR in the parietal cells and its ability to directly modulate acid secretion independently from secretagogues.

Acknowledgements

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FIGURE LEGENDS

Figure 1

Immunolocalization of $\alpha$ and $\beta$ subunits of the gastric H$^+/K^+$-ATPase in human stomach. Samples of human stomach obtained from the antral part were stained with specific antibodies against the $\alpha$ subunit (A,B) and the $\beta$ subunit (C) of the gastric H$^+/K^+$-ATPase. The distribution and localization was specific only to a subset of cells in the neck region of the glands resembling parietal cells as described before (15). (D) High magnification shows localization of the $\beta$H$^+/K^+$-ATPase in tubular intracellular compartments consistent with the described localization of the H$^+/K^+$-ATPase in tubulo-vesicular structures. Magnification 200x (A-C) and 800x (D).

Figure 2. H$^+/K^+$-ATPase activity measurements in freshly isolated human gastric glands. Single human gastric glands were prepared, loaded with the pH-sensitive dye BCECF, and intracellular pH (pH$_i$) measured over single parietal cells. H$^+/K^+$-ATPase was calculated from the omeprazole-sensitive pH$_i$ recovery rate after an acid load using the NH$_4$Cl prepulse. (A) Original pH$_i$ tracing of a control gland. (B) Stimulation of pH$_i$ recovery by incubation of glands with histamine (100 $\mu$M). (C) Inhibition of the histamine stimulated pH$_i$ recovery by the H$^+/K^+$-ATPase inhibitor omeprazole (100 $\mu$M) demonstrates that the histamine stimulated pH$_i$ recovery is mediated by the gastric H$^+/K^+$-ATPase. (D) Bar graph
summarizing the data as mean ± SEM (control: n = 60 cells, 4 glands, 4 patients, histamine: n = 50 cells, 5 glands, 2 patients, histamine + omeprazole: n = 65 cells, 3 glands, 4 patients).

**Figure 3. Localization of CaSR in human stomach.** Human stomach samples obtained from fasted patients were used to localize CaSR. (A) Staining of a subset of cells was observed with an antiserum raised against CaSR (red). (B) Staining against the βH+/K+-ATPase subunit to identify acid-secreting parietal cells (green). (C) Overlay of stainings against CaSR (red) and βH+/K+-ATPase (green) demonstrates expression of both proteins in the same cells. (D, E) High magnification pictures showing that CaSR (red) is localized to a different subcellular compartment than the βH+/K+-ATPase subunit (green) in resting parietal cells. (F, G, H) Overlay of stainings against the βH+/K+-ATPase subunit (green) and using pre-immune serum against CaSR (F), preincubation of the anti-CaSR with the immunizing peptide (G), or omission of the anti-CaSR antibody (all in red) and use of only the secondary antibody which demonstrate that no signal similar to the CaSR could be seen. A-C and F-H 400 x magnification, D 600 x magnification, E 800 x magnification.

**Figure 4. Acid secretion is modulated by CaSR.** (A) A reduction of extracellular cations from 1 mM Ca²⁺ and 1.2 mM Mg²⁺ to 100 µM Ca²⁺ and 0 mM Mg²⁺, respectively, abolished the stimulatory effect of histamine on intracellular
alkalinization ($\text{H}^+/$$\text{K}^+$-ATPase activity) ($n$ = 61 cells, 9 glands, 9 patients). (B) Addition of the divalent cation receptor agonist Gd$^{3+}$ (100 $\mu$M) even in a low cation containing solution induced an increase of the rate of alkalinization both in the presence or absence of histamine ($n$ = 68 cells, 8 glands, 6 patients). (C) Bar graph summarizing the effects of low and high divalent cations and Gd$^{3+}$ on H$^+/$$\text{K}^+$-ATPase activity. * marks significant differences between experimental treatments and control, # marks difference between 1 mM Ca$^{2+}$ plus 100 $\mu$M histamine and 5 mM Ca$^{2+}$ plus 100 $\mu$M histamine.

**Figure 5. CaSR stimulates H$^+/$$\text{K}^+$-ATPase activity and does not require H$_2$ receptors.** Inhibition of H$^+/$$\text{K}^+$-ATPase activity with the specific inhibitor omeprazole (100 $\mu$M) abolished the stimulatory effect of Gd$^{3+}$ on the rate of intracellular pH$_i$ recovery demonstrating that Gd$^{3+}$/CaSR activates H$^+$-extrusion via the H$^+/$$\text{K}^+$-ATPase ($n$ = 58 cells, 6 glands, 4 patients). Blocking of H$_2$ histamine receptors with cimetidine (100 $\mu$M) did not influence the Gd$^{3+}$ stimulated pH recovery ruling out the involvement of histamine in the effect on H$^+/$$\text{K}^+$-ATPase activity ($n$ = 34 cells, 4 glands, 2 patients).

**Figure 6. Activation of the CaSR by Gd$^{3+}$ increases the intracellular calcium concentration.** (A) Original tracing of intracellular calcium concentration [Ca$^{2+}$]$_i$ measurements using FURA-2 in a single parietal cell showing that exposure to Gd$^{3+}$ (100 $\mu$M) led to a sustained increase in the intracellular Ca$^{2+}$ concentration.
(B) Bar graph summarizing intracellular calcium measurements in the absence (48.8 ± 2.3 nM Ca^{2+}) and presence (77.6 ± 4.4 nM Ca^{2+}) of Gd^{3+} (n = 27 cells, 3 glands, 2 patients).
REFERENCES


Figure 2

A. Control

B. Histamine 100 µM, 1mM Ca²⁺

C. 100 µM Histamine, 100 µM Omeprazole

D. ∆pHi/min

Control Histamine 100 µM + Omeprazole 100 µM
Figure 4

A  Histamine 100 µM, 100 µM Ca²⁺, 0 Mg²⁺

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B  Gadolinium 100 µM, 100 µM Ca²⁺, 0 Mg

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C  ∆pH/min

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* p < 0.05
Figure 5

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![Graph showing changes in pH/min for different conditions. The x-axis represents different concentrations and conditions, including 100 µM Ca²⁺, 0 µM Mg²⁺, 100 µM Gd³⁺, 100 µM Omp, and 100 µM Cimetidine. The y-axis represents ΔpH/min, with values ranging from 0.02 to 0.12. The graph shows significant changes in pH/min for different conditions.]
Figure 6

A

100 µM Ca²⁺, 0 Mg

HEPES

Gd³⁺

B

100 µM Ca²⁺, 0 Mg

100 µM Gd³⁺