Original Paper

Cellular Physiology and Biochemistry

Cell Physiol Biochem 2010;25:195-202

Accepted: November 12, 2009

Ethanol Inhibits Gastric Acid Secretion in Rats Through Increased AMP-Kinase Activity

Sascha Kopic[1,2,*], Stefanie Corradini[1,2,*], Shafik Sidani[1], Michael Murek[1], Artur Vardanyan[1], Michael Föller[1,3], Markus Ritter[2] and John P. Geibel[1]

[1]Department of Surgery, Yale University, School of Medicine, New Haven, [2]Institute of Physiology and Pathophysiology, Paracelsus Medical University, Salzburg, [3]Institute of Physiology, Eberhard-Karls-University Tuebingen, Tuebingen, *S. Kopic and S. Corradini contributed equally

Key Words

Stomach • Parietal cell • H,K-ATPase • pH • BCECF • Alcohol

Abstract

The effects of ethanol on gastric acid secretion remain controversial. The present study examines the effect of low-dose (2%) short term (15-20min) ethanol exposure on gastric acid secretion via a potential interaction with AMP-activated protein kinase (AMPK). Real-time fluorescence digital imaging was used to provide functional evidence for the interaction of ethanol and AMPK in modulating secretagogue-induced acid secretion. Individual rat gastric glands were loaded with the pH-sensitive dye BCECF and the secretagogues carbachol (200 μM) or histamine (200 μM) were added to induce secretion. Rates of pH recovery were calculated as $\Delta pH_i/\Delta t$. In one series of experiments, secretagogue-induced acid secretion was inhibited by 2% ethanol, or the AMPK activator AICAR monophosphate (AICAR) (20 mM). In a separate series, 2% ethanol was added in combination with compound C (20 μM), an AMPK inhibitor, to prevent activation of AMPK. 2% ethanol significantly suppressed stimulated acid secretion. In order to confirm modulation of AMPK activity by ethanol, the specific AMPK inhibitor compound C was used, which reversed the inhibitory effects of ethanol on stimulated acid secretion. This study demonstrates that low dose ethanol (2%) inhibits secretagogue-dependent acid secretion by activation of the AMPK pathway in rat gastric parietal cells.

Introduction

The stomach produces large quantities of hydrochloric acid necessary for digestion. This production of acid is dependent on the delivery of protons (H^+) into the lumen of the gastric gland, where they combine with secreted Cl^- ions to produce concentrated acid (0.16 M). In contrast to the passive Cl^- flow through apical Cl^- channels [1], the proton extrusion by the parietal cell is an ATP-dependent process, performed by H^+,K^+-ATPase [2]. Upon stimulation by any one of the many different secretagogues, the parietal cell goes from the quiescent form to the active acid secretory form where the H^+,K^+-ATPase is trafficked from cytosolic tubulo-vesicular

KARGER

Fax +41 61 306 12 34
E-Mail karger@karger.ch
www.karger.com

1015-8987/10/0253-0195$26.00/0

Accessible online at:
www.karger.com/cpb

John P. Geibel, M.D., D.Sc.
Department of Surgery, Yale School of Medicine
BML 265, 310 Cedar Street, New Haven, 06511, CT (USA)
E-mail john.geibel@yale.edu

structures and is inserted into the apical membrane [3]. Three major pathways are known to activate H^+,K^+-ATPase in the parietal cell: 1) a neuronal, via the stimulation of the vagus nerve and the release of acetylocholine; 2) a hormonal, via gastrin-stimulated histamine release from ECL cells; and 3) a recently discovered pathway depending on stimulation of the calcium-sensing receptor (CaSR) on the basolateral membrane of the parietal cell [2, 4-8].

There has been a great deal of controversy as to the effects of ethanol on gastric acid secretion from being a mild stimulant to being a potent inhibitor [9-12]. However, the general consensus implicated that alcoholic beverages stimulate gastric acid secretion. Indeed, a series of groups observed a rise in histamine, gastrin and carbachol levels following wine and beer ingestion [13-16]. Paradoxically, this increase in secretagogue concentrations did not occur after consumption of equivalent amount of pure ethanol. The stimulatory effect of the beverages was therefore attributed to other ingredients than ethanol [13-16]. Various groups have thus set out to answer the question of whether pure ethanol influences gastric acid secretion. In summary, the results of this effort proved to be very controversial, but also indicated a dose dependency in the effects of ethanol on parietal cell function. Chacin et al. postulated that low-dose (2-20%) ethanol stimulates gastric acid secretion, whereas high concentrations (>20%) were shown to have an inhibitory effect [9]. A second group applying different concentrations (0,2-5% and >10%, respectively) came to the same conclusion [12]. In sharp contrast to these findings, other investigations concluded that acid secretion is reduced under low-dose conditions. Del Valle et al. not only demonstrated cessation of acid secretion following low-dose (1-20%) ethanol exposure, but also decreased ATP and cAMP concentrations in the affected parietal cells [10]. Niki et al. observed similar inhibition of carbachol- and histamine-triggered proton extrusion [11]. A concomitant ethanol-induced elevation in intracellular Ca^{2+} concentration was suggested, via desensitizing to the Ca^{2+} response triggered by carbachol, to be responsible for the inhibitory effects of ethanol on cholinergic stimulation [17, 18]. This model, however, does not explain impairment of histamine-induced acid secretion, which is mediated via the second messenger cAMP.

Ethanol metabolism mainly takes place in the liver, where it was recently described to have an effect on the activity of AMP-activated protein kinase (AMPK) [19]. AMPK is a multi-subunit protein kinase, activated by an increase in the AMP/ATP-ratio or as a response to other stress factors such as hypoxia, exercise, ischemia and inflammation [20, 21]. AMPK thereby acts as a cellular "energy sensor" that prevents ATP-depletion of the cell by down-regulating energy-consuming key-enzymes and by inducing pathways that generate ATP [20, 21]. Chronic ethanol exposure (4 weeks) was shown to inhibit AMPK in rat liver both *in vitro* and *in vivo*, with no major changes in the AMP/ATP-ratio [19]. However, after low-dose acute ethanol treatment, the liver AMP/ATP-ratio was reported to increase immediately, suggesting activation of AMPK [22].

Recently, we have observed that AMPK is involved in the inhibition of acid secretion in mouse stomach [23]. Preincubation of isolated gastric glands with 5-aminoimidazole-4-carboxamide 1-β-D-ribofuranoside monophosphate (AICAR), a potent activator of AMPK that mimics high intracellular AMP concentrations, resulted in significantly reduced secretagogue-induced acid secretion, whereas the reversible ATP-competitive AMPK inhibitor (6-(4-(2-piperidin-1-ylethoxy)phenyl))-3-pyridin-4-ylpyrazolo(1,5-a)pyrimidine (compound C) restored it. These data serve as evidence that AMPK is a physiological off-switch for gastric acid secretion in the murine parietal cell [23]. Since ethanol was shown to influence AMPK activity in the liver, we hypothesize that it modulates AMPK in the parietal cell and thereby affects acid secretion.

In order to address the controversy about the effects of ethanol on acid secretion, the present study seeks to answer if ethanol has direct effects on acid secretion which are linked to modulations in AMPK activity.

Materials and Methods

Animals

Sprague-Dawley rats 150-250 g (Charles River Laboratory) 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. Prior to experiments, animals were fasted for 12-18 h with free access to water.

Isolation of gastric glands

Animals were killed with an overdose of isoflurane and an abdominal incision was made. Following removal, the stomach was cut longitudinally; the corpus was isolated, sliced into 0.5 cm square sections and washed with ice-cold HEPES to remove residual food particles. The tissues were transferred to the stage of a dissecting microscope, and were prepared for dissection. Individual glands were isolated using a hand-dis-

section technique as described previously [4, 24, 25]. Following isolation, individual isolated glands were transferred to and allowed to adhere to 22x50mm cover slips that had been pre-coated with Cell-Tak (Collaborative Research, Bedford, MA) and were then transferred to the stage of an inverted microscope.

Digital imaging for intracellular pH measurements in isolated gastric glands

Isolated gastric glands were incubated in a HEPES-buffered Ringer's solution containing 10 µM of the pH-sensitive dye BCECF-AM [2',7'-bis-(2-carboxyethyl)-5-(and-6)-carboxyfluorescein, acetoxymethyl ester (Molecular Probes, Eugene, OR)] for 10 min as described previously [4, 24]. Following dye-loading, the chamber was flushed with a HEPES solution to remove all non-deesterfied dye. The perfusion chamber was mounted on the stage of an inverted microscope (Olympus IX50), which was used in the epifluorescence mode with a 60x objective. Individual regions of interest were outlined and simultaneously monitored during the course of the study. A minimum of seven cells or regions were selected per gland.

BCECF-loaded glands were excited at 440 nm ± 10nm and 490 nm ± 10nm, respectively, while monitoring the emission at 530 nm ± 10nm every 15 seconds. The ratio data were used to calculate the pH_i using the high K^+/nigericin calibration technique [4, 24, 26]. H^+ extrusion by individual parietal cells was monitored as recovery of pH_i, after acid loading the cells with a Na^+-free HEPES solution containing 20 mM NH_4Cl [24, 25]. Na^+-free conditions were chosen in order to exclude contribution of Na^+-dependent proton extrusion mechanisms such as the Na^+/H^+-Exchanger (NHE), thereby monitoring only H^+,K^+-ATPase activity as the only potential H^+ extrusion pathway. Intracellular pH_i recovery rates were calculated from the same initial starting pH to eliminate potential variation in the individual intracellular buffering power of the cells under different experimental conditions. The recovery rates are expressed as the $\Delta pH_i/\Delta t$, and were calculated over the pH range of 6.6 - 6.8. Trypan blue and a fluorescent LIVE/DEAD Cell Vitality Assay (Molecular Probes) were used to confirm that 2% ethanol did not destroy membrane integrity (data not shown). The composition of all solutions used is given in table 1. All experiments were performed in the nominal absence of bicarbonate. All chemicals were obtained from Sigma Chemicals unless otherwise stated.

Isolated perfused whole stomach pH measurements

Total gastrectomy was performed on wild-type rats and intragastral pH was measured as described previously [27, 28]. Briefly, gastrectomy was performed following anesthesia with isoflurane and ligation of both the gastroesophageal and gastroduodenal junctions. After excision, 500 µl of non-buffered saline was injected into the lumen of the isolated stomach. The whole stomach was placed in an oxygenated and heated (37°C) bath containing HEPES-buffered Ringer solution at pH 7.4. 200 µM histamine was added to the bath and ethanol at concentrations of 1, 2 and 5%, respectively, was added to the non-buffered saline used for injection. Following incubation for 60 minutes, the injected non-buffered saline solution was aspirated from the stomach and its pH was measured with a pH probe.

	Control	Na^+-free	NH_4Cl	High-K^+ calibration
NaCl	125	-	-	-
NMDG	-	125	125	125
NH_4Cl	-	-	20	-
KCl	3	3	3	105
$MgSO_4$	1.2	1.2	1.2	1.2
$CaCl_2$	1	1	1	1
Glucose	5	5	5	-
HEPES	32.2	32.2	32.2	32.2
pH (37 °C)	7.4	7.4	7.4	7.0

Table 1. Composition of solutions used for pH_i measurements. All concentrations are given in mM. NMDG is N-Methyl-D-Glucosamine. All solutions were titrated to pH 7.4 at 37 °C using either NaOH or KOH. NMDG was titrated with HCl.

Immunoblotting

Glands from the corpus of rat stomach were isolated as described elsewhere. The cells were disrupted using a sonicator and lysed in a lysis buffer composed of NaCl (150 mM), NaF (1 mM), Na_3VO_4 (1 mM), Triton X-100 (1%), sodium deoxycholate (0.5%), SDS (0.1%), Tris HCl (50 mM) pH 8; containing protease inhibitor [protease inhibitor cocktail tablets (Roche Diagnostics, Mannheim, Germany)]. The samples in lysis buffer were mixed with the appropriate volume of 5x Lämmli buffer (final concentration: 2% (w/v) SDS, 50 mM Tris/HCl, pH 6.8, 0.2 mg/ml bromphenol blue, 0.1 M DTT, 10% (v/v) glycerol) and incubated for 5 min at 95°C. Equal amounts of lysate protein (60 µg per lane) were separated by 8% discontinuous SDS-PAGE. Thereafter, proteins were transferred to polyvinylidene fluoride (PVDF) membranes (Millipore, Billerica, MA). Protein transfer was controlled by Ponceau red staining (not shown). After blocking with 5% nonfat milk in TBST (TBS-0.1% Tween 20) at room temperature for 1 h, the blots were probed overnight at 4°C with a commercial polyclonal rabbit anti-AMPKα antibody (Cell Signaling Technology, Danvers, MA; 1:1000 dilution in TBS-0.1% Tween 20-5% nonfat milk). After three times of washing with TBST for 10 min, the blots were incubated with a secondary anti-rabbit antibody (1:2000 dilution) conjugated with horseradish peroxidase (Amersham, Freiburg, Germany) for 1 h at room temperature. After three times of washing with TBST for 10 min, antibody binding was detected with the enhanced chemiluminescence (ECL, Amersham).

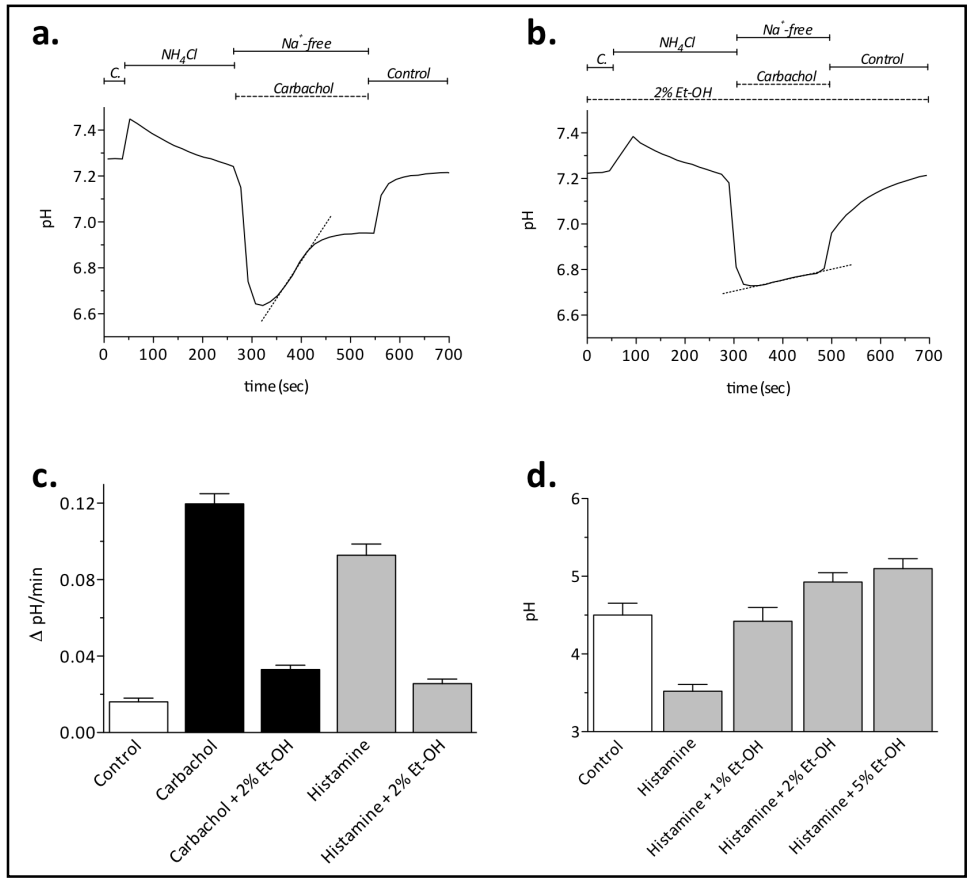

Fig. 1. Ethanol inhibits gastric acid secretion. a,b. Original tracings of intracellular pH measurements in single parietal cells of isolated rat gastric glands. Hand-dissected single rat gastric glands were loaded with the pH-sensitive dye BCECF and the recovery rate of pH_i was calculated from the slope (dashed line) after an acid load, using a NH_4Cl prepulse in the absence of Na^+. 200 µM carbachol has a stimulatory effect on gastric acid secretion (a.). Carbachol-stimulated acid secretion (200 µM) can be inhibited by 2% ethanol which was added to all solutions and the 10-min incubation bath prior to perfusion. c. Bar graph summarizing the inhibitory effects of ethanol on carbachol- and histamine-induced acid secretion in isolated rat gastric glands. The control shows a low basal proton efflux of the resting parietal cell in the absence of stimulatory agents (0.016 ± 0.001 ΔpH units/min) (n=63 cells/7 glands/7 rats). Carbachol (200 µM) causes a strong intracellular alkalinization (0.119 ± 0.005 ΔpH units/min) (n=129 cells/12 glands/12 rats). Histamine (200 µM) causes a lower rate of acid secretion (0.092 ± 0.005 ΔpH units/min) (n=53 cells/6 glands/5 rats). Carbachol-stimulated acid secretion (200 µM) is significantly reduced by addition of 2% ethanol to all solutions (0.033 ± 0.002 ΔpH units/min) (n=75 cells/9 glands/8 rats) ($p < 0.001$, respectively). Histamine-dependent proton efflux (200 µM) is also significantly reduced by 2% ethanol (0.025 ± 0.002 ΔpH units/min) (n=66 cells/8 glands/5 rats) ($p < 0.001$). Data are presented as mean ± SEM. d. Bar graph summarizing the inhibitory effects of ethanol on histamine-stimulated acid secretion in whole stomach preparations. Intragastric pH without secretagogue stimulation was 4.50 ± 0.154 (n=10 stomachs). Addition of the secretagogue histamine (200 µM) to the bath solution resulted in acidification of the intraluminal content to a pH of 3.52 ± 0.086 (n=41 stomachs). Despite stimulation of acid secretion by histamine (200 µM), injection of 1, 2 and 5% percent ethanol into the gastric lumen increased intragastric pH to 4.42 ± 0.177 (n=10 stomachs), 4.93 ± 0.119 (n=11 stomachs) and 5.10 ± 0.126 (n=10 stomachs), respectively. Data are presented as mean ± SEM.

Immunohistochemistry

Male Sprague-Dawley rats (150-250 g) were anesthetized with ketamine and perfused through the left ventricle with PBS followed by 4% paraformaldehyde–lysine–periodate (PLP) fixative [29]. Stomachs were removed, cleaned from food residues and fixed by immersion with PLP overnight at 4°C. Stomachs were subsequently washed three times with PBS and 5 µm cryosections were cut after cryoprotection with 2.3 M sucrose in PBS solution for at l east 12 h. Immunostaining was carried out as described previously [30]. 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 polyclonal rabbit anti-AMPKα antibody (Cell Signaling Technology) was diluted 1:100 in PBS and applied overnight at 4°C. Sections were then washed twice for 5 min with high-NaCl-PBS (PBS + 2.7% NaCl) and once with PBS and then incubated with a secondary anti-rabbit antibody at a dilution of 1:200 for 1 h at room temperature. Sections were again washed twice with high-NaCl-PBS and once with PBS before being mounted with Vecta-Mount (Vector Laboratories, Burlingame, CA). The specimens were viewed with a Zeiss LSM410 confocal laser scanning microscope.

Statistical analysis

All data are summarized as means of ΔpH_i/min of individual cells ± SEM. Unpaired Student's *t*-test was used to test for significant differences in pH_i recovery rates. Statistical significance was assumed at $p< 0.05$.

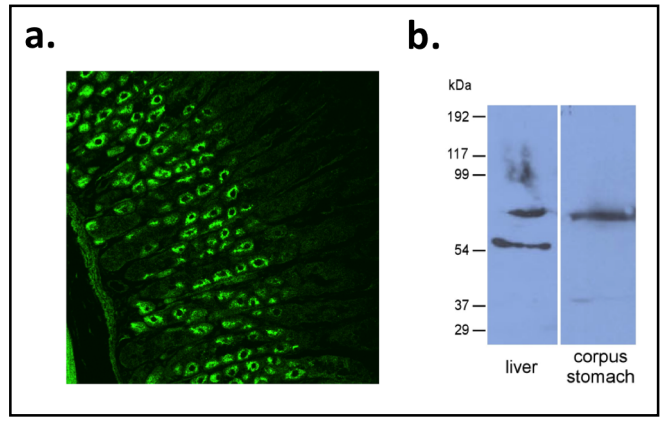

Fig. 2. Expression of AMPK in the corpus region of rat stomach. a. Immunohistochemistry in the corpus section of rat stomach. Immunolocalization of AMPK α-subunit (green) in the cytosol of gastric parietal cells. b. Western blot analysis demonstrating the presence of AMPK in rat stomach mucosa. A multiple tissue Western blot of proteins (~60 µg total protein per lane) isolated from gastric mucosa of rat corpus and parenchyma of rat liver. AMPK detection is reflected by a band of ~62 kDa in both samples.

Results

Stimulation of gastric acid secretion by secretagogues

Hand-dissected rat gastric glands were used for intracellular pH measurements to investigate the effects of ethanol on gastric acid secretion. In the absence of acid secretagogues the pH_i recovery rate is 0.016 ± 0.001 pH units/min (n=63 cells/7 glands/7 rats) (Fig. 1c). As shown in Figures 1a and c, in the presence of carbachol (200 µM) (n=129 cells/12 glands/12 rats) or histamine (200 µM) (n=53 cells/6 glands/5 rats) the pH_i recovery rate increases to 0.119 ± 0.005 and 0.092 ± 0.005 pH units/min, respectively, due to stimulated H^+,K^+-ATPase activity.

Inhibition of gastric acid secretion by ethanol in isolated gastric glands

Acute (10-20 min), low-dose (1-2%) treatment with ethanol significantly reduces gastric acid secretion. In the presence of 1% or 2% ethanol, the carbachol (200 µM) induced H^+ extrusion rate reduces to 0.036 ± 0.004 and 0.033 ± 0.002 pH units/min for 1% ethanol (n=43 cells/5 glands/3 rats) and 2% ethanol (n=75 cells/9 glands/8 rats) (Fig. 1b and c), respectively. There is no significant difference between inhibition with 1% or 2% ethanol (p = 0.908)(data not shown). In a separate series of experiments using histamine-stimulated (200 µM) cells, addition of 2% ethanol lowered H^+ extrusion to 0.025 ± 0.002 pH units/min (n=66 cells/8 glands/5 rats) (Fig. 1c).

Inhibition of gastric acid secretion by ethanol in isolated whole stomachs

Isolated stomach measurements were performed to investigate whether the inhibitory effects of ethanol observed at the single gland level are reproducible at the whole stomach level. Increasing concentrations of ethanol progressively increased luminal pH of the stomach (Fig. 1d). Intraluminal pH in the presence of 200 µM histamine reached 4.42 ± 0.177 (n=10 stomachs) for an ethanol concentration of 1%, 4.93 ± 0.119 (n=11 stomachs) for 2% ethanol and 5.10 ± 0.126 (n=10 stomachs) for 5% ethanol, respectively. Intraluminal pH in untreated stomachs was 4.50 ± 0.154 (n=10 stomachs). Addition of histamine to the bath reduced intraluminal pH to a value of 3.52 ± 0.086 (n=41 stomachs).

Expression and localization of AMPK in rat stomach

Western blot analysis of isolated rat gastric mucosa confirmed expression of AMPK in rat parietal cells (Fig. 2b). Controls were taken from rat liver, which expresses high amounts of AMPK. Identical molecular weight bands (~62kDa) were found in the proteins isolated from rat stomach and liver. The band at ~54kDa in the liver control is speculated to be a break-down product of AMPK. For localization of AMPK, immunohistochemistry was performed and showed a clear staining for AMPK (α-subunit) in the cytosol of rat parietal cells (Fig. 2a).

Inhibition of gastric acid secretion by AICAR

As observed previously in mouse parietal cells [23], 10 min preincubation with 20 mM AICAR, a specific activator of AMPK, significantly reduced carbachol-induced acid secretion to a rate of 0.015 ± 0.002 pH units/min (n=33 cells/4 glands/3 rats) (Fig. 3a and d).

Restoration of acid secretion by compound C and ATP

The specific AMPK inhibitor compound C (20 µM) was used in order to confirm modulation of AMPK activity by ethanol. Glands were simultaneously incubated with carbachol (200 µM) and 2% ethanol. As illustrated in Fig. 3b and d, 10 minute preincubation with compound C

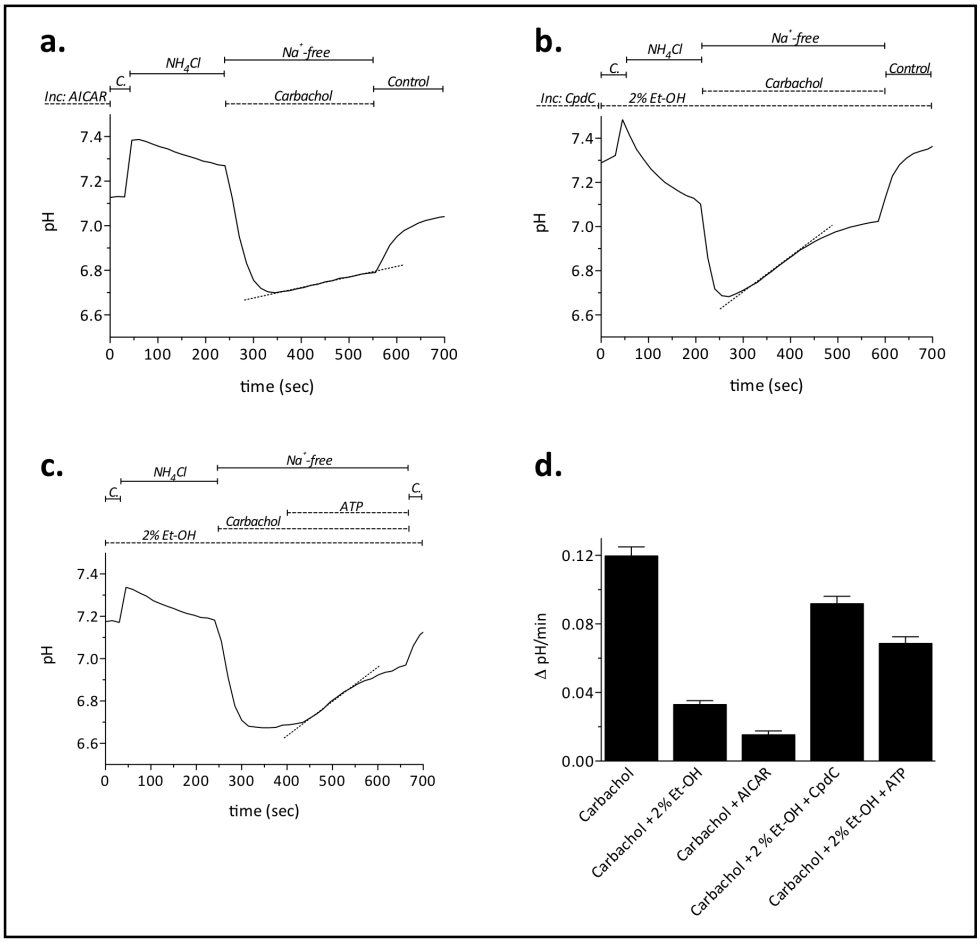

Fig. 3. Ethanol exerts its inhibitory effects through AMPK. a,b,c. Original tracings of intracellular pH measurements in single parietal cells of isolated rat gastric glands. AMPK acts as a molecular off-switch for gastric acid secretion: 10 min preincubation with the specific AMPK activator AICAR (20 mM) in the presence of carbachol (200 µM) completely inhibits stimulated acid secretion (a.). The inhibitory effects of 2% ethanol can be reversed by preincubating for 10min with the specific AMPK inhibitor Compound C (20 µM) (b.). ATP (2 mM) has an immediate effect on inhibited acid secretion (c.). Upon inhibition of acid secretion with 2% ethanol, ATP (2 mM) was added to the bath. Acid secretion was stimulated after a few seconds and resulted in an increase of intracellular pH. Carbachol (200 µM) was present through all experiments. d. Bar graph summarizing the reactivation of acid secretion by modulation of AMPK activity in isolated rat gastric glands. Carbachol-induced acid secretion (200 µM) (0.119 ± 0.005 ΔpH units/min) (n=129 cells/12 glands/12 rats) is significantly reduced by 2% ethanol (0.033 ± 0.002 ΔpH units/min) (n=75 cells/9 glands/8 rats) and a 10 min preincubation with the AMPK activator AICAR (20mM) (0.015 ± 0.002 ΔpH units/min) (n=33 cells/4 glands/3 rats) ($p < 0.001$, respectively). Inhibition of AMPK by a 10 min preincubation with compound C (20 µM) stimulated intracellular alkalinization and elevated pH_i (0.091 ± 0.004 ΔpH units/min) (n=91 cells/ 10 glands/6 rats). Addition of ATP (2mM) induced a rapid proton efflux (0.068 ± 0.003 ΔpH units/min) (n=60 cells/6 glands/4 rats). Data are presented as mean ± SEM.

reversed the inhibitory effect of ethanol. Under these conditions the recovery rate was 0.091 ± 0.004 pH units/min (n=91 cells/ 10 glands/6 rats).

A similar effect could be elicited by addition of 2 mM ATP in the presence of 2% ethanol and 200 µM carbachol. Activation of AMPK is a result of an increased AMP/ATP ratio and results in an inhibition of energy-consuming key-enzymes, such as H^+,K^+-ATPase [20, 21, 23]. As illustrated in Fig. 3c, d, addition of ATP to the bath solution immediately reverses the inhibitory effect of 2% ethanol on carbachol induced proton extrusion to a rate of 0.068 ± 0.003 pH units/min (n=60 cells/6 glands/4 rats).

Fig. 3d summarizes the mean carbachol-induced H^+ extrusion rates in the absence and presence of the different drugs.

Discussion

Alcohol consumption is a regular occurrence in a substantial portion of the population. As a consequence, numerous attempts have been made in the past to study its effects on the ability of the stomach to produce acid [9-12]. However, the results of these studies were very ambiguous in that no consensus has been reached as to whether ethanol inhibits or stimulates gastric acid secretion [9-12]. Beside the observation that there might be a biphasic effect of ethanol distinguishing between acute and chronic exposure [31, 32], the fact that many groups have employed remarkably high doses of ethanol (with up to 20% being classified as low-dose exposure) and different experimental approaches, ranging from

studies in cultured cells to observations on the whole-tissue level, may account for the heterogeneous past results [9-12]. In the present study we were able to demonstrate that ethanol at doses as low as 1% decreased acid secretion significantly both on the single-cell/gland and on the whole-stomach level. A toxic effect of ethanol on parietal cells at this dose was excluded directly by trypan blue or fluorescent LIVE/DEAD assay staining and indirectly by the reversibility of the ethanol-induced inhibition of gastric acid secretion.

Our group has previously demonstrated the role of AMPK in the inhibition of acid secretion in mouse stomach [23]. AMPK serves as a master regulatory protein kinase, sensing intracellular energy levels and down-regulating energy-consuming pathways upon imminent ATP-depletion, reflected by a high AMP/ATP-ratio [20, 21]. We suggested that AMPK serves as a physiological off-switch for gastric acid secretion in mouse parietal cells by decreasing the activity of the energy consuming H^+,K^+-ATPase [23]. Ethanol is known to be metabolized in both liver and stomach and was shown to interact with AMPK in the liver by decreasing its activity after long-term (4 weeks) exposure [19, 22, 33-35]. However, it has also been reported that acute ethanol exposure results in an immediate rise in AMP concentrations in liver cells [22, 36], which is known to act as an activation stimulus for AMPK [20, 21]. Since no scientific evidence for a concomitant activation of AMPK in response to this acute rise in the AMP/ATP ratio is available, one can only speculate about a putative biphasic effect of ethanol on AMPK activity. In light of these observations we therefore hypothesized that the inhibitory effect of ethanol on gastric acid secretion may be mediated by AMPK activation.

In the present study, expression of AMPK in rat gastric mucosa is confirmed through Western Blot analysis and immunohistochemical processing which demonstrated a cytosolic localization pattern of AMPK in the parietal cells. Exposure to the specific AMPK activator AICAR substantially decreased H^+,K^+-ATPase activity, suggesting that AMPK is significantly involved in the control of acid secretion, which confirms our previous findings in murine parietal cells [23]. Acute low-dose perfusion with ethanol (2%) inhibited acid secretion to a comparable level as did direct AMPK activation through AICAR. This series of studies was followed by treating glands simultaneously with 2% ethanol and the specific AMPK inhibitor compound C, with the aim of linking the observed ethanol-induced reduction of acid secretion to an interaction with AMPK. Following inhibition of AMPK by treatment with compound C, proton extrusion rates remained high despite exposure to ethanol. It was thus possible to demonstrate that the inhibitory effects of ethanol on gastric acid secretion can be reversed by impairing the activity of AMPK.

The results of this study therefore allow the assumption that short-term acute ethanol exposure increases AMPK activity and consequentially impairs acid secretion in rat parietal cells. Whether this effect is caused by a direct interaction between ethanol and AMPK or by an ethanol-induced decreased availability of ATP, which in consequence stimulates AMPK, remains subject of further investigations. However, ethanol and its metabolite acetate were shown to reduce available ATP and to increase AMP-levels in both liver and parietal cells [10, 22, 36] which putatively suggests AMPK activation by an ethanol-induced elevation in the AMP/ATP ratio. In the present study we were also able to overcome ethanol-induced, AMPK-mediated inhibition of acid secretion by increasing intracellular ATP concentrations. Although the exact mechanism of this ATP-stimulated reactivation of H^+,K^+-ATPase via AMPK remains to be elucidated, we speculate in accordance with other groups [10, 22, 36] that ethanol may increase the AMP/ATP ratio in the parietal cell, which acts as a primary activator for AMPK. This could explain why it was possible to overcome ethanol-induced inhibition of acid output by increasing intracellular ATP and thereby presumably lowering the intracellular AMP/ATP ratio.

In the present study, acute (15-20 min) low-dose (1-2%) ethanol was demonstrated to inhibit acid secretion in rat parietal cells. The inhibitory effect of ethanol could be reversed by inhibition of AMPK with compound C and high intracellular ATP concentrations. Given the high prevalence of dietary ethanol consumption in many populations, the finding that ethanol decreases gastric acid secretion has a direct implication for pharmacological agents whose pharmacokinetics are dependent on certain gastric pH conditions. Ethanol induced changes in these conditions could thereby result in an impaired uptake of these drugs. We thus emphasize the role of acute low-dose ethanol as a potent inhibitor of parietal cell proton extrusion and confirm the importance of AMPK as the physiological off-switch for acid secretion and a possible future pharmacological target.

References

1 Malinowska DH, Kupert EY, Bahinski A, Sherry AM, Cuppoletti J: Cloning, functional expression, and characterization of a pka-activated gastric cl- channel. Am J Physiol 1995;268:C191-200.

2 Shin JM, Munson K, Vagin O, Sachs G: The gastric hk-atpase: Structure, function, and inhibition. Pflugers Arch 2009;457:609-622.

3 Okamoto CT, Forte JG: Vesicular trafficking machinery, the actin cytoskeleton, and h+-k+-atpase recycling in the gastric parietal cell. J Physiol 2001;532:287-296.

4 Geibel JP, Wagner CA, Caroppo R, Qureshi I, Gloeckner J, Manuelidis L, Kirchhoff P, Radebold K: The stomach divalent ion-sensing receptor scar is a modulator of gastric acid secretion. J Biol Chem 2001;276:39549-39552.

5 Hebert SC, Cheng S, Geibel J: Functions and roles of the extracellular ca2+-sensing receptor in the gastrointestinal tract. Cell Calcium 2004;35:239-247.

6 Hersey SJ, Sachs G: Gastric acid secretion. Physiol Rev 1995;75:155-189.

7 Prinz C, Kajimura M, Scott D, Helander H, Shin J, Besancon M, Bamberg K, Hersey S, Sachs G: Acid secretion and the h,k atpase of stomach. Yale J Biol Med 1992;65:577-596.

8 Sachs G, Prinz C, Loo D, Bamberg K, Besancon M, Shin JM: Gastric acid secretion: Activation and inhibition. Yale J Biol Med 1994;67:81-95.

9 Chacin J, Cardenas P, Lobo P, Hernandez I: Secretory and metabolic effects of ethanol in the isolated amphibian gastric mucosa. Gastroenterology 1991;100:1288-1295.

10 del Valle JC, Salvatella M, Rossi I, Andrade R, Gutierrez Y, Pereda C, Samper B, Feliu JE: Impairment of h+-k+-atpase-dependent proton transport and inhibition of gastric acid secretion by ethanol. Am J Physiol Gastrointest Liver Physiol 2001;280:G1331-1340.

11 Niki S, Nakamura K, Rokutan K, Saitoh T, Aoike A, Kawai K: [effects of ethanol on acid secretion by isolated parietal cells from guinea pig]. Nippon Shokakibyo Gakkai Zasshi 1991;88:2094-2100.

12 Reichstein BJ, Okamoto C, Forte JG: Effect of ethanol on acid secretion by isolated gastric glands from rabbit. Gastroenterology 1986;91:439-447.

13 Intorre L, Bertini S, Luchetti E, Mengozzi G, Crema F, Soldani G: The effect of ethanol, beer, and wine on histamine release from the dog stomach. Alcohol 1996;13:547-551.

14 Peterson WL, Barnett C, Walsh JH: Effect of intragastric infusions of ethanol and wine on serum gastrin concentration and gastric acid secretion. Gastroenterology 1986;91:1390-1395.

15 Singer MV, Eysselein V, Goebell H: Beer and wine but not whisky and pure ethanol do stimulate release of gastrin in humans. Digestion 1983;26:73-79.

16 Singer MV, Leffmann C, Eysselein VE, Calden H, Goebell H: Action of ethanol and some alcoholic beverages on gastric acid secretion and release of gastrin in humans. Gastroenterology 1987;93:1247-1254.

17 Mutsukawa H, Zhao WL, Saitoh T, Natomi H, Sugano K: Ethanol elevates intracellular calcium in rabbit parietal cells. Gastroenterol Jpn 1991;26:544.

18 Niki S, Rokutan K, Nakamura K, Ogihara S, Kutsumi H, Saitoh T, Aoike A, Kawai K: [calcium-dependent signaling of acid secretion in isolated parietal cells from guinea pigs and its modification by ethanol]. Nippon Shokakibyo Gakkai Zasshi 1992;89:1484-1490.

19 You M, Matsumoto M, Pacold CM, Cho WK, Crabb DW: The role of amp-activated protein kinase in the action of ethanol in the liver. Gastroenterology 2004;127:1798-1808.

20 Hardie DG, Scott JW, Pan DA, Hudson ER: Management of cellular energy by the amp-activated protein kinase system. FEBS Lett 2003;546:113-120.

21 Hardie DG, Carling D, Carlson M: The amp-activated/snf1 protein kinase subfamily: Metabolic sensors of the eukaryotic cell? Annu Rev Biochem 1998;67:821-855.

22 Kono H, Bode JC, Martini GA: Effect of acute and chronic ethanol administration on the content of coenzymes linked to energy transfer in the liver of rats fed standard or low-protein diet. Gastroenterol Jpn 1979;14:226-232.

23 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.

24 Waisbren SJ, Geibel J, Boron WF, Modlin IM: Luminal perfusion of isolated gastric glands. Am J Physiol 1994;266:C1013-1027.

25 Waisbren SJ, Geibel JP, Modlin IM, Boron WF: Unusual permeability properties of gastric gland cells. Nature 1994;368:332-335.

26 Singh SK, Binder HJ, Geibel JP, Boron WF: An apical permeability barrier to nh3/nh4+ in isolated, perfused colonic crypts. Proc Natl Acad Sci U S A 1995;92:11573-11577.

27 Busque SM, Kerstetter JE, Geibel JP, Insogna K: L-type amino acids stimulate gastric acid secretion by activation of the calcium-sensing receptor in parietal cells. Am J Physiol Gastrointest Liver Physiol 2005;289:G664-669.

28 Kirchhoff P, Dave MH, Remy C, Kosiek O, Busque SM, Dufner M, Geibel JP, Verrey F, Wagner CA: An amino acid transporter involved in gastric acid secretion. Pflugers Arch 2006;451:738-748.

29 McLean IW, Nakane PK: Periodate-lysine-paraformaldehyde fixative. A new fixation for immunoelectron microscopy. J Histochem Cytochem 1974;22:1077-1083.

30 Wagner CA, Finberg KE, Stehberger PA, Lifton RP, Giebisch GH, Aronson PS, Geibel JP: Regulation of the expression of the cl-/anion exchanger pendrin in mouse kidney by acid-base status. Kidney Int 2002;62:2109-2117.

31 Treffot MJ, Laugier R, Bretholz A, Voirol M, Sarles H: Increased gastrin release in chronic calcifying pancreatitis and in chronic alcoholism. Horm Metab Res 1980;12:240-242.

32 Treffot MJ, Tiscornia OM, Palasciano G, Hage G, Sarles H: Chronic alcoholism and endogenous gastrin. Am J Gastroenterol 1975;63:29-32.

33 DiPadova C, Worner TM, Julkunen RJ, Lieber CS: Effects of fasting and chronic alcohol consumption on the first-pass metabolism of ethanol. Gastroenterology 1987;92:1169-1173.

34 Lim RT, Jr., Gentry RT, Ito D, Yokoyama H, Baraona E, Lieber CS: First-pass metabolism of ethanol is predominantly gastric. Alcohol Clin Exp Res 1993;17:1337-1344.

35 Yin SJ, Liao CS, Wu CW, Li TT, Chen LL, Lai CL, Tsao TY: Human stomach alcohol and aldehyde dehydrogenases: Comparison of expression pattern and activities in alimentary tract. Gastroenterology 1997;112:766-775.

36 Zydowo MM, Smolenski RT, Swierczynski J: Acetate-induced changes of adenine nucleotide levels in rat liver. Metabolism 1993;42:644-648.