EPITHELIAL TRANSPORT

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An amino acid transporter involved in gastric acid secretion

Abstract Gastric acid secretion is regulated by a variety of stimuli, in particular histamine and acetyl choline. In addition, dietary factors such as the acute intake of a protein-rich diet and the subsequent increase in serum amino acids can stimulate gastric acid secretion only through partially characterized pathways. Recently, we described in mouse stomach parietal cells the expression of the system L heteromeric amino acid transporter comprised of the LAT2-4F2hc dimer. Here we address the potential role of the system L amino acid transporter in gastric acid secretion by parietal cells in freshly isolated rat gastric glands. RT-PCR, western blotting and immunohistochemistry confirmed the expression of 4F2-LAT2 amino acid transporters in rat parietal cells. In addition, mRNA was detected for the B^0AT1, ASCT2, and $ATB(0+)$ amino acid transporters. Intracellular pH measurements in parietal cells showed histamine-induced and omeprazole-sensitive H^+-extrusion which was enhanced by about 50% in the presence of glutamine or cysteine (1 mM), two substrates of system L amino acid transporters. BCH, a non-metabolizable substrate and a competitive inhibitor of system L amino acid transport, abolished the stimulation of acid secretion by glutamine or cysteine suggesting that this stimulation required the uptake of amino acids by system L. In the absence of histamine glutamine also stimulated H^+-extrusion, whereas glutamate did not. Also, phenylalanine was effective in stimulating H^+/K^+-ATPase activity. Glutamine did not increase intracellular Ca^{2+} levels indicating that it did not act via the recently described amino acid modulated Ca^{2+}-sensing receptor. These data suggest a novel role for heterodimeric amino acid transporters and may elucidate a pathway by which protein-rich diets stimulate gastric acid secretion.

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Introduction

Gastric acid secretion is a tightly regulated process of H^+-extrusion by the gastric H^+/K^+-ATPase localized in parietal cells. Classically, two stimulatory pathways have been defined: (1) a neuronal via the vagus nerve through release of acetyl choline and (2) a biphasic endocrine pathway [21, 26, 32]. The endocrine pathway depends on the release of gastrin from antral G cells leading to the stimulation of histamine-containing enterochromaffin-like (ECL) cells [21, 26, 32]. ECL cells then release histamine, which in turn initiates the direct insertion and activation of H^+/K^+-ATPases into the apical membrane of parietal cells and subsequent acid secretion mainly via a cAMP and PKA-dependent pathway. The exposure to histamine, however, also causes a simultaneous rise in intracellular Ca^{2+} in parietal cells [5]. This elevation in intracellular Ca^{2+} has been associated with increased acid secretion. In addition to these classical pathways, several other pathways are able to stimulate or enhance gastric acid secretion. Activation of the divalent cation receptor, CaSR or SCAR, has recently been described to stimulate gastric acid secretion independent of other pathways [9, 10]. A protein-rich meal, and the subsequent rise in blood

amino acid levels, is another stimulus known to increase gastric acid secretion [11, 13, 15, 19]. This stimulatory effect does not increase serum gastrin levels and is fully operative even after vagotomy [13, 15]. These results imply that amino acids may have direct effects on parietal cells and may modulate gastric acid secretion through hitherto unrecognized mechanisms.

Recently, we have described that in mouse stomach, several amino acid transporter subunits are expressed on mRNA and protein level [8]. All these subunits belong to the novel family of heteromeric amino acid transporters. The structural and functional hallmarks of these transporters are that they consist of two subunits, a light chain and a heavy chain, and that these transporters mostly obey an antiport mechanism leading to the exchange of intracellular against extracellular amino acids [6, 28–30]. Thus, the activity of these transporters does not lead to the accumulation of amino acids but rather to a widening of the range of transported amino acids and equilibration of intra- and extracellular amino acids. The main subunits identified in mouse stomach were the heavy chain 4F2hc (also named CD98, SLC3A2) and the associated heavy chain LAT2 (SLC7A8) which together form one isoform of the system L transport activity. The activity of system L has been characterized as the Na^+-independent transport of large neutral amino acids in exchange for other intracellular neutral amino acids [17]. Pharmacologically, system L amino acid transport activity can be competitively inhibited by BCH [6, 14, 20, 30] which itself is a transport substrate but cannot be metabolized. The localization of 4F2hc and LAT2 in mouse stomach was restricted to the acid secretory parietal cells in agreement with earlier reports describing system L-like amino acid transport activity in these cells [24].

In order to address the role of system L amino acid transport in parietal cells, we examined the influence of amino acids transported by the system L on the main function of parietal cells, the extrusion of H^+ by the H^+/K^+-ATPase. Similar to mouse stomach, system L amino acid transporter subunits were identified in rat stomach parietal cells. Addition of glutamine or cysteine, substrates of system L, stimulated H^+-extrusion by the gastric H^+/K-ATPase in freshly isolated gastric glands as confirmed by omeprazole sensitivity. The stimulation was prevented by BCH, a known competitive inhibitor of this transport system. These results suggest that system L amino acid transporter may be involved in modulating gastric acid secretion after a protein-rich meal and may represent part of a sensing mechanism for amino acid concentrations in blood.

Material and methods

Animals

Wistar Rats 250–300 g (Charles River) were housed in climate- and humidity-controlled, light-cycled rooms, fed standard chow with free access to water, and handled according to the humane practices of animal care established by the Yale Animal Care and Use Committee and the Swiss Kantonales Veterinäramt, Zürich. Prior to experiments, animals were fasted for 12 h with free access to water.

RNA extraction and RT-PCR

Male Wistar rats were killed, and stomach, kidney, and brain were collected. Stomach mucosa was scraped off on ice and all the samples were rapidly frozen. Total mRNA was extracted from 30 mg of tissue using the RNA Aqueous 4PCR kit (Ambion) according to the manufacturer's instruction. For RNA extraction, tissue was thawed in RNALater solution (Ambion), transferred to lysis buffer, and homogenized on ice with an Elvehjem potter. RNA was bound on columns and treated with DNAse for 15 min at 30°C temperature to reduce genomic DNA contamination. The quantity and purity of the total eluted RNA were assessed by spectrometry and on agarose gels. Each RNA sample was diluted to 200 ng/μl and 1 μl used as a template for reverse transcription using the Taqman reverse transcription kit (Applied Biosystems, USA).

For reverse transcription, 200 ng RNA template was diluted in a 20-μl reaction mix that contained (final concentrations): RT buffer (1x), $MgCl_2$ (5.5 mM), random hexamers (2.5 μM), RNAse inhibitor (0.4 U/μl), the multiscribe reverse transcriptase enzyme (1.25 U/μl), deoxyNTP mix (500 μM each) and RNAse-free water.

PCR primers were designed using the primer express program to yield amplicons between 50 and 450 bps (Table 1). Taqman universal PCR Mastermix (2× concentrated) was used (Applied Biosystems 4304437) containing AmpliTaq Gold DNA Polymerase, dNTPs with dUTP and optimized buffer components. One microlitre cDNA (200 ng), 1.4 μl primers (each 25 μM), and 10 μl mastermix were mixed with water to a total reaction volume of 20 μl. PCR products were mixed with 10× Bromophenol blue dye and loaded on a 4% ethidium bromide/agarose gel and bands were visualized under UV light.

Membrane preparation and western blot analysis

After removing the kidneys, brain, and stomach from Wistar rats, gastric mucosa was scraped off and then suspended in an ice-cold K-HEPES buffer (200 mM mannitol, 80 mM K-HEPES, 41 mM KOH, pH 7.5) with pepstatin, leupeptin, K-EDTA, and phenylmethylsulfonyl fluoride (PMSF) added as protease inhibitors. To obtain crude membrane fractions, the kidneys, brain, and gastric mucosa were homogenized on ice and centrifuged at 1,000 g for 10 min; the supernatant was collected and centrifuged at 100,000 g for 60 min at 4°C. The pellets containing the plasma membranes were resuspended in a homogenization buffer and stored at

Table 1 Primers used to amplify the 4F2hc, LAT1, LAT2, B°AT1, ASCT2, and ATB(0+) gene products from total RNA isolated from rat stomach mucosa, kidney, and brain

Gen	Forward primer	Reverse primer	Accession number/expected size
4F2hc (SLC3A2)	5'-GTATTTGAATGCCACTGGCA (1141–1160)	5'-AAAGGATGTCAGGAGTCCTGC (1190–1210)	AB015433 68 bp
LAT1 (SLC7A5)	5'-CCTGCTCGGCTTCATCCAGAT (702–722)	5'-GGACAACTTCGGTGCAGGTT (754–774)	AB015432 73 bp
LAT2 (SLC7A8)	5'-TCCACATTTGGTGGAGTCAA (1576–1595)	5'-TGGATCATGGCTAACACG CT (1657–1676)	AB024400 111 bp
ASCT2 (SLC1A5)	5'-GCCTGATCGGAGGTGCAGCC (457–476)	5'-CGGGTAAAGAGGAAGTAGATG (1085–1106)	AJ132846 649 bp
B0AT1 (SLC6A19)	5'-CCCAACATCTTCTGGCAAGT (1174–1193)	5'-CAGGGTACTCCAGCCACAAT (1360–1379)	ENSRNOG00000026501 205 bp
ATB(0+) (SLC6A14)	5'-ATCCGGAAGCACTAGCTCAA (1426–1445)	5'-GAACCCTCATGCGTTTCATT (1568–1587)	XM_233305 161 bp

−80°C until use. Fifty microgram of membrane proteins was solubilized in a Laemmli sample loading buffer and separated on an 8% SDS polyacrylamide gel. The proteins were transferred to PVDF membranes, with unspecific protein binding blocked by PBS containing 5% non-fat dry milk. The membranes were incubated with either a polyclonal rabbit anti-LAT1 (1:5,000) (friendly gift of N. Thompson, Brown University, RI, USA [3], rabbit anti-LAT2 antibody (1:5,000) [8, 20] or goat anti-4F2hc (Santa Cruz Biotech, 200 μg/ml, 1:1,000 [8]) for 2 h at room temperature. The membranes were then washed three times, blocked for 1 h and again incubated for 1 h at room temperature with the secondary goat anti-rabbit or donkey anti-goat antibodies 1:5,000 linked to alkaline phosphatase (Promega). The protein signal was detected with the CDP Star chemilumescensce system (Roche Diagnostics).

Immunohistochemistry

Male Wistar rats (200–250 g) were anesthetized with ketamine/xyalzine i.p. and perfused through the left ventricle with PBS followed by paraformaldehyde–lysine–periodate (PLP) fixative [16]. The stomachs were removed, cleaned from food residues, and fixed overnight at 4°C by immersion in PLP. Stomachs were then washed three times with PBS and the sections were cut at a thickness of 5 μm after cryoprotection with 2.3 M in PBS for at least 12 h. Immunostaining was carried out as described previously [12]. Sections were incubated with 1% SDS for 5 min, washed three 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-human β gastric H^+/K^+-ATPase (Affinity Bioreagents, CA, USA) and rabbit anti-LAT1 (affinity purified, friendly gift of N. Thompson, Brown University, RI, USA [3]), rabbit anti-mouse LAT2 [20] and goat anti-4F2hc (Santa Cruz Biotech, 200 μg/ml [8]) were diluted 1:50, 1:200 or 1:400, respectively, in PBS and applied overnight at 4°C. 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, donkey–anti-goat Alexa 488 or donkey–anti-mouse Alexa 488, Molecular Probes, Oregon) at a dilution of 1:1,000 and 1:200, respectively, for 1 h at room temperature. Sections were then washed twice with high NaCl PBS and once with PBS before mounting with VectaMount (Vector laboratories, Burlingame, CA). The specimens were viewed with a Nikon E-800 microscope or a Leica SP1 UV CLSM confocal microscope.

Isolation of gastric glands

The stomach was opened longitudinally after removal and the corpus and antrum were isolated and sliced into

Table 2 Composition of solutions used for intracellular pH measurements in single parietal cells in freshly isolated rat gastric glands

All concentrations are given in mM. NMDG is N-methyl-D-glucamine, all solutions were titrated to pH 7.4 at 37°C using either NaOH or KOH. NMDG was titrated with HCl

	Standard HEPES	Na$^+$-free HEPES	Na$^+$-free HEPES + NH$_4$Cl	High K$^+$ calibration
NaCl	125	–	–	–
NMDG	–	125	105	32.8
NH$_4$Cl	–	–	20	–
KCl	3	3	3	105
MgSO$_4$	1.2	1.2	1.2	1.2
CaCl$_2$	1	1	1	1
KH$_2$PO$_4$	2	2	2	–
Glucose	5	5	5	–
HEPES	32.2	32.2	32.2	32.2
pH	7.4	7.4	7.4	6.5, 7.0

0.5 cm^2 sections, and washed with cold Ringer solution to remove residual food particles. The tissues were transferred to the stage of a dissecting microscope. Individual glands were isolated using a hand-dissection technique as described previously [12]. Following isolation, individual isolated glands were either allowed to adhere to cover slips that had been pre-coated with Cell-Tak (Collaborative Research, Bedford, MA) and were transferred to the stage of an inverted microscope.

Digital imaging for intracellular pH and Ca^{2+} measurements

Coverslips with isolated gastric glands were transferred to a thermostatically controlled perfusion chamber ($\approx$3 ml/min flow rate), maintained at 37°C on an inverted microscope (Zeiss Axiovert 200), and equipped with a video imaging system (Visitron, Munich, Germany) for the duration of the experiment. Isolated gastric glands were incubated in a HEPES-buffered Ringer solution containing either 10 μM of the pH-sensitive dye BCECF-AM (2′,7′)-bis-(2-carboxyethyl)-5-(and-6)-carboxy-fluorescin, aceto-methyl ester or 10 mM of the Ca^{2+}-sensitive dye Fluo-4 (Molecular Probes, Eugene, OR) for 10 min as described previously [12] . Following loading, the chamber was flushed with a HEPES buffered Ringer solution to remove all non-deesterfied dye. BCECF and Fluo-4 were successively excited at 440 ± 10 nm and 490 ± 10 nm for BCECF and at 488 ± 10 nm, and the resultant fluorescent signal was monitored at 535 ± 10 nm and 520 ± 10 nm, respectively, using an intensified CCD camera. Individual regions of interest were outlined and simultaneously monitored during the course of the study. A minimum of seven cells or regions was selected per gland. All data including the individual images for all wavelengths were recorded to the hard disk which allowed us to return to the individual images after the experiment for further analysis.

Intensity ratio data of 490/440 were converted to pH values by using the high K$^+$/nigericin calibration technique [25]. Data are expressed as ΔpH/min, percentages were calculated using Gauss' law of error propagation, and tested for significance using the Student's t test.

All the experiments were performed in the nominal absence of bicarbonate. The initial solution was a HEPES-buffered Ringer solution (125 mM NaCl, 3 mM KCl, 1 mM CaCl$_2$, 1.2 mM MgSO$_4$, 2 mM KH$_2$PO$_4$, 32.2 mM HEPES, pH 7.4). Cells were acidified by using the NH$_4$Cl (20 mM) prepulse technique and washed into a Na$^+$-free solution (Na$^+$ was replaced by equimolar concentrations of N-methyl-D-glucamine) as described previously [12]. The composition of all solutions used is given in Table 2.

All chemicals were obtained from Sigma Chemicals; Omeprazole was a kind gift of Dr. K. Andersson AstraZeneca, Mölndal, Sweden.

Results

Expression of system L amino acid transporters in rat stomach

RT-PCR was used to test for the presence of mRNA encoding subunits of system L heteromeric amino acid transporters in rat stomach mucosa. Total RNA was extracted from rat stomach mucosa, kidney, and brain. The kidney and brain were included as positive controls as these organs are known to express several subunits of system L heteromeric amino acid transporters [8, 30]. RT-PCR products of the expected size were found for all three known subunits, the 4F2hc heavy chain and the LAT1 and LAT2 light chains in all three organs were tested (Fig. 1a). In addition, we tested for the presence of other amino acid transporters capable of transporting amino acids with similar substrates as system L such as ASCT2 (SLC1A5), B^{0}AT1 (SLC6A19), and ATB(0+) (SLC6A14) [1]. mRNA for all three transporters was detected in rat stomach. The presence of mRNAs of 4F2hc, LAT1, LAT2, and ATB(0,+) in gastric mucosa has been described similarly in mouse or human stomach tissue [8, 23].

Western blotting with rat brain, kidney, and stomach mucosa confirmed the expression of 4F2hc and LAT2 protein in rat stomach mucosa yielding again bands of the expected size (Fig. 1b). These results demonstrated the expression of the subunits 4F2hc and LAT2 subunits

forming the system L heteromeric amino acid transporter in rat stomach mucosa.

Localization of system L amino acid transporters in rat parietal cells

Immunohistochemistry confirmed the presence of 4F2hc and LAT2 in rat stomach, whereas no specific signal for LAT1 could be detected (Fig. 2). The localization of 4F2hc and LAT2 was restricted to a subset of cells and to the basolateral side of these cells. Double-labelling for LAT2 and the gastric H^+/K^+-ATPase β subunit showed clearly that LAT2 is expressed together with the H^+-extruding ATPase in parietal cells as described previously in mouse stomach [8].

Acid extrusion is increased by system L amino acid transporter substrates

Freshly isolated rat gastric glands were used for intracellular pH measurements to assess the activity of the gastric H^+/K^+-ATPase as the rate of intracellular alkalinization ($\Delta pH/min$) in the absence of bicarbonate and Na^+ after an acid load (20 mM NH_4Cl prepulse) as described previously [31]. Glands were already preincubated for 10 min with histamine (100 µM) prior to the measurements to stimulate H^+/K^+-ATPase activity which resulted in an alkalinization rate of

0.023 ± 0.001 units pH/min (Fig. 3a, c, Table 3, $n = 33$ cells, five glands, four animals). Histamine was present in all solutions during the experiments.

Addition of 1 mM glutamine or 1 mM cysteine to all solutions (including the 10 min preincubation with histamine) resulted in an increase of the alkalinization rate by about 100–200% (0.039 ± 0.003 U pH/min for glutamine and 0.068 ± 0.006 U pH/min for cysteine, respectively).

Gastric glands were stimulated with histamine and incubated with omeprazole, a specific inhibitor of the H^+-extruding H^+/K^+-ATPase, to confirm that glutamine increased the rate of H^+-extrusion via this pump. Omeprazole reduced the rate of pH_i recovery to 0.002 ± 0.002 U pH/min demonstrating that glutamine stimulated H^+/K^+-ATPase activity (Fig. 4, $n = 37$ cells, six glands, four animals).

Thus, these results suggested that substrates of the system L amino acid transporter can modulate histamine-stimulated gastric H^+/K^+-ATPase activity and enhance proton excretion.

Glutamine or phenylalanine alone stimulate H^+/K^+-ATPase activity

Stimulation of H^+/K^+-ATPase activity was also observed in the presence of glutamine (1 mM) even without prior activation of H^+-extrusion by histamine (Fig. 5). Control glands without exposure to histamine
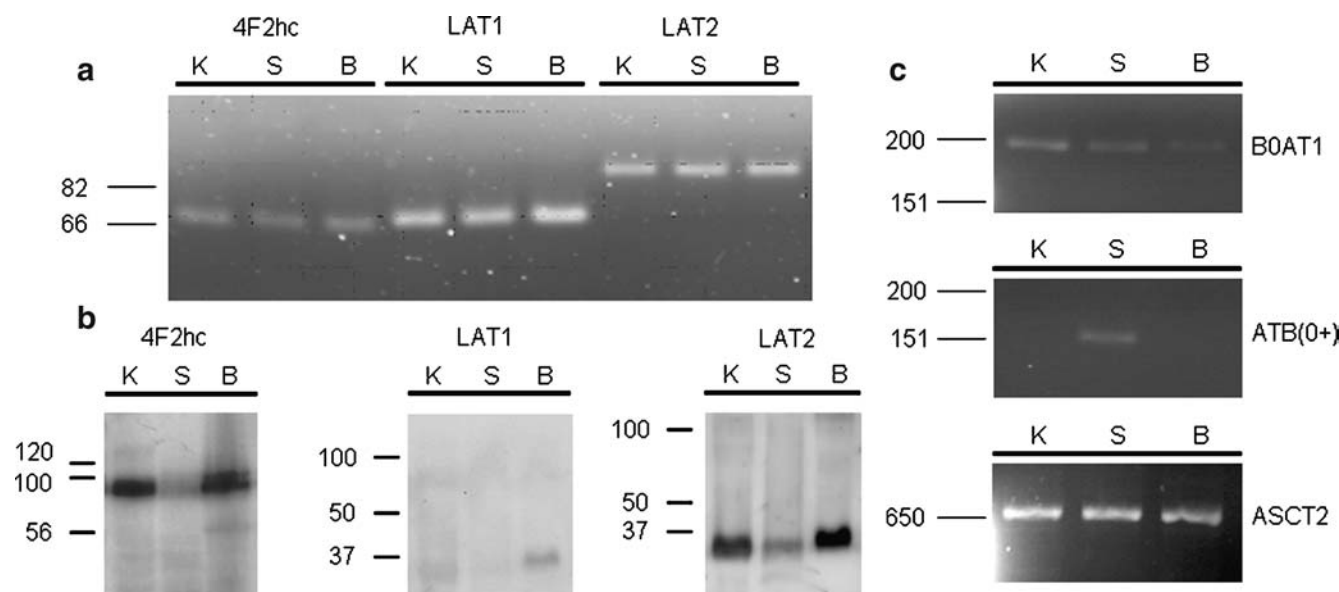

Fig. 1 Expression of system L amino acid transporter subunits in rat stomach. a mRNA was isolated from rat kidney (K), stomach mucosa (S), and brain (B), and RT-PCR was performed for the three subunits 4F2hc, LAT1, and LAT2. Only single PCR products were found for each gene in all three organs. b Western blotting of crude membranes (50 µg protein/lane) prepared from rat kidney (K), stomach mucosa (S), and brain (B). Only 4F2hc and LAT2 could be detected in stomach whereas LAT1 was only observed in rat brain. The weak bands detected in kidney and stomach are probably not LAT1 and unspecific. c PCR products for the B⁰AT1, ATB(0+), and ASCT2 transporters in kidney, stomach, and brain. mRNA for all three transporters was detected in stomach. B⁰AT1 and ASCT2 were also present in brain and kidney, whereas ATB(0+) was not

Fig. 2 Localization of system L subunits 4F2 and LAT2 in rat gastric parietal cells. **a–c** Localization of 4F2hc (*green*) (**a**) and LAT2 (*red*) (**b**) in rat stomach gastric glands. (**c**) overlay of 4F2hc and LAT2. Original magnification 200×. **d, e** High magnification (400× (**d**), 600× (**e**)) of single parietal cells showing the colocalization of LAT2 (*red*) and the β subunit of the H^+/K^+-ATPase (*green*) in the same cells. LAT2 localizes to the basolateral membrane, whereas the H^+/K^+-ATPase subunit is seen in intracellular vesicles and tubulovesicular structures as previously described. Stomachs were taken from fasted animals
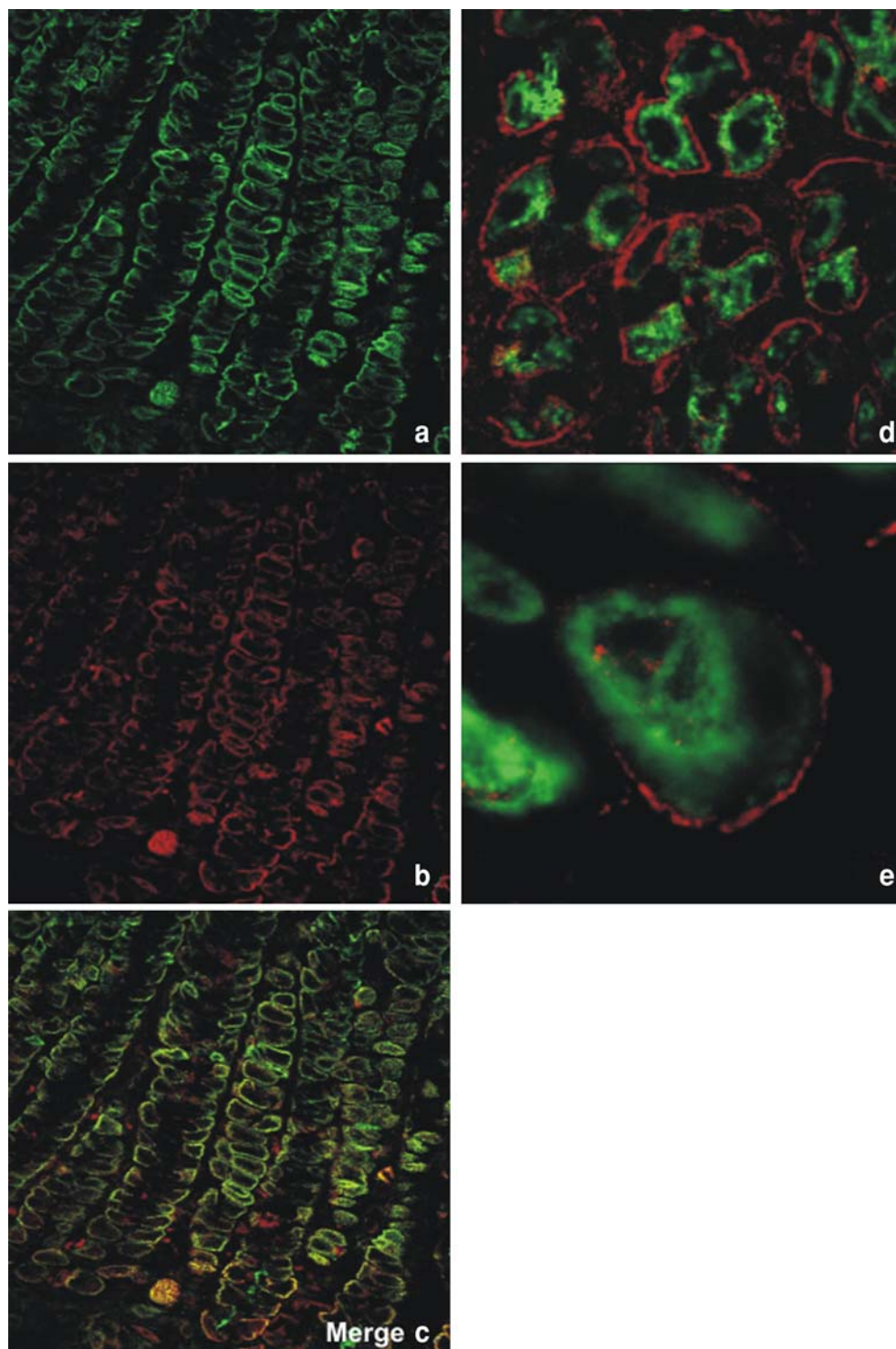
or glutamine showed an intracellular alkalinization rate of 0.007 ± 0.001 U pH/min, whereas an addition of glutamine increased this rate to 0.052 ± 0.005 U pH/min. Also L-phenylalanine, another substrate of system L, increased the intracellular alkalinization rate to 0.066 ± 0.003 U pH/min. (Fig. 5, Table 3). In contrast, addition of 1 mM L-glutamate which is not transported by system L to all solutions had no effect on H^+/K^+-ATPase activity (Fig. 5, Table 3). It has been previously suggested that amino acids stimulate gastric acid secretion by releasing histamine from neighbouring ECL cells and thus activate parietal cells. To test for glutamine-induced histamine-mediated stimulation of H^+/K^+-ATPase activity, gastric glands were preincubated for 10 min with the H_2-receptor antagonist cimetidine (100 μM) and all the experiments were performed in the
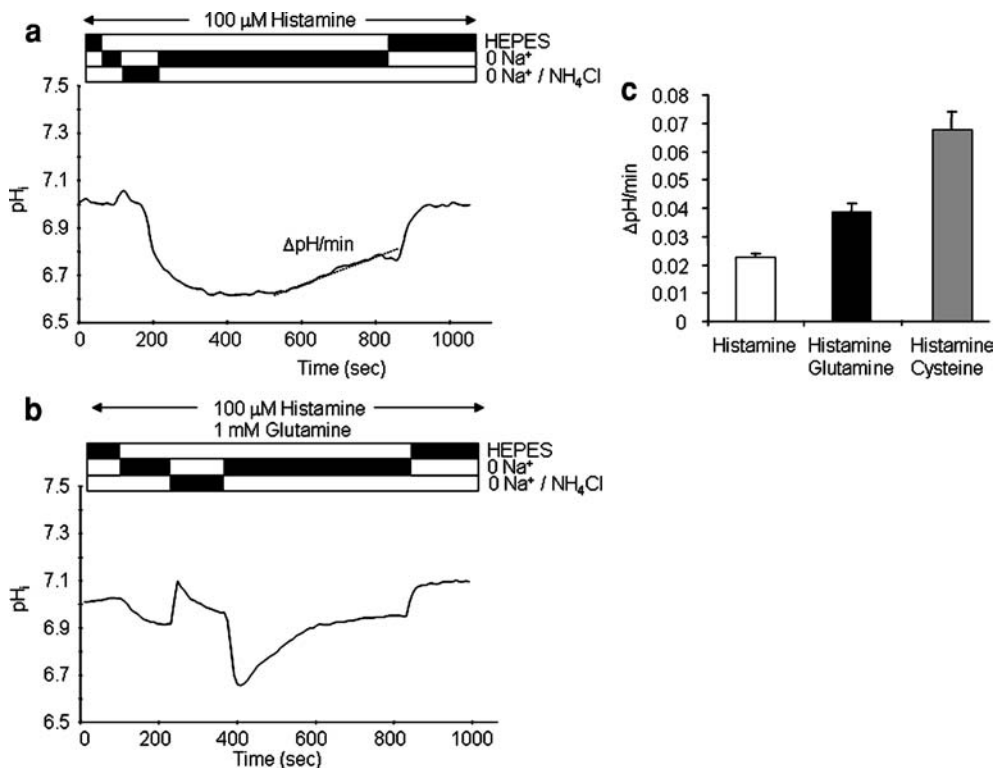
Fig. 3 Stimulation of intracellular alkalinization by amino acids in gastric parietal cells. **a** Original tracing of intracellular pH measurement in a single parietal cell in rat isolated gastric gland. Glands were stimulated with 100 μM histamine and cells acidified with NH$_4$Cl in the absence of Na$^+$. The rate of intracellular Na$^+$-independent alkalinization (*dashed line*) was calculated (ΔpH/min). **b** Original tracing of intracellular pH measurement of a parietal cell stimulated with 1 mM glutamine. **c** Summary *bar* of the rate of intracellular alkalinization of histamine stimulated rat gastric parietal cells in the absence and presence of 1 mM glutamine or cysteine. Data are presented as mean ± SEM

continuous presence of cimetidine. Addition of glutamine (1 mM) stimulated H$^+$-extrusion to a similar extent in the absence and presence of cimetidine (0.044 ± 0.004 U pH/min.) (Fig. 6, Table 3) demonstrating that the stimulatory effect of glutamine does not require H$_2$ receptors or release of histamine from ECL cells.

Stimulation of acid extrusion by glutamine and cysteine requires system L amino acid transport activity

The stimulatory effect of glutamine and cysteine on H$^+$/K$^+$-ATPase activity was further examined in the presence of BCH, a specific inhibitor of system L amino acid transport activity. BCH while being transported itself cannot be metabolized [6, 14, 18, 20, 30] and thus acts as a competitive inhibitor. BCH (10 mM) was used in excess over glutamine or cysteine (both 1 mM) and completely prevented the stimulatory effect of both amino acids on pH$_i$-recovery (H$^+$-extrusion)(Fig. 7, Table 3). These results thus, indicate that both the amino acids need to be transported by a system L-like amino acid transporter to stimulate H$^+$/K$^+$-ATPase

activity and that they may act from an intracellular site either as intact amino acids or after being metabolized. BCH does not surrogate for glutamine or cysteine at this intracellular site.

Intracellular Ca^{2+} measurements in parietal cells

We have recently shown that the activation of the divalent cation receptor, CaSR, can also stimulate H$^+$/K$^+$-ATPase activity even in the absence of any other stimulus such as histamine [9]. The affinity of the CaSR for divalent cations and its subsequent activation can be modulated by L-amino acids binding to the receptor and shifting the activation curve to the left, i.e., activation occurs already at lower concentrations of divalent cations [2, 7]. Activation of the Ca^{2+}-sensing receptor in turn increases intracellular Ca^{2+}, a stimulus known to be involved in the activation and stimulation of the pump [4, 5]. In order to examine if glutamine stimulated the activity of the gastric H$^+$/K$^+$-ATPase by increasing intracellular Ca^{2+}, intracellular Ca^{2+} was measured using the Ca^{2+}-sensitive dye Fluo-4. Glands were not stimulated with histamine. Superfusion of isolated gas-

Table 3 Summary of intracellular pH measurements in single parietal cells in freshly isolated rat gastric glands

	pH_i recovery $\Delta pH/min$	n
Control	0.007 ± 0.001	93 cells 10 glands 7 animals
L-Glutamine	0.052 ± 0.005	95 cells 8 glands 3 animals
L-Phenylalanine	0.066 ± 0.003	66 cells 5 glands 3 animals
L-Glutamate	0.003 ± 0.001	108 cells 8 glands 3 animals
L-Glutamine + cimetidine	0.044 ± 0.004	101 cells 6 glands 3 animals
Histamine	0.023 ± 0.001	33 cells 5 glands 4 animals
Histamine + L-Glutamine	0.039 ± 0.003	82 cells 10 glands 7 animals
Histamine + L-Cysteine	0.068 ± 0.006	76 cells 8 glands 4 animals
Histamine + L-GlutamineOmeprazole	0.002 ± 0.002	37 cells 6 glands 4 animals
Histamine + L-GlutamineBCH	0.015 ± 0.002	45 cells 4 glands 3 animals
Histamine + L-CysteineBCH	0.012 ± 0.002	42 cells 5 glands 4 animals

All data are given as mean $\pm$ SEM

tric glands with 1 mM glutamine did not alter the intensity of the Fluo-4 signal. However, when 500 µM Gd^{3+}, a potent activator of the Ca^{2+}-sensing receptor in gastric parietal cells [4, 9], was applied a rapid increase in intensity of the Fluo-4 signal was observed, indicative of an increase in intracellular Ca^{2+} [4, 9] (Fig. 8). Thus, glutamine at this low concentration does not stimulate H^+/K^+-ATPase activity by acting on the Ca^{2+}-sensing receptor.

Discussion

Gastric acid secretion is stimulated through a variety of factors including neurotransmitters, hormones, metabolic factors, and several nutrients including amino acids [32]. The mechanism by which nutrients stimulate and regulate gastric acid secretion is not fully understood. A protein-rich diet or the intravenous infusion of a solution containing either single amino acids or a mix of different amino acids has been shown to represent a potent stimulus for gastric acid secretion [11, 13, 15, 19]. This stimulatory effect is observed only with L-amino acids but not with their D-stereomers [13, 15]. In addition, gastric acid secretion occurs in the absence of gastrin release and after vagotomy [13, 15]. Taken together, these results suggest a direct effect of amino acids on acid-secretory parietal cells by a mechanism recognizing the stereo-specificity of amino acids.

The recent identification of system L amino acid transporter in parietal cells provided a novel pathway. System L amino acid is stereo-selective accepting only L-isomers and transports most neutral amino acids that have been found to stimulate gastric acid secretion upon intravenous infusion, namely, phenylalanine, cysteine, glutamine, and leucine. Our results demonstrate that system L amino acid transport could indeed be involved in amino acid stimulated gastric acid secretion: (1) system L amino acid transporter subunits 4F2hc and LAT2 are specifically expressed in parietal cells in mouse and rat stomach, (2) glutamine, phenylalanine, and cysteine stimulated gastric H^+/K^+-ATPase activity, (3) the stimulatory effect is specific for system L substrates and does not occur with glutamate, (4) stimulation is abolished by BCH, an inhibitor of system L amino acid transport, (5) the effect did not require H_2-receptors ruling out the involvement of ECL cells, and (6) amino acids did not act via the recently described Ca^{2+}-sensing receptor as evident from intracellular Ca^{2+}-measurements. However, it should also be noted that phenylalanine, glutamine, and cysteine are substrates of B°AT1 and ATB(0+) and glutamine and cysteine of ASCT2. Only ATB(0+) is sensitive to BCH whereas B°AT1 and ASCT2 not. Thus, the data on amino acid selectivity and
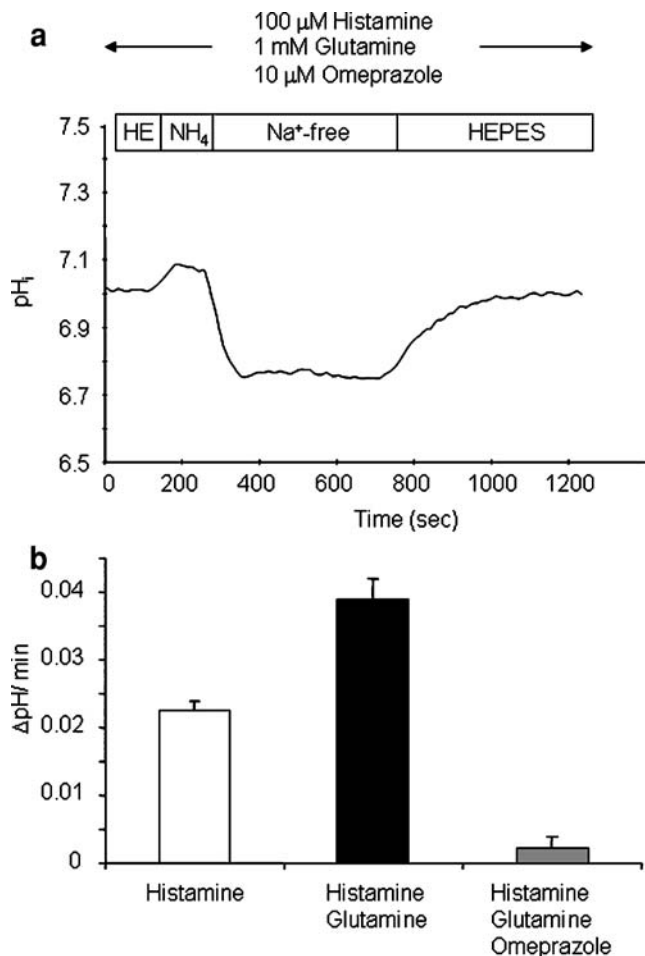
Fig. 4 Amino acids stimulate omeprazole-sensitive H^+/K^+-ATPase activity. **a** Original tracing of an intracellular pH measurement of a histamine-stimulated rat parietal cell in the presence of 1 mM glutamine and the specific H^+/K^+-ATPase inhibitor omeprazole (100 μM). No intracellular Na^+-independent alkalinization could be observed. **b** *Bar graph* summarizing intracellular pH-recovery rates (ΔpH/min) of histamine stimulated rat parietal cells in the presence of 1 mM glutamine or 1 mM glutamine/100 μM omeprazole. The data for histamine and histamine plus glutamine from Fig. 3 are shown again for better comparison, mean ± SEM

Fig. 5 Glutamine or phenylalanine alone stimulates H^+/K^+-ATPase activity. The intracellular Na^+-independent alkalinization rate was measured in rat gastric glands left either untreated (control), incubated with 1 mM glutamine, 1 mM phenylalanine or 1 mM glutamate. Incubation with 1 mM glutamine or phenylalanine strongly stimulated the Na^+-independent alkalinization rate whereas glutamate had no effect, mean ± SEM

as a metabolic fuel for ATP synthesis and subsequently contribute to H^+/K^+-ATPase activity. Glucose, lactate, and some amino acids have previously been shown to support aminopyridine accumulation in parietal cells possibly as substrates for ATP synthesis [22].

Thus, the system L amino acid transporter may be part of a sensing mechanism for which it represents the amino acid uptake system across the plasma membrane, whereas the intracellular mechanism that senses the amino acids remains to be identified. The fact that a mammalian plasma membrane transporter forms part of a metabolic-sensing mechanism has been extensively studied in the insulin secreting β-cells of the pancreas

inhibition by BCH fit best to system L but do not completely rule out the involvement of the three above-mentioned amino acid transporters.

The mechanism by which amino acids transported by system L stimulate acid secretion remains unclear. It appears, however, that the sensor mechanism must be localized intracellularly as it requires the uptake of amino acids into parietal cells which can be blocked by BCH. The sensing mechanism might involve the metabolism of the transported amino acids as BCH is transported by system L but cannot be metabolized. Interestingly, BCH even decreased the acid secretion below control. This could be due to the exchange function of LAT2-4F2hc that mediates the efflux of amino acids stimulated by the uptake of BCH. The amino acids tested here could at least in the case of glutamine also act
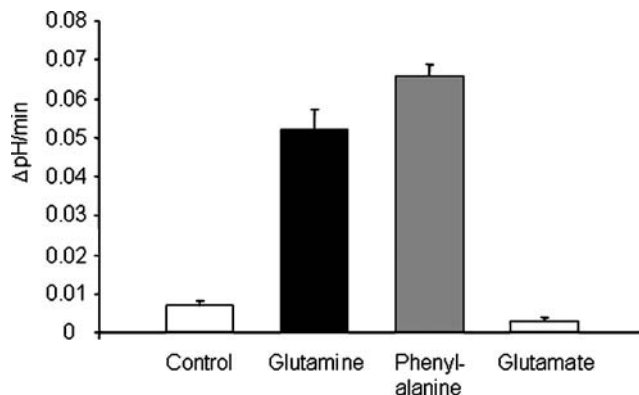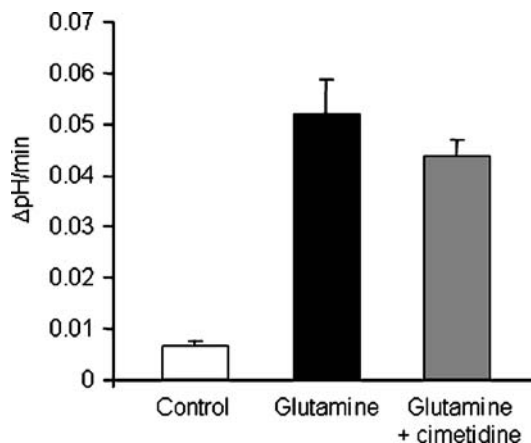

Fig. 6 H_2-receptors are not involved in the effect of glutamine on parietal cell acid secretion. Rat gastric glands were left untreated (control) or incubated with 1 mM glutamine in the absence or presence of the H_2-receptor antagonist cimetidine (100 μM). Cimetidine did not affect the stimulation by glutamine demonstrating that H_2-receptors are not mediating the effect. The data of control and glutamine are shown again for better comparison, mean ± SEM
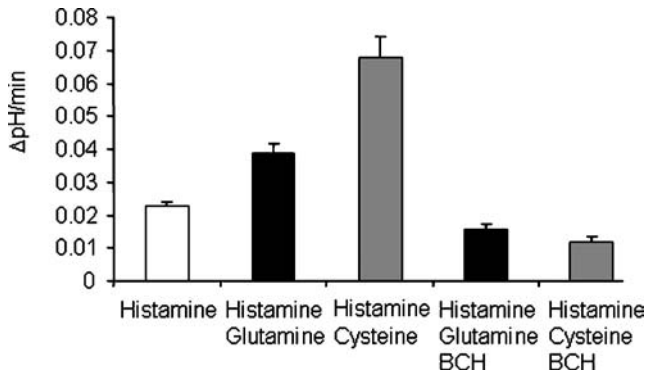
Fig. 7 Inhibition of system L amino acid transport activity with BCH prevents stimulatory effect of amino acids on H^+/K^+-ATPase activity. Incubation of rat gastric glands with BCH (10 mM), a competitive inhibitor of system L amino acid transport activity, abolished the stimulatory effect of glutamine (1 mM) and cysteine (1 mM) on the rate of intracellular pH alkalinization. The data for histamine, histamine plus glutamine and histamine plus cysteine are shown again for better comparison, mean ± SEM
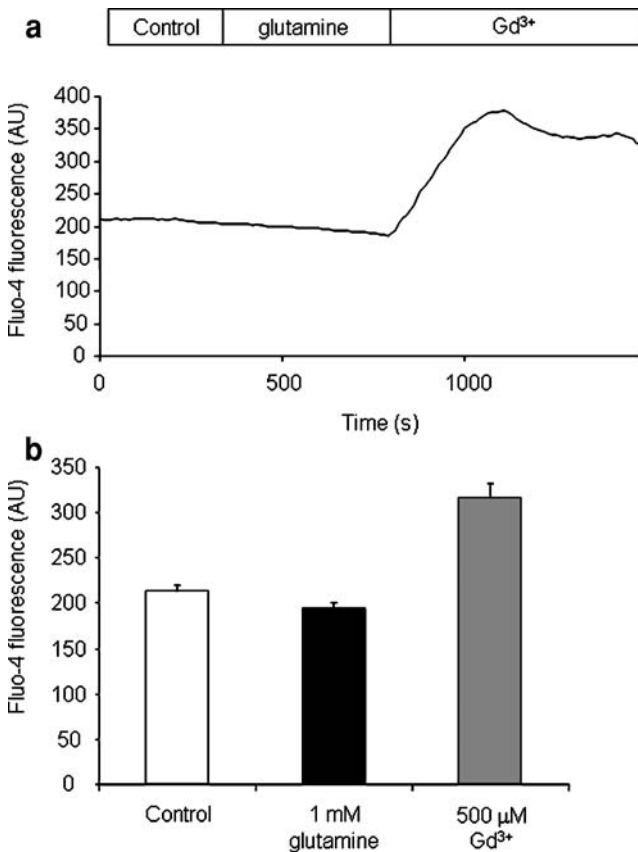

Fig. 8 Glutamine does not increase intracellular Ca^{2+}. Intracellular Ca^{2+} was measured in rat parietal cells using Fluo-4 and glands were superfused with 1 mM glutamine. No increase in the Fluo-4 intensity could be observed. However, upon addition of 500 μM Gd^{3+}, a known activator of the calcium-sensing receptor, a rise in intracellular Ca^{2+} could be observed. **a** Original tracing of intracellular Ca^{2+} measurement. **b** *Bar graph* summarizing intracellular Ca^{2+} measurements as mean ± SEM, $n = 64$ cells from four glands, three different animals

where glucose is taken up via the GLUT2 glucose transporter and then metabolized to generate an ATP-based signal initiating insulin secretion [27]. The mechanism described here in gastric parietal cells may be of a similar nature and the system L mediated uptake of amino acids into parietal cells may provide the initial step mediating the increased gastric acid secretion after the ingestion of a protein-rich meal.

In summary, an isoform of the system L amino acid transporter is expressed in acid-secreting gastric parietal cells and its substrates stimulate H^+/K^+-ATPase activity in isolated gastric glands. The stimulatory effect requires transport of system L-specific amino acids and does not depend on H_2 or CaSR receptors. This defines a novel pathway mediating the stimulatory effect of amino acids on gastric acid secretion.

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