Ca²⁺ Activated K⁺ Channel K_{ca}3.1 as a Determinant of Gastric Acid Secretion

Anand Rotte^{1*}, Venkanna Pasham^{1*}, Andreas F. Mack², Madhuri Bhandaru¹, Syed M. Qadri¹, Melanie Eichenmüller¹, Peter Ruth³ and Florian Lang¹

Departments of ¹Physiology, ²Anatomy, ³Pharmacology & Toxicology of the Institute of Pharmacy, University of Tübingen, *contributed equally and thus share first authorship

Key Words

Stomach • K⁺ channels • K_{ca}3.1 • H⁺/K⁺ ATPase • K⁺ recycling • H⁺ secretion • Carbachol

Abstract

The Ca²⁺ activated K⁺ channel K_{ca}3.1 is expressed in a variety of tissues. In the gastric gland it is expressed in the basolateral cell membrane. To determine the functional significance of K_{ca}3.1 activity for gastric acid secretion, gastric acid secretion was determined in isolated glands from gene targeted mice lacking functional K_{ca}3.1 (K_{ca}3.1^{-/-}) and from their wild type littermates (K_{ca}3.1^{+/+}). According to BCECF-fluorescence cytosolic pH in isolated gastric glands was similar in K_{ca}3.1^{-/-} and K_{ca}3.1^{+/+} mice. Na⁺-independent pH recovery (ΔpH/min) following an ammonium pulse, a measure of H⁺/K⁺ ATPase activity, was, however, significantly faster in K_{ca}3.1^{-/-} than in K_{ca}3.1^{+/+} mice. Accordingly, the luminal pH was significantly lower and the acid content significantly higher in K_{ca}3.1^{-/-} than in K_{ca}3.1^{+/+} mice. The abundance of mRNA encoding H⁺/K⁺ ATPase and KCNQ1 was similar in both genotypes. Increase of extracellular K⁺ concentrations to 35 mM (replacing Na⁺/NMDG) and treatment with histamine (100 μM) significantly increased ΔpH/min to a larger extent in

K_{ca}3.1^{+/+} than in K_{ca}3.1^{-/-} mice and dissipated the differences between the genotypes. Carbachol (100 μM) increased ΔpH/min in both genotypes but did not abolish the difference between K_{ca}3.1^{-/-} and K_{ca}3.1^{+/+} mice. In K_{ca}3.1^{+/+} mice the K_{ca}3.1 opener DCEBIO (100 μM) did not significantly alter basal ΔpH/min but significantly blunted ΔpH/min in the presence of carbachol. In conclusion, K_{ca}3.1 activity suppresses carbachol stimulated gastric acid secretion.

Copyright © 2011 S. Karger AG, Basel

Introduction

The intermediate Ca²⁺ activated K⁺ channel K_{ca}3.1 is expressed in several tissues and participates in the regulation of diverse functions [1-4]. A functional role of K_{ca}3.1 during gastric acid secretion has, however, not been explored.

Proton secretion in gastric glands is accomplished by the H⁺/K⁺ ATPase, which pumps H⁺ into the gastric lumen in exchange of K⁺ [5-8]. The luminal K⁺ is replenished by recirculation of K⁺ through luminal KCNQ1/KCNE K⁺ channels [9-11]. Accordingly, gastric acid secretion is abolished by genetic knockout of KCNQ1 [12]. The channels are activated by cAMP [13-

16], which thus stimulates gastric acid secretion [6, 8, 17].

The present study explored, whether $K_{ca}3.1$ contributes to the regulation of gastric acid secretion. To this end, gastric acid secretion was determined in gene targeted mice lacking functional $K_{ca}3.1$ and their wild type littermates.

Materials and Methods

Animals

The $K_{ca}3.1$ deficient mice were described earlier [18]. Sex and age matched mice with age more than 3 months were used for the experiments. All animal experiments were conducted according to the German law for the care and use of laboratory animals and were approved by local authorities.

Treatments

Mice had free access to a standard mouse diet (C1310, Altromin, Lage, Germany) and tap water. Effects of histamine (100 μ M), carbachol (100 μ M), DCEBIO (100 μ M) or Tram 34 (1 μ M) were determined by adding the substances directly to the perfusing solutions at the indicated final concentrations. The substances were obtained from Sigma (Taufkirchen, Germany). The stock solutions of DCEBIO and Tram 34 were prepared in DMSO, whereas histamine and carbachol were dissolved in distilled water. Sham experiments were performed by adding DMSO or plain distilled water to the perfusing solution at a final dilution of 0.1 %.

Determination of serum gastrin and histamine

To determine the serum gastrin and histamine levels, the animals were fasted overnight for 16 hours with free access to tap water. About 150 μ l of blood specimens were withdrawn into heparinized hematocrit capillaries by retro-orbital puncture during light anesthesia with diethylether (Roth, Karlsruhe, Germany). Total serum gastrin concentrations were determined by a RIA kit (DiaSorin, Dietzenbach, Germany) and serum histamine concentrations by an ELISA kit (IBL international, Hamburg, Germany) according to the manufacturer's instructions.

Gastric H^+ secretion

For isolation of gastric glands animals were fasted for 16 hours prior to experiments on wire grids with free access to tap water. After sacrificing the animals the stomach was removed and cut longitudinally. After washing with standard HEPES solution the fundic and pyloric regions were discarded and the gastric corpus was sliced into 0.3 cm² sections. The tissues were transferred onto the cooled stage of a dissecting microscope and individual glands were carefully detached from the gastric wall by snapping off the tissue using sharpened microdissection tweezers. Care was taken not to touch the apical part of the glands. The glands were attached to a glass coverslip precoated with Cell-Tak adhesive (BD Biosciences).

The solutions, flow lines and perfusion chamber were maintained at 37°C by a thermostatically controlled heating system. The volume of the perfusion chamber was 600 μ l and the flow rate was 4 ml/min for all solutions. For digital imaging of cytosolic pH_i isolated individual glands were incubated in a HEPES-buffered Ringer solution containing 10 μ M BCECF-AM (Molecular Probes, Leiden, The Netherlands) for 15 min at 37°C. After loading, the chamber was flushed for 5 min with Ringer solution to remove any deesterified dye sticking to the outside of the glands. The perfusion chamber was mounted on the stage of an inverted microscope (Zeiss Axiovert 135), which was used in the epifluorescence mode with a 40 x oil immersion objective (Zeiss Neoplan, Germany). BCECF was successively excited at 490/10 and 440/10 nm and the resultant fluorescent signal was monitored at 535/10 nm using an intensified charge-coupled device camera (Proxitronic, Germany) and specialized computer software (Metafluor, USA). Between 8 and 20 parietal cells were outlined and monitored during the course of the measurements. The results from each cell were averaged and taken for final analysis. Intensity ratio (490/440) data were converted into pH values using the high- K^+ /nigericin calibration technique [19]. To this end the glands were perfused at the end of each experiment for 5 minutes with standard high- K^+ /nigericin (10 μ g/ml) solution (pH 7.0). The intensity ratio data thus obtained were converted into pH values using the r_{max} , r_{min} , pK_a values previously generated from calibration experiments performed in isolated gastric glands to generate a standard nonlinear curve (pH range 5 to 8.5). The intensity ratio data were converted into pH values using the following equation: $pH = pK_a [\log((ratio_{test}/ratio_{pH\ 7.0}) - r_{min}) / (r_{max} - (ratio_{test}/ratio_{pH\ 7.0}))]$.

For acid loading, cells were transiently exposed to a solution containing 20 mM NH_4Cl leading to marked initial alkalization of cytosolic pH (pH_i) due to entry of NH_3 and binding of H^+ to form NH_4^+ [20]. The acidification of cytosolic pH upon removal of ammonia allowed calculating the mean intrinsic buffering power (β) of the cells [20]. Assuming that NH_4^+ and NH_3 are in equilibrium in cytosolic and extracellular fluid and that ammonia leaves the cells as NH_3 :

$$\beta = \Delta[NH_4^+]_i / \Delta pH_i,$$

where ΔpH_i is the decrease of cytosolic pH (pH_i) following ammonia removal and $\Delta[NH_4^+]_i$ is the decrease of cytosolic NH_4^+ concentration, which is identical to the concentration of $[NH_4^+]_i$ immediately before the removal of ammonia. The pK for NH_4^+/NH_3 is 8.9 [21] and at an extracellular pH (pH_o) of 7.4 the NH_4^+ concentration in extracellular fluid ($[NH_4^+]_o$) is 19.37 $[20/(1+10^{pH_o-pK})]$ mM. The intracellular NH_4^+ concentration ($[NH_4^+]_i$) was calculated from:

$$[NH_4^+]_i = 19.37 \cdot 10^{pH_o-pH_i} \text{ mM}$$

The calculation of the buffer capacity required that NH_4^+ exits completely. After the initial decline, pH_i indeed showed little further change in the absence of Na^+ and presence of omeprazole, indicating that there was no relevant further exit of NH_4^+ . The pH recovery during and following the NH_4^+ pulse was determined in the absence of Na^+ to prevent cellular realkalinization by the Na^+/H^+ exchanger.

To calculate the $\Delta pH/min$ during re-alkalinization, a manual linear fit was placed over a narrow pH range (pH 6.7 to 6.9) which could be applied to all measured cells.

The solutions were composed of (in mM): standard HEPES: 115 NaCl, 5 KCl, 1 CaCl₂, 1.2 MgSO₄, 2 NaH₂PO₄, 10 glucose, 32.2 HEPES; sodium free HEPES: 132.8 NMDG⁺Cl⁻, 3 KCl, 1 CaCl₂, 1.2 MgSO₄, 2 KH₂PO₄, 32.2 HEPES, 10 mannitol, 10 glucose (for sodium free ammonium chloride 10 mM NMDG⁺Cl⁻ and mannitol were replaced with 20 mM NH₄Cl); high K⁺ for calibration: 105 KCl, 1 CaCl₂, 1.2 MgSO₄, 32.2 HEPES, 10 mannitol, 10 µg/ml nigericin. The pH of the solutions was titrated to 7.4 or 7.0 with HCl/NaOH, HCl/NMDG and HCl/KOH, respectively, at 37°C. Where indicated the K⁺ concentration was increased at the expense of Na⁺/NMDG to 35 mM.

Determination of pH and acid content of the gastric lumen

The pH of the luminal stomach contents depends on the amount of acid secreted. Activation of gastric gland leads to decrease in luminal pH and conversely inhibition leads to increase in pH. The luminal pH of the overnight fasted mice therefore serves as a useful indicator of basal gastric acid secretion. To determine the basal intraluminal pH, a previously described method was slightly modified and used [22-24]. Briefly, the mice were fasted overnight on wire grids with free access to tap water and sacrificed under slight ether anaesthesia. The whole stomach was then carefully removed and cut open and the luminal contents were collected by washing the stomach with 10 ml unbuffered isotonic sodium chloride solution (containing additionally 5 mM KCl and 10 mM glucose). The washings were then centrifuged at 150 g for 5 minutes to remove the particulate matter and the pH of the supernatant solution was measured by a standard pH meter (Knick, Germany). The acid content in the solution was measured by titration of the above obtained supernatant solution with 0.01 N NaOH to an endpoint of pH 7.0 by monitoring the pH with a pH meter and additionally adding a universal pH indicator (Sigma, Taufkirchen, Germany) to the solution. The acid content thus obtained was then corrected by the acid content of the blank isotonic sodium chloride solution and used for further statistical analysis.

Real-Time Reverse transcription-PCR (RT-PCR, Light Cycler)

Total RNA was extracted from mouse tissue in Trizol (Pqlab, Erlangen, Germany) according to the manufacturer's instructions. After DNase digestion reverse transcription of total RNA was performed using random hexamers (Roche Diagnostics, Penzberg, Germany) and SuperScript II reverse transcriptase (Invitrogen, Carlsbad, CA, USA). Polymerase chain reaction (PCR) amplification of the respective genes were set up in a total volume of 20 µl using 40 ng of cDNA, 500 nM forward and reverse primer and SensiMix™ Lite Kit (Quantace Bioline GmbH, Luckenwalde, Germany) according to the manufacturer's protocol. Cycling conditions were performed as follows: initial denaturation at 95°C for 5 min, followed by 40 cycles of 95°C for 30 sec, 55°C for 30 sec and 72°C for 30 sec and terminal elongation at 72°C for 5 min. For the amplification we used the following primers (5'→3' orientation):

Kcnq1, fw GCC ACC ATC AAG GTC ATC AG rev TGA CAT CTC GCA CGT CGT AG;

Atp4b, fw CCC AAT GGC ACC TTC AGT CT rev GGA GCT

TGG CTG CCA CC;

Tbp, fw CAC TCC TGC CAC ACC AGC TT rev TGG TCT TTA GGT CAA GTT TAC AGC C.

Specificity of PCR products was confirmed by analysis of a melting curve. Real-time PCR amplifications were performed on a Light Cycler (Roche Diagnostics) and all experiments were done in duplicate. Amplification of the house-keeping gene *Tbp* was performed to standardize the amount of sample RNA. Relative quantification of gene expression was performed using the Δct method as described earlier [25].

Statistics

Intracellular pH, pH recovery and buffer capacity of all parietal cells from one gland were averaged and the respective mean values were used for further statistical analysis. Data are provided as arithmetic means $\pm$ SEM, *n* represents the number of mice used. All data were tested for significance using Student's t-test with Welch's correction or ANOVA (Dunnets test), where applicable, and only results with *p* < 0.05 were considered statistically significant.

Results

To study the functional significance of the Ca²⁺ activated K⁺ channel K_{ca}3.1 in stomach, K_{ca}3.1 knockout (*K_{ca}3.1^{-/-}*) mice were compared to their wild type littermates (*K_{ca}3.1^{+/+}*). The mice were fasted overnight and the body weight and the weight of empty stomach were determined. The body weight was similar in *K_{ca}3.1^{-/-}* mice (21.6 $\pm$ 0.9 g, *n* = 6) and *K_{ca}3.1^{+/+}* mice (20.3 $\pm$ 1.7 g, *n* = 6). The stomach weight was again similar in *K_{ca}3.1^{-/-}* mice (0.203 $\pm$ 0.01 g, *n* = 6) and *K_{ca}3.1^{+/+}* mice (0.235 $\pm$ 0.02 g, *n* = 6). The expression of K_{ca}3.1 channel in parietal cells was studied by immunofluorescence using H⁺/K⁺ ATPase staining as a counter stain to identify the parietal cells. Mouse parietal cells were seen to show positive immunoreactivity towards K_{ca}3.1 channel indicating the presence of the channel in the cells (Fig. 1).

BCECF fluorescence was employed to determine cytosolic pH. Basal cytosolic pH prior to the ammonium pulse was similar in isolated gastric glands from *K_{ca}3.1^{-/-}* and from *K_{ca}3.1^{+/+}* mice and was not significantly different between the various experimental conditions (Table 1). The buffer capacity was also not significantly different between gastric gland cells from *K_{ca}3.1^{-/-}* and *K_{ca}3.1^{+/+}* mice and was again not significantly different between the various experimental conditions (Table 1). Accordingly, the effect of H⁺ movement across the cell membrane is reflected by the respective changes of cytosolic pH in gastric glands from *K_{ca}3.1^{-/-}* and *K_{ca}3.1^{+/+}* mice.

Table 1. Gastric acid secretion in $K_{ca}3.1^{-/-}$ mice and $K_{ca}3.1^{+/+}$ mice under basal and stimulated conditions. *-indicates significant difference to respective wild type mice. #-indicates significant difference to respective control (untreated or 0.1 % DMSO treated mice). §- indicates significant difference to presence and absence of DCEBIO during carbachol treatment.

		Rate of pH recovery (delta pH units/minute)	Intracellular pH (units)	Buffer capacity (mM/pH unit)	number of mice	number of cells
Control (Untreated)	$K_{ca}3.1^{+/+}$	0.041 ± 0.011	7.22 ± 0.03	39.5 ± 7.2	8	68
	$K_{ca}3.1^{-/-}$	0.107 ± 0.018*	7.26 ± 0.04	37.3 ± 4.3	11	121
High K^{+}	$K_{ca}3.1^{+/+}$	0.311 ± 0.076#	7.29 ± 0.05	37.4 ± 7.1	5	66
	$K_{ca}3.1^{-/-}$	0.275 ± 0.058#	7.27 ± 0.05	35.1 ± 4.0	6	60
Histamine	$K_{ca}3.1^{+/+}$	0.287 ± 0.081#	7.31 ± 0.08	29.0 ± 6.1	6	65
	$K_{ca}3.1^{-/-}$	0.241 ± 0.070#	7.25 ± 0.07	39.5 ± 11.6	5	69
Carbachol	$K_{ca}3.1^{+/+}$	0.223 ± 0.038#	7.44 ± 0.06#	34.7 ± 7.1	7	79
	$K_{ca}3.1^{-/-}$	0.374 ± 0.054#*	7.39 ± 0.05	30.6 ± 5.8	6	64
Sham (0.1 % DMSO)	$K_{ca}3.1^{+/+}$	0.040 ± 0.010	7.23 ± 0.03	33.8 ± 4.5	5	31
	$K_{ca}3.1^{-/-}$	0.161 ± 0.059*	7.27 ± 0.04	31.1 ± 4.9	4	27
DCEBIO	$K_{ca}3.1^{+/+}$	0.039 ± 0.008	7.23 ± 0.05	33.4 ± 11.3	3	34
	$K_{ca}3.1^{-/-}$	0.133 ± 0.075*	7.28 ± 0.05	30.4 ± 4.5	3	25
Carbachol + 0.1 % DMSO	$K_{ca}3.1^{+/+}$	0.210 ± 0.039#	7.37 ± 0.06	33.9 ± 6.8	7	91
	$K_{ca}3.1^{-/-}$	0.307 ± 0.035#*	7.39 ± 0.08	27.0 ± 7.0	7	57
Carbachol + DCEBIO	$K_{ca}3.1^{+/+}$	0.097 ± 0.020#§	7.31 ± 0.04	31.8 ± 3.9	9	111
	$K_{ca}3.1^{-/-}$	0.290 ± 0.011*	7.34 ± 0.07	28.6 ± 8.4	5	51
Tram 34	$K_{ca}3.1^{+/+}$	0.150 ± 0.047#	7.28 ± 0.11	43.0 ± 11.1	3	40
Carbachol + DCEBIO + Tram34	$K_{ca}3.1^{+/+}$	0.302 ± 0.009#	7.38 ± 0.08	24.1 ± 6.8	4	45

Fig. 1. Expression of $K_{ca}3.1$ in the parietal cells. Immunofluorescence of $K_{ca}3.1$ and H^{+}/K^{+} -ATPase expression in parietal cells from $K_{ca}3.1^{+/+}$ mice. Antibodies are directed against $K_{ca}3.1$ (green fluorescence) and the β -subunit of the H^{+}/K^{+} -ATPase (red fluorescence).

The Na^{+} -independent pH recovery following an ammonium pulse ($\Delta pH/min$) was taken as a measure of H^{+}/K^{+} ATPase activity. As illustrated in Fig. 2, $\Delta pH/min$ was significantly (~ 2 -fold) faster in gastric glands from $K_{ca}3.1^{-/-}$ mice than in gastric glands from $K_{ca}3.1^{+/+}$ mice. Omeprazole (100 μM) treatment virtually abolished the $\Delta pH/min$ in both genotypes and abrogated the differences between the genotypes (Fig. 2).

To elucidate the significance of luminal K^{+} concentration, experiments were performed in a solution

containing a high K^{+} concentration. An increase of the bath K^{+} concentration to 35 mM (replacing $Na^{+}/NMDG$) increased $\Delta pH/min$ to a greater extent in $K_{ca}3.1^{+/+}$ mice than in $K_{ca}3.1^{-/-}$ mice (Fig. 2). Accordingly, an increase of extracellular K^{+} concentration abolished the statistically significant difference of gastric acid secretion between $K_{ca}3.1^{+/+}$ mice and $K_{ca}3.1^{-/-}$ mice.

The enhanced gastric acid secretion in $K_{ca}3.1^{-/-}$ mice was further evident from pH and acid content of the gastric lumen. As shown in Fig. 3, the pH of the solution

Fig. 2. pH recovery in parietal cells following an ammonium pulse at normal and increased extracellular K^+ concentration. Alterations of cytosolic pH (ΔpH) in parietal cells following an ammonium pulse. To load the cells with H^+ , 20 mM NH_4Cl was added and Na^+ removed (replaced by NMDG, '0' Na^+) in a first step (see bars below each original tracing), NH_4Cl removed in a second step, Na^+ added in a third step and nigericin (pH_0 '7') applied in a fourth step to calibrate each individual experiment. a. Time dependent alterations of pH (arithmetic means $\pm$ SEM of 6-10 cells each) in representative single experiments in $K_{ca}3.1^{+/+}$ mice (left panels) and $K_{ca}3.1^{-/-}$ mice (right panels) at 5 mM K^+ (upper panels), or 35 mM K^+ (middle panels) in the absence (upper and middle panels) and presence (lower panels) of 100 μM omeprazole (omp). b. Arithmetic means $\pm$ SEM of Na^+ -independent alterations of cytosolic pH ($\Delta pH/min$) in parietal cells from $K_{ca}3.1^{-/-}$ mice (closed bars) and $K_{ca}3.1^{+/+}$ mice (open bars) at 5 mM K^+ ($n = 8-11$ mice each group, left bars) and at 35 mM K^+ ($n = 5-6$ mice each group, middle bars) and in presence of 100 μM omeprazole ($n = 3-4$ mice each group, right bars). * indicates significant difference between $K_{ca}3.1^{-/-}$ mice and $K_{ca}3.1^{+/+}$ mice, # indicates significant difference between control (5 mM K^+) and respective treatment.

Fig. 3. Intraluminal pH and acid content of stomach from overnight fasted mice. Arithmetic means $\pm$ SEM of ($n = 5-6$ mice each group) of (a) the pH of the luminal contents and (b) acid equivalents in the luminal contents of stomach from $K_{ca}3.1^{-/-}$ mice (closed bars) and $K_{ca}3.1^{+/+}$ mice (open bars). * indicates significant difference between $K_{ca}3.1^{-/-}$ mice and $K_{ca}3.1^{+/+}$ mice.

containing the luminal aspirates from $K_{ca}3.1^{-/-}$ mice was significantly lower than that from $K_{ca}3.1^{+/+}$ mice. Along those lines the acid content was significantly (~ 2 -fold) higher in the gastric lumen from $K_{ca}3.1^{-/-}$ mice.

Gastric acid secretion is accomplished by the H^+/K^+ ATPase, which requires the apical K^+ channel KCNQ1 for recycling of K^+ into the lumen. To verify the possible genomic upregulation of H^+/K^+ ATPase and/or KCNQ1

Fig. 4. Expression of KCNQ1 and of the β -subunit of the H^+/K^+ ATPase in gastric tissue. a. Arithmetic means $\pm$ SEM ($n = 4$ mice each group) of the relative expression of KCNQ1 mRNA in the gastric tissue from $K_{ca}3.1^{-/-}$ mice (closed bars) and $K_{ca}3.1^{+/+}$ mice (open bars). b. Arithmetic means $\pm$ SEM ($n = 4$ mice each group) of the relative expression of the H^+/K^+ ATPase β -subunit in gastric tissue from $K_{ca}3.1^{-/-}$ mice (closed bars) and $K_{ca}3.1^{+/+}$ mice (open bars).

in the $K_{ca}3.1^{-/-}$ mice, real time PCR analysis was utilized to quantify KCNQ1 and H^+/K^+ ATPase β -subunit transcript levels in $K_{ca}3.1^{-/-}$ and $K_{ca}3.1^{+/+}$ mice. As illustrated in Fig. 4, the transcript levels were similar in $K_{ca}3.1^{-/-}$ and $K_{ca}3.1^{+/+}$ mice.

Plasma gastrin and histamine levels were determined to explore, whether the increased gastric acid secretion in $K_{ca}3.1^{-/-}$ mice was due to abnormal stimulation by

Fig. 5. Effects of histamine and carbachol on pH recovery in parietal cells. a. Time dependent alterations of pH (arithmetic means $\pm$ SEM of 6-10 cells each) in representative single experiments in $K_{ca}3.1^{+/+}$ mice (left panels) and $K_{ca}3.1^{-/-}$ mice (right panels) in the absence of stimulators (upper panels) and presence of 100 μ M histamine (middle panels) or of 100 μ M carbachol (lower panels). b. Arithmetic means $\pm$ SEM (n = 4-7 mice each group) of Na^+ -independent alterations of cytosolic pH (Δ pH/min) in parietal cells following an ammonium pulse in the presence of histamine (left bars) or carbachol (right bars) in gastric glands from $K_{ca}3.1^{-/-}$ mice (closed bars) and $K_{ca}3.1^{+/+}$ mice (open bars). * indicates significant difference between $K_{ca}3.1^{-/-}$ mice and $K_{ca}3.1^{+/+}$ mice, # indicates significant difference between absence (control, fig. 2) and presence of respective secretagogue treatment.

Fig. 6. Effects of DCEBIO in presence and absence of carbachol on pH recovery in parietal cells. a. Time dependent alterations of pH (arithmetic means $\pm$ SEM of 6-10 cells each) in representative single experiments in $K_{ca}3.1^{+/+}$ mice in the absence (upper panels) and presence (lower panels) of 100 μ M carbachol in the absence (left panels) and presence (right panels) of 100 μ M DCEBIO. b. Arithmetic means $\pm$ SEM (n = 4-7 mice each group) of Na^+ -independent alterations of cytosolic pH (Δ pH/min) in parietal cells following an ammonium pulse in $K_{ca}3.1^{+/+}$ mice in the absence of drugs (white bar) and presence of DCEBIO alone (light grey bar), carbachol alone (black bar) or carbachol and DCEBIO together (dark grey bar). # indicates significant difference to absence of drugs, § indicates significant difference to carbachol alone.

gastrin or histamine. Plasma gastrin levels were slightly lower in $K_{ca}3.1^{-/-}$ (22.6 ± 1.5 pM, n = 10) as compared to $K_{ca}3.1^{+/+}$ (25.1 ± 0.9 pM, n = 13) mice, pointing to inhibition of gastrin release in $K_{ca}3.1^{-/-}$ mice. Histamine levels were similar in $K_{ca}3.1^{-/-}$ (3.3 ± 0.8 ng/ml, n = 9) and $K_{ca}3.1^{+/+}$ (3.9 ± 0.6 ng/ml, n = 9) mice.

To elucidate the effect of secretagogue treatment on the gastric acid secretion, further experiments were conducted in the glands treated with histamine or

carbachol. Treatment of gastric glands with 100 μ M histamine or with 100 μ M carbachol was followed by an increase of Δ pH/min in both genotypes. In the presence of carbachol but not in the presence of histamine Δ pH/min remained significantly higher in $K_{ca}3.1^{-/-}$ mice than in $K_{ca}3.1^{+/+}$ mice (Fig. 5). The difference between the genotypes was thus abolished in the presence of histamine but not in the presence of carbachol.

To further elucidate the role of $K_{ca}3.1$ channels in

the regulation of gastric acid secretion, the gastric glands were treated with the specific $K_{ca}3.1$ opener DCEBIO (100 μ M). In $K_{ca}3.1^{+/+}$ mice DCEBIO did not significantly affect Δ pH/min under control conditions, but significantly blunted the upregulation of Δ pH/min by carbachol (Fig. 6). Accordingly, opening of $K_{ca}3.1$ channels blunted the stimulation of gastric acid secretion by the secretagogue. Conversely, treatment of the gastric glands from $K_{ca}3.1^{+/+}$ mice with the $K_{ca}3.1$ inhibitor Tram 34 (1 μ M) significantly increased Δ pH/min (Table 1). Additionally, Tram 34 treatment abolished the inhibitory effect of DCEBIO on the carbachol stimulated H^+ -ion secretion (Table 1).

Finally experiments were also performed in gastric glands from $K_{ca}3.1^{-/-}$ mice following treatment with carbachol in presence and absence of DCEBIO (100 μ M). However, DCEBIO treatment did not have any significant effect on the carbachol induced gastric acid secretion (Table 1).

Discussion

The present study discloses a novel mechanism regulating gastric acid secretion. Gastric acid secretion is enhanced in gene targeted mice lacking the intermediate conductance Ca^{2+} activated K^+ channel $K_{ca}3.1$. Accordingly, $K_{ca}3.1$ activity apparently blunts the gastric acid secretion. $K_{ca}3.1$ has been reported to be inserted into the basolateral membrane [26]. Activation of K^+ channels in the basolateral membrane is expected to hyperpolarize that cell membrane. Considering the permeability of the paracellular shunt [27], the hyperpolarization of the basolateral cell membrane leads to a circular current hyperpolarizing the apical membrane. The hyperpolarization of the apical cell membrane in turn limits the electrochemical driving force for K^+ exit into the gastric lumen (Fig. 7). To the extent that opening of basolateral K^+ channels hyperpolarizes both, basolateral and apical membrane potential, the activation of $K_{ca}3.1$ could thus decrease the K^+ exit across the apical cell membrane through KCNQ1. Along those lines, an increase of extracellular K^+ concentration to 35 mM is expected to depolarize the cell membranes thus enhancing K^+ exit across the apical membrane. K^+ recycling through KCNQ1 has previously been shown to be critically important for the stimulation of gastric acid secretion [12]. The basolateral cell membrane harbours in addition Cl^- channels [28], which on the one hand similarly affect cell membrane potential and on the other render cytosolic Cl^-

Fig. 7. Model of parietal cells including basolateral K^+ channels.

activity sensitive to alterations of cell membrane potential.

It should, be pointed out, however, that $K_{ca}3.1$ may, at least in theory, influence the regulation of gastric acid secretion by other mechanisms such as altered release of secretagogues. The plasma levels of gastrin and histamine were, however, rather decreased in $K_{ca}3.1$ deficient mice, an observation explained by the enhanced luminal acidity in the stomach of those mice.

The role of $K_{ca}3.1$ appears at first glance counterintuitive, as increase of cytosolic Ca^{2+} activity is well known to stimulate gastric acid secretion [8]. Possibly, the stimulatory effect of increased cytosolic Ca^{2+} activity would be more pronounced, if it would not be blunted by the simultaneous stimulation of $K_{ca}3.1$. In any case, the fine tuning of K^+ recirculation and thus gastric acid secretion by $K_{ca}3.1$ adds to the complexity of regulation. Whereas stimulation of KCNQ1 K^+ channels should enhance gastric acid secretion, stimulation of $K_{ca}3.1$ K^+ channels counteracts gastric acid secretion. Besides Ca^{2+} and cAMP [8, 29, 30], phosphoinositide 3 kinase (PI3K) dependent signaling participates in the regulation of gastric acid secretion [31–34].

In conclusion, the gastric acid secretion is significantly enhanced in $K_{ca}3.1^{-/-}$ mice as compared to $K_{ca}3.1^{+/+}$ mice. Thus, $K_{ca}3.1$ contributes to the complex regulation of gastric acid secretion.

Abbreviations

BCECF (2',7'-bis-(2-carboxyethyl)-5-(and -6)-carboxyfluorescein, acetoxymethyl este); DMSO (Dimethylsulfoxide); NMDG (N-methyl D-glucamine); HEPES (4-(2-hydroxyethyl)-1-piperazineethanesulfonic acid); ATP4b (β -subunit of H^+/K^+ ATPase); Tbp (TATA box binding protein); PI3K (phosphoinositol 3 kinase).

Acknowledgements

The authors gratefully acknowledge the meticulous preparation of the manuscript by Tanja Loch. This work

was supported by grants from the DFG (SFB 773). All authors of this manuscript declare that they have no conflicts of interests.

References

- Bradding P, Wulff H: The K⁺ channels K(Ca)_{3.1} and K(v)_{1.3} as novel targets for asthma therapy. *Br J Pharmacol* 2009;157:1330-1339.
- Dufer M, Gier B, Wolpers D, Krippeit-Drews P, Ruth P, Drews G: Enhanced glucose tolerance by SK4 channel inhibition in pancreatic beta-cells. *Diabetes* 2009;58:1835-1843.
- Hoffmann EK: Intracellular signalling involved in volume regulatory decrease. *Cell Physiol Biochem* 2000;10:273-288.
- Tharp DL, Bowles DK: The intermediate-conductance Ca²⁺-activated K⁺ channel (KCa3.1) in vascular disease. *Cardiovasc Hematol Agents Med Chem* 2009;7:1-11.
- Hofer AM, Machen TE: K-induced alkalization in all cell types of rabbit gastric glands: a novel K/H exchange mechanism. *J Membr Biol* 1992;126:245-256.
- Hou W, Schubert ML: Gastric secretion. *Curr Opin Gastroenterol* 2006;22:593-598.
- Lee HC, Breitbart H, Berman M, Forte JG: Potassium-stimulated ATPase activity and hydrogen transport in gastric microsomal vesicles. *Biochim Biophys Acta* 1979;553:107-131.
- Yao X, Forte JG: Cell biology of acid secretion by the parietal cell. *Annu Rev Physiol* 2003;65:103-131.
- Dedek K, Waldegger S: Colocalization of KCNQ1/KCNE channel subunits in the mouse gastrointestinal tract. *Pflugers Arch* 2001;442:896-902.
- Heitzmann D, Grahammer F, von Hahn T, Schmitt-Graff A, Romeo E, Nitschke R, Gerlach U, Lang HJ, Verrey F, Barhanin J, Warth R: Heteromeric KCNE2/KCNQ1 potassium channels in the luminal membrane of gastric parietal cells. *J Physiol* 2004;561:547-557.
- Heitzmann D, Koren V, Wagner M, Sterner C, Reichold M, Tegtmeier I, Volk T, Warth R: KCNE beta subunits determine pH sensitivity of KCNQ1 potassium channels. *Cell Physiol Biochem* 2007;19:21-32.
- Vallon V, Grahammer F, Volkl H, Sandu CD, Richter K, Rexhepaj R, Gerlach U, Rong Q, Pfeifer K, Lang F: KCNQ1-dependent transport in renal and gastrointestinal epithelia. *Proc Natl Acad Sci USA* 2005;102:17864-17869.
- Dong MQ, Lau CP, Gao Z, Tseng GN, Li GR: Characterization of recombinant human cardiac KCNQ1/KCNE1 channels (I (Ks)) stably expressed in HEK 293 cells. *J Membr Biol* 2006;210:183-192.
- Kurokawa J, Chen L, Kass RS: Requirement of subunit expression for cAMP-mediated regulation of a heart potassium channel. *Proc Natl Acad Sci USA* 2003;100:2122-2127.
- Marx SO, Kurokawa J, Reiken S, Motoike H, D'Armiento J, Marks AR, Kass RS: Requirement of a macromolecular signaling complex for beta adrenergic receptor modulation of the KCNQ1-KCNE1 potassium channel. *Science* 2002;295:496-499.
- Potet F, Scott JD, Mohammad-Panah R, Escande D, Baro I: AKAP proteins anchor cAMP-dependent protein kinase to KvLQT1/IsK channel complex. *Am J Physiol Heart Circ Physiol* 2001;280:H2038-H2045.
- Ammar DA, Zhou R, Forte JG, Yao X: Syntaxin 3 is required for cAMP-induced acid secretion: streptolysin O-permeabilized gastric gland model. *Am J Physiol Gastrointest Liver Physiol* 2002;282:G23-G33.
- Sausbier M, Matos JE, Sausbier U, Beranek G, Arntz C, Neuhuber W, Ruth P, Leipziger J: Distal colonic K(+) secretion occurs via BK channels. *J Am Soc Nephrol* 2006;17:1275-1282.
- Waisbren SJ, Geibel J, Boron WF, Modlin IM: Luminal perfusion of isolated gastric glands. *Am J Physiol* 1994;266:C1013-C1027.
- Roos A, Boron WF: Intracellular pH. *Physiol Rev* 1981;61:296-434.
- Boyarsky G, Ganz MB, Sterzel RB, Boron WF: pH regulation in single glomerular mesangial cells. I. Acid extrusion in absence and presence of HCO₃⁻. *Am J Physiol* 1988;255:C844-C856.
- Gawenis LR, Greeb JM, Prasad V, Grisham C, Sanford LP, Doetschman T, Andringa A, Miller ML, Shull GE: Impaired gastric acid secretion in mice with a targeted disruption of the NHE4 Na⁺/H⁺ exchanger. *J Biol Chem* 2005;280:12781-12789.
- Sidani S, Kopic S, Socrates T, Kirchhoff P, Foller M, Murek M, Capasso A, Geibel JP: AMP-activated protein kinase: a physiological off switch for murine gastric acid secretion. *Pflugers Arch* 2009;459:39-46.
- Sidani SM, Kirchhoff P, Socrates T, Stelter L, Ferreira E, Caputo C, Roberts KE, Bell RL, Egan ME, Geibel JP: DeltaF508 mutation results in impaired gastric acid secretion. *J Biol Chem* 2007;282:6068-6074.
- Pfaffl MW: A new mathematical model for relative quantification in real-time RT-PCR. *Nucleic Acids Res* 2001;29:e45.
- Heitzmann D, Warth R: Physiology and pathophysiology of potassium channels in gastrointestinal epithelia. *Physiol Rev* 2008;88:1119-1182.
- Grady TP, Cheung LY: Microelectrode studies of Necturus antral mucosa: electrical potentials and resistances. *Am J Physiol* 1983;244:G71-G75.
- Ikari A, Sakai H, Tanaka A, Ikeda A, Inoue K, Takeguchi N: Prostaglandin E(2)-activated housekeeping Cl⁻ channels in the basolateral membrane of rat gastric parietal cells. *Jpn J Physiol* 1999;49:365-372.
- Kopic S, Corradini S, Sidani S, Murek M, Vardanyan A, Foller M, Ritter M, Geibel JP: Ethanol inhibits gastric acid secretion in rats through increased AMP-kinase activity. *Cell Physiol Biochem* 2010;25:195-202.
- Schubert ML: Gastric exocrine and endocrine secretion. *Curr Opin Gastroenterol* 2009;25:529-536.
- Lang PA, Schniepp R, Kirchhoff P, Socrates T, Sidani SM, Geibel JP: PI3 kinase dependent stimulation of gastric acid secretion by dexamethasone. *Cell Physiol Biochem* 2007;20:527-534.
- Rotte A, Bhandaru M, Foller M, Biswas R, Mack AF, Friedrich B, Rexhepaj R, Nasir O, Ackermann TF, Boini KM, Kunzelmann K, Behrens J, Lang F: APC sensitive gastric acid secretion. *Cell Physiol Biochem* 2009;23:133-142.
- Rotte A, Mack AF, Bhandaru M, Kempe DS, Beier N, Scholz W, Dicks E, Potzsch S, Kuhl D, Lang F: Pioglitazone induced gastric acid secretion. *Cell Physiol Biochem* 2009;24:193-200.
- Rotte A, Pasham V, Bhandaru M, Eichenmüller M, Yang W, Qadri SM, Kempe DS, Puchchakayala G, Pearce D, Birnbaum MJ, Lang F: Regulation of gastric acid secretion by PKB/Akt2. *Cell Physiol Biochem* 2010;25:695-704.