L-Type amino acids stimulate gastric acid secretion by activation of the calcium-sensing receptor in parietal cells

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Busque, Stephanie M., Jane E. Kerstetter, John P. Geibel, and Karl Insogna. L-Type amino acids stimulate gastric acid secretion by activation of the calcium-sensing receptor in parietal cells. Am J Physiol Gastrointest Liver Physiol 289: G664–G669, 2005. First published June 16, 2005; doi:10.1152/ajpgi.00096.2005.—Parietal cells are the primary acid secretory cells of the stomach. We have previously shown that activation of the calcium-sensing receptor (CaSR) by divalent (Ca2+) or trivalent (Gd3+) ions stimulates acid production in the absence of secretagogues by increasing H+·K+-ATPase activity. When overexpressed in HEK-293 cells, the CaSR can be allosterically activated by l-amino acids in the presence of physiological concentrations of extracellular Ca2+ (Ca2+0; 1.5–2.5 mM). To determine whether the endogenously expressed parietal cell CaSR is allosterically activated by l-amino acids, we examined the effect of the amino acids l-phenylalanine (l-Phe), l-tryptophan, and l-leucine on acid secretion. In ex vivo whole stomach preparations, exposure to l-Phe resulted in gastric luminal pH significantly lower than controls. Studies using d-Phe (inactive isomer) failed to elicit a response on gastric pH. H+·K+-ATPase activity was monitored by measuring the intracellular pH (pHi) of individual parietal cells in isolated rat gastric glands and calculating the rate of H+ extrusion. We demonstrated that increasing Ca2+0 in the absence of secretagogues caused a dose-dependent increase in H+ extrusion. These effects were amplified by the addition of amino acids at various Ca2+ concentrations. Blocking the histamine-2 receptor with cimetidine or inhibiting system l-amino acid transport with 2-amino-2-norbornane-carboxylic acid did not affect the rate of H+ extrusion in the presence of l-Phe. These data support the conclusion that amino acids, in conjunction with a physiological Ca2+ concentration, can induce acid secretion independent of hormonal stimulation via allosteric activation of the stomach CaSR.

stomach; pH; H-K-ATPase; rat; mouse; gastric glands

THE REGULATION OF ACID SECRETION is complex, requiring many receptor-ligand interactions. In the classical pathway of acid secretion, gastrin release is induced from antral G cells, causing a subsequent efflux of histamine from enterochromaffin-like cells. Histamine then binds to its cognate receptor (H2R) on the basolateral membrane of the parietal cell. The resultant cascade of events include a rise in cytosolic Ca2+ (Ca2+0) and cAMP levels, which trigger both chloride and H+ efflux from the apical cell surface via a chloride conductance channel and the H+·K+-ATPase (13, 14, 22, 25, 29). Upon activation by secretagogues (gastrin, histamine, and acetylcholine), the H+·K+-ATPase, located on tubular vesicular elements within the cytosol, traffic to the apical cell surface where the vesicles subsequently fuse with canaliculi along the apical membrane resulting in HCl secretion into the lumen of the gland (11, 13, 22, 25, 29).

The role of nutrients in regulating parietal cell acid production is less well understood. Previous work has suggested that intragastric or intraduodenal administration of l- but not d-amino acids can modulate gastric acid secretion in the absence of classic hormonal intermediaries such as gastrin and histamine in humans and animals (15–17, 23). Intravenous infusion of amino acids stimulated acid secretion before elevated serum gastrin levels were detected; this effect was also observed in human subjects who had undergone a vagotomy (15, 16, 19). Serum amino acid levels in these subjects were comparable to those observed postprandially after a steak meal, suggesting that physiological amino acid concentrations affect acid production in vivo (19).

The calcium-sensing receptor (CaSR) is a member of family C of G protein-coupled receptors and is expressed on many tissues throughout the body, with high expression in gastric parietal cells (3–5, 12, 20). Recent studies have confirmed that the CaSR is also expressed in gastrin-secreting G cells and plays an active role in gastrin release (2, 4, 5). The CaSR functions as a “biosensor” for divalent and trivalent ions, such that small changes in the concentrations of these ions can alter receptor activity. In addition to its biosensor function, it has also been shown that certain antibiotics as well as polyamines can activate the receptor. Conigrave et al. (7, 9) demonstrated that CaSR activity can be regulated by aromatic l-amino acids at substimulatory concentrations of extracellular Ca2+ (Ca2+0), as evidenced by a rise in Ca2+0 concentration, suggesting allosteric modification. In HEK-293 cells overexpressing the CaSR, l-phenylalanine (l-Phe), l-tryptophan (l-Trp), or l-histidine in the presence of physiological Ca2+0 concentrations (1.5–2.5 mM) caused a significant rise in Ca2+0, indicative of receptor activation. The d-isofoms, as well as branched chain amino acids such as l-leucine (l-Leu), had little or no effect (7, 9).

Although the above data establish a mechanism by which amino acids could theoretically modulate parietal cell acid secretion, the ability of amino acids to stimulate gastric acid secretion in parietal cells has not been experimentally determined. Therefore, we sought to determine whether the endogenously expressed CaSR could be allosterically activated by physiological levels of amino acids. In this study, we chose to use phenylalanine and tryptophan (two of the more potent amino acids in regards to CaSR activation), in the presence of various Ca2+0 concentrations, to determine the effects on pari-
etal cell acid secretion. We demonstrate by measuring gastric pH ex vivo in whole stomach preparations and acid secretory rates in parietal cells in the presence of L-Phe or L-Trp and physiological levels of Ca\(^{2+}\) that allosteric activation of the CaSR by amino acids led to enhanced H\(^+\),K\(^{+}\)-ATPase activity independent of the hormonal status of the tissue.

**METHODS**

*Ex vivo whole stomach pH measurements.* Male Sprague-Dawley rats weighing ~200–300 g were housed in climate- and humidity-controlled, light-cycled rooms and fed standard chow with free access to water. Before experimentation, animals were fasted for 18–24 h to reduce basal acid secretion. Animals were killed with an overdose of ether or isoflurane, and an abdominal incision was made. The stomach was ligated at the esophageal and duodenal junctures and excised. With the use of a syringe, 1 ml of nonbuffered, isotonic saline (140 mM) was infused into the lumen of the stomach. This volume of saline did not distend the stomach, thus avoiding potential stimulation of acid secretion by stretch. The preparations were then placed in either oxygenated HEPES-buffered Ringer solution (HEPES) or in the same solution containing 50 mM L- or D-Phe (pH 7.4) and maintained at 37°C. When amino acids were added to the solutions, we adjusted the mannitol concentration and reduced the glucose concentration to 5 mM to maintain a final osmolarity of 290 mosM (see Table 1). All solutions were checked on a freezing point osmometer (Precision Systems; Natick, MA). Decreasing the glucose concentration by half did not adversely affect tissue viability or acid secretion. After a 1-h incubation period, the stomach contents were aspirated, and the pH was recorded. It was necessary to add an additional 1 ml of nonbuffered saline to the stomach aspirate after incubation to ensure sufficient volume to accurately measure pH.

*In vitro pH measurements in parietal cells of freshly isolated gastric glands.* Male Sprague-Dawley rats were killed as described in *Ex vivo whole stomach pH measurements*; stomachs were excised and later transferred to the stage of a dissecting microscope where individual gastric glands were hand-dissected using microforceps and kept on ice until use. Small segments of the stomach mucosa were prepared and later transferred to the perfusion chamber main- 

Table 1. Composition of solutions

<table>
<thead>
<tr>
<th></th>
<th>Na(^{+})-Free HEPES</th>
<th>Na(^{+})-Free HEPES + NH(_4)Cl</th>
<th>High-K(^{+}) Calibration</th>
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<tr>
<td>NaCl</td>
<td>115</td>
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<td>132.8</td>
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<td>NMDG</td>
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<td>KHCl</td>
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<td>1.2</td>
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<tr>
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<td>1</td>
<td>1</td>
</tr>
<tr>
<td>Mannitol</td>
<td>*</td>
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</tr>
<tr>
<td>Glucose</td>
<td></td>
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<td>32.2</td>
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<tr>
<td>pH(37°C)</td>
<td>7.4</td>
<td>7.4</td>
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</tr>
</tbody>
</table>

*Values are in millimolar. The final osmolarity of all solutions was 290 mosM. Glucose concentration was reduced to 5 mM when L-phenylalanine, L-tryptophan, L-leucine, or D-phenylalanine were added to a solution.* *As detailed in METHODS, all solutions were adjusted with mannitol to maintain a constant osmolarity.*

Proton extrusion by individual parietal cells was monitored by observing the recovery of intracellular pH (pH\(_i\)) after cells were acid loaded with a Na\(^{+}\)-free HEPES solution containing 20 mM NH\(_4\)Cl. Parietal cells were subsequently superfused with Na\(^{+}\)-free HEPES, which abolished all Na\(^{+}\)/H\(^{+}\) exchanger activity, trapping H\(^{+}\) within the cytosol and initiating an immediate drop in pH\(_i\). Under these conditions, the only potential H\(^{+}\)-extrusion pathway is via H\(^{+}\),K\(^{+}\)-ATPase activation. pH\(_i\) recovery rates were measured in Na\(^{+}\)-free HEPES solutions containing 1) 30 mM L-Phe plus 0.5, 1, 2, or 5 mM Ca\(^{2+}\); 2) 0.5, 1, 2, or 5 mM Ca\(^{2+}\) only; 3) 30 mM L-Trp, L-Leu, or D-Phe plus 2 mM Ca\(^{2+}\); 4) 3 mM L-Phe and 2 mM Ca\(^{2+}\) with or without 10 mM 2-amino-2-norbornane-carboxylic acid (BCH; Sigma); or 5) 30 mM L-Phe plus 2 mM Ca\(^{2+}\) with or without 100 \mu M cimetidine or omeprazole. As noted, with the addition of amino acid to these solutions, the glucose and mannitol concentrations used in Table 1 were reduced to maintain a constant osmolarity. pH\(_i\) recovery rates were calculated from the same initial starting pH to eliminate potential variations in the individual intracellular buffering power of cells under the different experimental conditions. The recovery rates are expressed as the change in pH (ΔpH) units per minute and were calculated over the pH range of 6.8–7.2.

*Statistical analysis.* An unpaired Student’s *t*-test was used to test for differences in pH of ex vivo whole stomach preparations and to test for significant differences in recovery rates for in vitro parietal cell pH experiments.

**RESULTS**

**L-Phe directly stimulates gastric acid production ex vivo.** To determine whether L-Phe could elicit changes in gastric acid secretion in the whole organ, we examined luminal pH in freshly isolated rat stomachs after incubation in HEPES or in the same solution in the presence of 50 mM L- or D-Phe. As illustrated in Fig. 1, in the presence of 50 mM L-Phe, mean luminal pH was significantly lower than in stomach preparations incubated in HEPES alone (2.69 ± 0.22 vs. 4.02 ± 0.37, *n* = 9 for each, *P* = 0.003). In the presence of 50 mM D-Phe, the inactive isomer, mean luminal pH was 3.49 ± 0.32 (*n* = 6), which was not significantly different from the control group (*P* = 0.13).

*In vitro pH measurements.* To explore the cellular mechanism by which L-Phe stimulates acid secretion, we measured pH\(_i\) in parietal cells of hand-dissected rat gastric glands. As shown in Fig. 2A, in the absence of acid secretagogues but in the presence of 2 mM Ca\(^{2+}\), there was little recovery of pH\(_i\) after parietal cells were exposed to an acid load in a Na\(^{+}\)-free, NH\(_4\)Cl-containing solution, resulting in a fall in pH\(_i\) to ~6.8. As illustrated in Fig. 2B, the addition of 30 mM L-Phe in the presence of 2 mM Ca\(^{2+}\) significantly increased the rate of pH\(_i\) recovery, indicative of H\(^{+}\)-K\(^{+}\)-ATPase activity and H\(^{+}\)-extrusion from the cell. When Ca\(^{2+}\) was reduced to 0.5 mM, as illustrated in Fig. 2C, the robust pH\(_i\) recovery induced by 30 mM L-Phe was completely abolished. Perfusion with 30 mM D-Phe, the inactive isomer, in the presence of 2 mM Ca\(^{2+}\) was reduced to 37°C on the stage of an inverted microscope (Olympus IX50) attached to a digital imaging system (Universal Imaging; Downingtown, PA) and perfused with HEPES to remove any deesterified dye. BCECF was excited at 490 ± 10 and 440 ± 10 nm, and the emitted fluorescence light was measured at 535 ± 10 nm using an intensified charge-coupled device camera (28). Data were collected every 15 s, and the ratio at 490/440 nm was initially recorded as arbitrary pH units, which were then converted to absolute pH using a high-K\(^{+}\)/nigericin calibration technique (26, 28).

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did not elicit pH recovery (Fig. 2D). These data demonstrate the need for a permissive level of Ca\textsuperscript{2+} for amino acids to exert their effect in vitro and that this effect is stereo specific (7, 9).

To more fully explore the interaction between amino acids and Ca\textsuperscript{2+} in parietal cells, studies were conducted in isolated glands exposed to 0.5, 1, 2, or 5 mM Ca\textsuperscript{2+} in the presence or absence of 30 mM L-Phe (Fig. 3). In the presence of physiological Ca\textsuperscript{2+} concentrations (1 or 2 mM) and L-Phe, the rates of H\textsuperscript{+} extrusion were comparable (0.074 ± 0.007 and 0.083 ± 0.005 ΔpH units/min, respectively, n = 32 and 51 cells). Removal of L-Phe and perfusion with 1 or 2 mM Ca\textsuperscript{2+} resulted in a reduction in the H\textsuperscript{+} extrusion rate from 0.074 ± 0.007 to 0.051 ± 0.007 ΔpH units/min and from 0.083 ± 0.005 to 0.041 ± 0.006 ΔpH units/min, respectively. Reducing Ca\textsuperscript{2+} concentration to 0.5 mM completely inhibited H\textsuperscript{+} extrusion (0.020 ± 0.008 ΔpH units/min, n = 16 cells). The addition of L-Phe had only a minimal effect on ΔpH (0.005 ± 0.004 ΔpH units/min, n = 29 cells). Exposing the glands to 5 mM Ca\textsuperscript{2+} alone significantly raised ΔpH (0.159 ± 0.02 ΔpH units/min, n = 32 cells). The ability of amino acids to augment acid secretion at sub-maximal Ca\textsuperscript{2+} concentrations is highly suggestive of allosteric activation.

To confirm and extend our findings with L-Phe, we tested additional amino acids that are known to either strongly activate or elicit weak modulatory effects on the CaSR (7–9). As shown in Fig. 4A, exposing parietal cells to 30 mM L-Trp, an amino acid known to activate the CaSR, resulted in rates of H\textsuperscript{+} extrusion by 10.220.33.2 on June 24, 2017 http://ajpgi.physiology.org/ Downloaded from
extrusion very similar to those observed with 30 mM L-Phe (0.082 ± 0.013 vs. 0.083 ± 0.005 ΔpH units/min). Perfusion with a weak activator, L-Leu, or the inactive isomer, D-Phe, resulted in pHi recovery rates significantly lower than those observed with L-Phe (0.024 ± 0.006 and 0.015 ± 0.003 vs. 0.083 ± 0.005 ΔpH units/min, respectively, $P < 0.0001$). These results also suggest allosteric activation of the CaSR by amino acids, because the profile for allosteric activation was identical in rank order potency to that shown by Conigrave et al. (7–9) in HEK-293 cells overexpressing the CaSR.

To confirm that L-Phe was acting via the CaSR and not via the system L-amino acid transporter, we used BCH, a known inhibitor of the L-amino acid transport system (6, 18, 24, 27). For these experiments, we used 3 mM L-Phe and 2 mM Ca$_{2+}^+$ in the presence or absence of 10 mM BCH, a concentration that competitively blocks the L-amino acid transport system. The mean H$^+$ extrusion rate in the presence of BCH was not different from the rate in the absence of BCH (0.059 ± 0.004 vs. 0.062 ± 0.006, $n = 33$ and 30 cells, respectively; see Fig. 4B).

Figure 5 summarizes mean H$^+$ extrusion rates in the presence of 30 mM L-Phe and either cimetidine or omeprazole. In the presence of 100 μM cimetidine, a H2R antagonist, the H$^+$ extrusion rate induced by 30 mM L-Phe was comparable with that seen with L-Phe alone (0.078 ± 0.007 vs. 0.083 ± 0.005 ΔpH units/min, $n = 30$ and 51, respectively). In a separate series of experiments, cells were perfused with 100 μM omeprazole in the presence of 30 mM L-Phe and 2 mM Ca$_{2+}^+$. The rate of H$^+$ extrusion was significantly reduced from 0.083 ± 0.005 to 0.03 ± 0.006 ΔpH units/min ($P < 0.0001$), confirming the role of the H$^+$-K$^+$-ATPase in this H$^+$ extrusion pathway. These data clearly demonstrate that L-Phe-induced H$^+$ extrusion from parietal cells occurs via H$^+$-K$^+$-ATPase activation, which is histamine independent and omeprazole sensitive.

**Fig. 3.** Ca$_{2+}^+$ dose-dependent effects on L-Phe-stimulated H$^+$ extrusion. Mean pH$^+$ recovery rates (y-axis) were calculated in Na$^+$-free HEPES solutions containing 0.5, 1, 2, or 5 mM Ca$_{2+}^+$ in the presence of absence of 30 mM L-Phe. At 1 and 2 mM Ca$_{2+}^+$, H$^+$ extrusion rates were comparable to each other (b and c, open bars; $n = 16$ and 25 cells, respectively). The addition of 30 mM L-Phe significantly increased pH$^+$ recovery rates under these conditions (b and c, solid bars; $n = 32$ and 51 cells, respectively). Reducing Ca$_{2+}^+$ to 0.5 mM resulted in a decrease in pH$^+$, and the addition of L-Phe did not significantly increase the H$^+$ extrusion rate at this low Ca$_{2+}^+$ concentration (a, open and solid bars; $n = 16$ and 29 cells, respectively). Perfusion with 5 mM Ca$_{2+}^+$ resulted in H$^+$ extrusion rates comparable with that seen with L-Phe plus 2 mM Ca$_{2+}^+$ (d, open bar, vs. c, solid bar), whereas the addition of L-Phe in the presence of 5 mM Ca$_{2+}^+$ resulted in a twofold increase in H$^+$ extrusion compared with 5 mM Ca$_{2+}^+$ alone (d, open vs. solid bars; $n = 17$ and 32 cells, respectively).

**Fig. 4.** Calcium-sensing receptor (CaSR)-mediated H$^+$ extrusion is amino acid and isomer specific. A: mean pH$^+$ recovery rates (y-axis) were calculated in Na$^+$-free HEPES solutions containing 2 mM Ca$_{2+}^+$ and the indicated amino acids all at 30 mM. Perfusion either of the aromatic amino acids L-Phe or L-tryptophan (L-Trp) resulted in comparable H$^+$ extrusion rates. Perfusion with weak activators, D-Phe or L-leucine (L-Leu), gave pH$^+$ recovery rates that were substantially lower than those of L-Phe or L-Trp ($n = 17$ or 58 cells, respectively, $^*P < 0.0001$). B: mean pH$^+$ recovery rates (y-axis) in the presence of 2 mM Ca$_{2+}^+$ and 3 mM L-Phe. Blocking system L-amino acid transport with 10 mM 2-amino-2-norbornane-carboxylic acid (BCH), a competitive inhibitor of the system L-amino acid transporter, did not effect H$^+$ extrusion rates induced by 2 mM Ca$_{2+}^+$ and 3 mM L-Phe ($n = 30$ and 33 cells, respectively).

**DISCUSSION**

Experimental work in animals and humans has established that amino acids can stimulate gastric acid secretion (15–17, 19, 23). However, the molecular mechanism(s) by which amino acids elicit this effect has not yet been fully elucidated. The CaSR is expressed in parietal cells of gastric glands and can be activated by divalent and trivalent cations to induce acid secretion in isolated rat gastric glands (3–5, 12, 20). The CaSR is also expressed in gastric G cells, where activation of the receptor results in gastrin release, ultimately stimulating gastric acid production (2, 4, 5).

The endogenous ligands for the CaSR in the stomach are not known. Our data strongly suggest that exposure to amino acids at physiological Ca$_{2+}^+$ concentrations enhance the rate of H$^+$ extrusion in a manner consistent with allosteric activation of...
the CaSR. Previous work in HEK-293 cells stably overexpressing the CaSR demonstrated that L-amino acids augmented the rise in Ca\(^{2+}\) observed in the presence of 1.5-2.5 mM Ca\(^{2+}\), supporting the notion that amino acids are allosteric activators of this receptor (7, 9). Our findings in parietal cells endogenously expressing the CaSR are entirely consistent with the same conclusion, namely, that CaSR-dependent acid secretion occurs via allosteric activation by L-amino acids.

Our group has recently identified a functional system L-heteromeric amino acid transporter comprised of the LAT2-4F2hc dimer in mouse parietal cells (J. P. Geibel, unpublished observations). We therefore felt it was important to exclude the possibility that amino acids were exerting their effect on H\(^+\) extrusion independent of CaSR activation, namely, via system L-amino acid transport. However, we did not observe an inhibitory effect of BCH on L-Phe-induced acid extrusion, excluding a contribution of this transporter to the enhanced H\(^+\) extrusion observed in the presence of amino acids.

Our data strongly support the conclusion that L-amino acids, specifically L-Phe and L-Trp, can regulate gastric acid secretion. We chose these particular amino acids because they have been shown to be potent allosteric activators of the CaSR in HEK-293 cells transfected with CaSR (7, 9). In our initial studies, we measured luminal pH in freshly isolated whole stomach preparations bathed in HEPES or the same solution with 50 mM L- or D-Phe. A concentration of 50 mM L-Phe was used to ensure that a sufficient amount of amino acids would diffuse across the stomach serosa to reach the basolateral aspect of the parietal cells, emulating the postprandial rise in serum amino acids (1, 10). We observed a significant decrease in pH of the gastric aspirate from preparations bathed in L-Phe compared with those in buffer alone. Incubation with 50 mM D-Phe, however, did not induce acid secretion.

To explore the cellular mechanism for the effect of amino acids on acid secretion observed in the above experiments, we used freshly isolated gastric gland preparations. Although, as noted, the CaSR is expressed in antral G cells of the gastric gland, we tested our hypothesis in parietal cells, the primary acid secretory cells of the stomach (2, 4, 5). In our experimental system, CaSR-mediated activation of the H\(^+\)-K\(^+\)-ATPase and thus H\(^+\) extrusion is observed as a rise in pHi (net proton extrusion from cell to lumen of the gland). In the absence of acid secretagogues, administration of 30 mM L-Phe or 30 mM L-Trp in the presence of 2 mM Ca\(^{2+}\) in the gastric gland perfusate produced a significant increase in pHi and the rate of H\(^+\) extrusion from acid-loaded parietal cells (Figs. 3c, solid bar, and 4). Reducing the Ca\(^{2+}\) concentration to 1 mM in the continued presence of L-Phe had no effect on H\(^+\) extrusion (Fig. 3, b vs. c, solid bars). Further decreasing Ca\(^{2+}\) to 0.5 mM reduced H\(^+\) extrusion to a rate significantly lower than that observed with either 1 or 2 mM Ca\(^{2+}\) alone (Fig. 3, a, solid bar, vs. b and c, open bars). The rate of H\(^+\) extrusion with 0.5 mM Ca\(^{2+}\) plus 30 mM L-Phe was significantly lower than the rate observed with either 1 or 2 mM Ca\(^{2+}\) plus 30 mM L-Phe (Fig. 3, a vs. b and c, solid bars). These data demonstrate a dependence of amino acid-induced acid secretion on L-amino acids. The finding that use of an inactive isomer, D-Phe, was devoid of any activity supports the specificity of this amino acid effect.

The molecular details of how amino acids interact with the CaSR are not known. Recent evidence indicates that the CaSR’s “Venus fly trap” domain is required for L-amino acid sensing by the CaSR, although it has not yet been directly determined that amino acids bind there (21). Nonetheless, the fact that we observed additivity of Ca\(^{2+}\) and amino acids even at high concentrations of Ca\(^{2+}\) is consistent with the idea of separate binding sites for calcium and amino acids (7, 9, 21). The possibility of two separate binding sites in no way precludes an allosteric interaction of amino acids and Ca\(^{2+}\) in activating this receptor. The enhanced rates of acid secretion at submaximal Ca\(^{2+}\) (Fig. 3), the parallel potencies to those observed by Conigrave et al. (Fig. 4A), and the absolute requirement for a permissive concentration of Ca\(^{2+}\) (Fig. 3) are all consistent with this idea.

To exclude any hormonal contribution to the effects observed with L-Phe, all in vitro experiments were performed in the absence of known acid secretagogues such as histamine and/or gastrin. Consistent with the conclusion that L-Phe is acting via a hormone-independent pathway, inhibiting the H2R with cimetidine did not affect the rate of H\(^+\) extrusion induced by 30 mM L-Phe and 2 mM Ca\(^{2+}\) (see Fig. 5). The fact that omeprazole blocked the effect of amino acids on H\(^+\) extrusion shows that the amino acid effect on the CaSR works via the H\(^+\)-K\(^+\)-ATPase (see Fig. 5).

Existence of a novel acid secretory pathway initiated by physiological levels of amino acids and Ca\(^{2+}\) illustrates the complexities of amino acid-stimulated acid secretion by parietal cells. It is apparent that no single pathway is solely responsible for acid secretion by the stomach. Inhibiting CaSR activity specifically or studying animals with conditional deletion of the receptor in the parietal cells may help to elucidate the importance of this pathway in acid secretion. The in vivo role of amino acids in modulating acid secretion may combine indirect effects of CaSR-mediated G cell gastrin release and direct effects on parietal cell CaSR acid secretion. This could ultimately explain why some individuals who consume high-protein diets experience symptoms of acid reflux, and, more importantly, why some patients experience breakthrough acid secretion despite acid suppression therapy with H2R antago-
nists. In light of these findings, clarifying the role of nutrient sensing by the CaSR may help to identify new treatments for acid reflux disease through dietary manipulation of CaSR activity.

GRANTS

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