Low pH enhances connexin32 degradation in the pancreatic acinar cell

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Reed AM, Kolodecik T, Husain SZ, Gorelick FS. Low pH enhances connexin32 degradation in the pancreatic acinar cell. Am J Physiol Gastrointest Liver Physiol 307: G24–G32, 2014. First published May 8, 2014; doi:10.1152/ajpgi.00010.2014.—Decreased extracellular pH is observed in a number of clinical conditions and can sensitize to the development and worsen the severity of acute pancreatitis. Because intercellular communication through gap junctions is pH-sensitive and modulates pancreatitis responses, we evaluated the effects of low pH on gap junctions in the rat pancreatic acinar cell. Decreasing extracellular pH from 7.4 to 7.0 significantly inhibited gap junctional intracellular communication. Acidic pH also significantly reduced levels of connexin32, the predominant gap junction protein in acinar cells, and altered its localization. Increased degradation through the proteasomal, lysosomal, and autophagic pathways mediated the decrease in connexin32 under low-pH conditions. These findings provide the first evidence that low extracellular pH can regulate gap junctional intercellular communication by enhancing connexin degradation.

cell pH; gap junctions; protein degradation; cell-cell interaction; pancreas; acinar cell

Acute and chronic reductions of extracellular pH are observed in a range of disease conditions and increase the risk and severity of acute pancreatitis. Using a cerulein model of pancreatitis, we have shown that reducing extracellular pH sensitizes to zymogen activation and cellular injury in vitro and pancreatitis in vivo (3). When exposed to acidic conditions, cells treated with a physiological, low dose of cerulein display pancreatitis responses similar to responses to supramaximal doses of cerulein (3, 39). However, the mechanism responsible for the injurious effects of lowering extracellular pH remains unclear. Low pH leads to enhanced connexin gating in many other cell types (28, 37) and inhibits GJIC in acinar cells (16). However, the effects of low pH on connexin protein levels are unknown. We investigated the hypothesis that low pH inhibits GJIC in pancreatic acinar cells by leading to enhanced Cx32 degradation.

MATERIALS AND METHODS

Preparation and stimulation of pancreatic acini. Groups of acinar cells (20–200 cells), called acini, or mechanically dissected pancreatic lobules, were isolated from the pancreas of rats that were euthanized in accordance with a protocol approved by the Veterans Affairs Animal Care and Use Committee and the Yale Institutional Animal Care and Use Committee, as described elsewhere (4). For Ca2+ signaling experiments and measurement of fluorescence recovery after photobleaching, smaller acinar groups of 5–15 cells were isolated as described elsewhere (39). For immunoblot and IF studies, acini were treated for 30 min with physiological (0.1 nM) or supramaximal (100 nM) cerulein in the presence or absence of 100 nM concanamycin, 5 μM MG-132, 10 μM lactacystin, 40 μM chloroquine, 500 nM cycloheximide, or 500 μM 3-methyladenine (all from Sigma, St. Louis, MO).

Fluorescence recovery after photobleaching. Isolated acini were loaded for 15 min with 5 μM 5(6)-carboxyfluorescein diacetate at room temperature and washed with HEPES buffer medium to prevent further dye loading. Cells were then placed on a perfusion slide that was mounted on the stage of a confocal microscope (LSM 510, Zeiss, Thornwood, NY) and perfused for 10 min with buffer at pH 7.4 or 7.0. A krypton-argon laser was used to excite the dye at 488 nm, and emission signals >515 nm were collected. The laser was briefly focused on a single acinar cell within an intact acinus to selectively photobleach the fluorescein in that cell. Then an image of the entire field was obtained each second for 5 min.

Fluorescence intensity in the photobleached acinar cell was normalized by fluorescence intensity measured in nonbleached reference cells in the same field of view to account for decreases in fluorescence due to dye loss from or bleaching of the entire field. The resulting fluorescence recovery pattern was fit to a single exponential curve using nonlinear least-squares regression with a quasi-Newton algorithm. The fluorescence recovery curve was of the following form: F(t) = F∞(1 − e−kt) + F0, where F(t) is fluorescence measured in the bleached acinar cell at time t, F∞ is fluorescence in that cell immediately after bleaching, F0 is the net amount of fluorescence recovered...
at steady state, and \( k \) is the constant that describes the rate of fluorescence recovery.

Detection of cellular \( \text{Ca}^{2+} \) signals. Acini were incubated for 30 min with the \( \text{Ca}^{2+} \)-sensing dye fluo 4-AM (6 \( \mu \)M; Molecular Probes, Eugene, OR). Acinar cells were then perfused with ceruline (0.1 nM) after a 5-min pretreatment with buffer at pH 7.4 or 7.0. \( \text{Ca}^{2+} \) signals were visualized using a confocal microscope (LSM 510 NLO, Zeiss) at \( \times 20 \) or \( \times 63 \) magnification and 1.4 numerical aperture. An argon laser was used to excite the dye at 488-nm wavelength, and emission signals >515 nm were collected. Variability of \( \text{Ca}^{2+} \) oscillation frequency was defined as follows: (highest-frequency oscillation – lowest-frequency oscillation)/highest-frequency oscillation within each intact acinus.

Immunoblot. At the end of the experiment, samples (500 \( \mu \)l of media and cells) were collected, and \( 6 \times \) SDS-PAGE loading buffer (100 \( \mu \)l) was added to each sample. Samples were heated to 95\(^\circ\)C for 5 min or until cell pellets were dissolved with gentle periodic mixing. Protein concentration was determined using a Pierce 660-nm protein assay reagent (Thermo Scientific, Rockford, IL) supplemented with ionic detergent compatibility reagent (Thermo Scientific). Within each experiment, equal protein (50 \( \mu \)g) for each sample (1, 9) was separated on SDS-polyacrylamide gels (AnyKyd, Bio-Rad, Hercules, CA) and transferred to Immobilon-P membranes (Millipore, Billerica, MA), which were blocked for 1 h at room temperature with Blotto (Tris-buffered saline (TBS), 5% nonfat dry milk, and 0.05% Tween 20). Membranes were then probed with anti-Cx32 (rabbit; Sigma, St. Louis, MO) or anti-LC3 (rabbit; Cell Signaling, Danvers, MA) antibody in Blotto for 1 h at room temperature, washed, and then probed with horseradish peroxidase-labeled goat anti-rabbit IgG (Sigma) for 1 h at room temperature. After treatment with the secondary antibody, membranes were washed in TBS, and autoradiography was performed using RapidStep ECL reagent (Calbiochem, a division of EMD Millipore).

Immunofluorescence. Lobules were described as described above and frozen in optimal temperature cutting compound (OCT, Sakura Finetek, Torrance, CA). Frozen sections were cut and fixed on individual slides with 4% paraformaldehyde in TBS. After fixation, samples were permeabilized with TBS containing 0.05% saponin [immunofluorescence (IF) buffer] for 15 min and then quenched/blocking in IF buffer with 0.5 M ammonium chloride and 3% goat serum for 30 min. Slides were then washed three times with IF buffer, and primary antibodies were added to IF buffer with 3% goat serum for 1 h at room temperature. Primary antibodies were as follows: Cx32 [rabbit (Sigma) or mouse (Millipore)] and double-labeled for LC3 (goat; Santa Cruz Biotechnology, Dallas, TX), ubiquitin (mouse; Enzo Life Sciences, Farmingdale, NY), or E-cadherin (BD Transduction Laboratories, San Jose, CA). Slides were then washed three times with IF buffer. Secondary antibody (goat anti-rabbit IgG labeled with Alexa 488, goat anti-mouse IgG labeled with Alexa 555, or donkey anti-rabbit labeled with Alexa 555) was then added for 1 h at room temperature, and sections were mounted using Vectashield hard-set fluorescent mounting medium with 4′,6-diamidino-2-phenylindole (Vector Laboratories, Burlingame, CA). Images were captured using a Spot camera mounted on a Zeiss Axioskop fluorescence microscope.

Electron microscopy. Lobules were described as described above and cut into 1- to 2-\( \mu \)m sections. Tissue samples were dissected out and fixed in 4% paraformaldehyde-0.1% glutaraldehyde in PBS for 30 min and then in 4% paraformaldehyde for 1 h. The samples were rinsed in PBS and placed in 2.3 M sucrose overnight on a rotor at 4\(^\circ\)C, transferred to aluminum pins, and frozen rapidly in liquid nitrogen. The frozen blocks were cut on an ultramicrotome (Leica Cryo-EMUC6 UltraCut), and 65-nm-thick sections were collected using the Tokoyasu method (42), placed on carbon/formvar-coated grids, and floated in a dish of PBS for immunolabeling.

Grids were placed section-side-down on drops of 0.1 M ammonium chloride to quench untreated aldehyde groups and then blocked for nonspecific binding on 1% fish skin gelatin in PBS. Single-labeled grids were incubated on a primary antibody, mouse anti-Cx32 (Sigma), at 1:200 dilution, which required a rabbit anti-mouse bridge (Jackson ImmunoResearch Laboratories, West Grove, PA). Protein A-gold (10 nM; Utrecht Medical Center) was used as a secondary antibody.

RESULTS

Low pH inhibits GJIC. To determine whether low pH inhibits GJIC, cell-to-cell dye transfer experiments were performed. Isolated acinar cells were loaded with 5 \( \mu \)M (6)-carboxyfluorescein diacetate, which is known to move between adjacent cells through gap junctions. Individual cells within intact acini were photobleached, and fluorescence recovery was assessed. Figure 1, A and B, depicts the fluorescence recovery in an individual acinar cell perfused with pH 7.4 buffer. Figure 1, C and D, shows that the percent recovery and rate constant are significantly lower in cells treated with pH 7.0 buffer than in cells treated with pH 7.4 buffer. Although 0.1 nM ceruline alone does not affect recovery characteristics compared with buffer, the percent recovery and rate constant were significantly lower in cells treated with 0.1 nM ceruline at pH 7.0 than in all other cells.

To confirm the inhibitory effects of low pH on GJIC, we developed an assay that quantifies \( \text{Ca}^{2+} \) signal synchronization (Fig. 1E). Ceruline-induced \( \text{Ca}^{2+} \) oscillations are normally synchronized within the acinus, as \( \text{Ca}^{2+} \) and inositol 1,4,5-trisphosphate diffuse through gap junctions (13). Therefore, inhibition of GJIC is expected to cause \( \text{Ca}^{2+} \) signal asynchrony in intact acini. To determine if acidic pH affected \( \text{Ca}^{2+} \) signal synchrony, individual cells within intact acini were examined for variability of oscillation frequency. As demonstrated in Fig. 1E, there was significantly more variability in the frequency of oscillations in cells treated with 0.1 nM ceruline at pH 7.0 (32%) than in cells treated at pH 7.4 (14%). These studies show that decreasing pH from 7.4 to 7.0 inhibits GJIC in acinar cells.

Low pH decreases Cx32 levels. GJIC is regulated by two factors: the number of gap junction channels expressed at the cell membrane and the gating of these channels. The number of channels is dynamic because of the short half-life of connexin, as depicted in Fig. 2A. Figure 2B illustrates the effects of low pH on Cx32 levels as evaluated by immunoblot analysis. Lowering buffer pH from 7.4 to 7.0 had no effect on unstimulated acini. However, low pH significantly decreased Cx32 levels in cells treated with 0.1 and 100 nM ceruline. To determine whether the effects of low pH were mediated by increased degradation of Cx32 or decreased Cx32 synthesis, the experiment was repeated in the presence of 500 nM cycloheximide, which inhibits protein synthesis (Fig. 2C). The pattern of Cx32 expression in the presence of cycloheximide was similar to that in the absence of cycloheximide, suggesting that low pH causes increased Cx32 degradation, rather than decreased synthesis. These results indicate that although low pH alone does not impact channel number, it sensitizes to connexin degradation.

The effects of low pH on Cx32 localization were evaluated by IF (Fig. 3) in acini labeled for Cx32 and the cell-cell adhesion protein E-cadherin, which localizes to the plasma membrane in control conditions. In unstimulated acini, at pH 7.4 and 7.0, punctate Cx32 labeling was seen at the appositional surface of the plasma membrane, with staining similar to that reported by others (8). A punctate pattern of Cx32 staining was observed at pH 7.0 with 0.1 nM ceruline.
at the plasma membrane was also seen in cells treated with 0.1 nM cerulein at pH 7.4. However, at pH 7.0, the normal physiological pattern of Cx32 expression was less prominent, and a hazy intracellular pattern of staining was seen. This pattern of Cx32 staining is similar to that observed under pathological conditions with high-dose cerulein at pH 7.4 and 7.0.

The effects of low pH on gap junction structure were also evaluated by immunoelectron microscopy (Fig. 4). In unstimulated controls at pH 7.4 and 7.0, dense gap junction complexes with abundant Cx32 labeling are seen bridging neighboring cells. This normal gap junction structure is also seen with 0.1 nM cerulein at pH 7.4. However, with 0.1 nM cerulein at pH 7.0, there is separation of adjacent acinar cells with loss of gap junction complexes at the plasma membrane. Cx32 labeling is sparse and often only present on one side of the plasma membrane. This lack of organized gap junctions is similar to that seen with supramaximal cerulein treatment.

Low pH enhances Cx32 degradation. Gap junction degradation can occur through several mechanisms, including the lysosomal, autophagic, and proteasomal pathways. Because low pH enhances Cx32 degradation, we evaluated the effects of low pH on various degradation pathways. Figure 5, A and B, shows a significant increase in Cx32 levels at pH 7.4 and 7.0 in untreated acini pretreated with the lysosomal inhibitors concanamycin and chloroquine, suggesting that the lysosomal pathway is involved in Cx32 turnover in the baseline state. Lysosomal inhibition also significantly increased Cx32 levels in cells subjected to physiological stimulation. The dramatic decrease in Cx32 levels in acini treated with 0.1 nM cerulein at

Fig. 1. Acidic extracellular pH inhibits gap junctional intercellular communication (GJIC). A: serial confocal images of an acinus before and after photobleaching demonstrate fluorescence recovery. B: fluorescence of the photobleached cell normalized to that of a nonbleached cell. Recovery pattern was described by 1st-order kinetics using nonlinear regression as described in MATERIALS AND METHODS. C: percent recovery of baseline fluorescence. D: rate constant (k). E: changes in synchrony of Ca2+ signaling among acinar cells within an intact acinus treated with 0.1 nM cerulein (CER). Variability is expressed as percentage of the fastest oscillation frequency within a cluster of intact acini. Values are means ± SE of 3 separate experiments. *P < 0.05 vs. corresponding treatment at pH 7.4.
pH 7.0 was abrogated with lysosomal inhibition. Similarly, there was a significant increase in Cx32 levels in cells treated with high-dose cerulein at pH 7.4 and 7.0. Because the lysosomal pathway appears to be involved in Cx32 degradation in physiological and pathological conditions, we also evaluated the autophagic pathway, which terminates in lysosomal proteolysis. Figure 6, A and B, shows that inhibition of the autophagic pathway with 3-methyladenine pretreatment leads to increases in Cx32 in all treatment groups, suggesting that autophagic degradation modulates Cx32 turnover in baseline, physiological, and pathological conditions in the acinar cell. To confirm the role of autophagy in Cx32 degradation, cells were double-labeled with Cx32 and LC3 and studied using IF (Fig. 6D). There was little Cx32-LC3 colocalization in control cells at pH 7.4 and 7.0 and cells treated with 0.1 nM cerulein at pH 7.4. However, colocalization of Cx32 and LC3 was increased in cells treated with 0.1 nM cerulein at pH 7.0 and 100 nM cerulein at pH 7.4 and 7.0, confirming the role of autophagic degradation in decreasing Cx32 levels under these conditions.

Finally, Fig. 7 evaluates the contribution of the proteasomal pathway to Cx32 degradation in two ways. First, in Fig. 7, A and B, cells were pretreated with the proteasomal inhibitors lactacystin and MG-132, and Cx32 was measured by immunoblot analysis. In untreated acini at pH 7.4 and 7.0, pretreatment with concanamycin, when cells were treated with 0.1 nM cerulein at pH 7.0 and 100 nM cerulein at pH 7.4 and 7.0, levels of LC3-II are reduced on immunoblot analysis. Therefore, in these conditions, the number of autophagosomes is reduced. This decrease could result from two distinct scenarios: lack of autophagy with decreased production of autophagosomes or increased autophagic flux with rapid consumption of LC3-II (31). To distinguish between these possibilities, cells were pretreated with concanamycin, which inhibits lysosomal degradation, thereby causing accumulation of LC3-II in conditions where autophagic flux is increased. In cells treated with 0.1 nM cerulein at pH 7.0 and 100 nM cerulein at pH 7.4 and 7.0, pretreatment with concanamycin caused an increase in LC3-II levels. These data suggest that the decrease in Cx32 levels in these treatment groups is mediated, in part, by increased autophagic consumption. To verify the role of autophagy in Cx32 degradation, cells were double-labeled with Cx32 and LC3 and studied using IF (Fig. 6D). There was little Cx32-LC3 colocalization in control cells at pH 7.4 and 7.0 and cells treated with 0.1 nM cerulein at pH 7.4. However, colocalization of Cx32 and LC3 was increased in cells treated with 0.1 nM cerulein at pH 7.0 and 100 nM cerulein at pH 7.4 and 7.0, confirming the role of autophagic degradation in decreasing Cx32 levels under these conditions.

Finally, Fig. 7A evaluates the contribution of the proteasomal pathway to Cx32 degradation in two ways. First, in Fig. 7A and B, cells were pretreated with the proteasomal inhibitors lactacystin and MG-132, and Cx32 was measured by immunoblot analysis. In untreated acini at pH 7.4 and 7.0, pretreatment with concanamycin, when cells were treated with 0.1 nM cerulein at pH 7.0 and 100 nM cerulein at pH 7.4 and 7.0, levels of LC3-II are reduced on immunoblot analysis. Therefore, in these conditions, the number of autophagosomes is reduced. This decrease could result from two distinct scenarios: lack of autophagy with decreased production of autophagosomes or increased autophagic flux with rapid consumption of LC3-II (31). To distinguish between these possibilities, cells were pretreated with concanamycin, which inhibits lysosomal degradation, thereby causing accumulation of LC3-II in conditions where autophagic flux is increased. In cells treated with 0.1 nM cerulein at pH 7.0 and 100 nM cerulein at pH 7.4 and 7.0, pretreatment with concanamycin caused an increase in LC3-II levels. These data suggest that the decrease in Cx32 levels in these treatment groups is mediated, in part, by increased autophagic consumption. To verify the role of autophagy in Cx32 degradation, cells were double-labeled with Cx32 and LC3 and studied using IF (Fig. 6D). There was little Cx32-LC3 colocalization in control cells at pH 7.4 and 7.0 and cells treated with 0.1 nM cerulein at pH 7.4. However, colocalization of Cx32 and LC3 was increased in cells treated with 0.1 nM cerulein at pH 7.0 and 100 nM cerulein at pH 7.4 and 7.0, confirming the role of autophagic degradation in decreasing Cx32 levels under these conditions.
LOW pH ENHANCES Cx32 DEGRADATION

Fig. 4. Acidic extracellular pH causes loss of gap junction complexes. Pancreatic lobules were treated with 0.1 or 100 nM cerulein for 30 min and then labeled with anti-Cx32 primary antibody and protein A-gold secondary antibody. Sixty fields per treatment were examined by electron microscopy. Representative images of gap junctions are shown.

DISCUSSION

Although many other studies have shown that low pH inhibits GJIC and enhances connexin gating, this study provides the first evidence that extracellular pH can regulate connexin levels. Specifically, our data show that low pH decreases Cx32 levels by enhancing Cx32 degradation. Although not addressed in this study, abundant data from studies of pancreatic acinar cells and other cell types demonstrate that low pH inhibits GJIC by causing pore closure in gap junction hemichannels composed of Cx32 or other connexins (7, 12, 16, 28, 37, 44, 46). Our fluorescence recovery after photobleaching and Ca^2+ signal asynchrony data confirm that low pH inhibits GJIC in acinar cells. Decreasing buffer pH from 7.4 to 7.0 in untreated acini leads to a significant decrease in percent recovery and rate constant. Because this treatment is not associated with a decrease in Cx32 levels, the effects of low buffer pH on GJIC can be attributed to enhanced Cx32 gating with low pH. In the other treatment groups, it is difficult to determine the individual contributions of increased gating and decreased Cx32 expression to the overall decrease in GJIC associated with low pH.

Our data on acini treated with low-dose cerulein provide the best illustration of the rapid and dramatic effects of low pH on Cx32 expression. Physiological stimulation with 0.1 nM cerulein at pH 7.4 produces a significant increase in Cx32 levels. It is possible that increased Cx32 expression is necessary to coordinate the robust secretory activities of the acinar cell in physiological conditions. The increased expression of Cx32 is likely mediated by an increase in Cx32 synthesis, given that cycloheximide treatment reduced the increase in Cx32 expression. However, cycloheximide did not entirely inhibit the increase in Cx32 seen with low-dose cerulein. Therefore, decreased Cx32 degradation, evidenced by the suppression of the proteasomal degradation pathway with low-dose cerulein, also likely contributes to the increase in Cx32 levels at pH 7.4. In contrast, low-dose cerulein at pH 7.0 causes a significant decrease in Cx32 expression to levels comparable to those seen...
with supraphysiological stimulation. Similarly, Cx32 IF and electron microscopy of gap junctions are similar in cells treated with low-dose cerulein at pH 7.4 and cells treated with supraphysiological doses of cerulein. Therefore, lowering pH from 7.4 to 7.0 converts a physiological pattern of Cx32 expression to a pathological one.

Low pH enhances degradation of Cx32 through the proteasomal, lysosomal, and autophagic pathways. In other cell types, low extracellular pH has been shown to sensitize to proteasomal degradation. In cultured myocytes, for example, decreasing pH from 7.4 to 7.1 increased mRNAs for ubiquitin and the C2 proteasomal unit. However, similar to our data, the

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**Fig. 6.** Acidic extracellular pH enhances Cx32 degradation through the autophagic pathway. 

A and B: isolated acini were pre-treated for 1 h with 500 μM 3-methyladenine (3-MA) and then for 30 min with 0.1 or 100 nM cerulein at pH 7.4 or 7.0. Samples were then probed by immunoblot with anti-Cx32 antibody. Values are means ± SE; n = 3. *P < 0.05 vs. corresponding treatment without 3-MA. C: isolated acini were treated for 30 min with 0.1 or 100 nM cerulein in the presence or absence of 100 nM concanamycin (1 h pretreatment) and then probed by immunoblot with anti-LC3 antibody. D: pancreatic lobules were treated with 0.1 or 100 nM cerulein at pH 7.4 or 7.0 for 30 min. Pancreatic tissue was then probed for Cx32 and LC3. Insets: higher magnification of areas in boxes. Representative images for 3 separate experiments are shown.
enhanced protein degradation associated with reduced extracellular pH required an additional stimulus (14). Extracellular acidification has also been shown to affect lysosomal trafficking in human mammary epithelial cells. In these studies, low pH altered lysosome number, size, and localization, with an overall effect of facilitating lysosomal exocytosis and enhancing secretion of degradative enzymes (10). Although our data suggest that low pH enhances autophagic degradation in the acinar cell, another study has shown that low pH can inhibit autophagy in other cell types (48). Whether this discrepancy involves the difference in pH used in the two studies, special regulation of Cx32, or other factors requires further investigation.

Although it is likely that low pH has direct effects on degradation pathways, indirect effects are also possible. We previously showed that low pH enhances Ca$^{2+}$ signals in the acinar cell in the presence of low-dose cerulein (39). Lysosomal and proteasomal degradation of Cx43 has been shown to be Ca$^{2+}$-dependent, involving PKC (24, 25, 27). Ca$^{2+}$-dependent phosphorylation of Cx32 by PKC (5) is also known to regulate Cx32 degradation. Effects of low pH on cerulein-induced Ca$^{2+}$ signaling may explain why decreasing extracellular pH affects Cx32 levels only in the presence of cerulein. Additionally, the cell adhesion molecule E-cadherin regulates connexin assembly at the plasma membrane in a Ca$^{2+}$-dependent manner (17, 40, 47). Our IF studies show that low pH causes E-cadherin disorganization, which may in turn affect gap junction structure and function.

Our studies, which mirror the magnitude pH changes in patients with acidosis, have relevance to acute pancreatitis.

**Fig. 7.** Acidic extracellular enhances Cx32 degradation through the proteasomal pathway. A and B: isolated acini were pretreated for 1 h with 10 μM lactacystin or 5 μM MG-132 and then for 30 min with 0.1 or 100 nM cerulein at pH 7.4 or 7.0. Samples were then probed by immunoblot with anti-Cx32 antibody. Values are means ± SE; n = 3. *P < 0.05 vs. corresponding treatment without lactacystin or MG-132. C: pancreatic lobules were treated with 0.1 or 100 nM cerulein at pH 7.4 or 7.0 for 30 min, and pancreatic tissue was probed for Cx32 and ubiquitin. Insets: higher magnification of areas in boxes. Representative images for 3 separate experiments are shown.
Acidosis increases the risk and severity of pancreatitis in humans and has been shown to sensitize to pancreatitis responses in vivo and in vitro animal studies (3, 19, 22, 35, 36, 38, 41). Disruption of acinar cell gap junctions by global Cx32 knockout increases the severity of acute cerulein-induced pancreatitis. The present study links these two observations. Low pH inhibits GJIC and disrupts gap junction structure and, therefore, may be one mechanism involved in the injurious effects of low pH on the acinar cell. This newly described pathway may be relevant to other tissues and disease processes. Thus metabolic acidosis increases the severity or the risk of a number of other diseases, including cardiac arrhythmias, osteoporosis, and chronic kidney disease, which also involve perturbed gap junction function/structure (11, 18, 21, 30, 34, 43).

In summary, the current work provides the first evidence that low pH can regulate gap junction structure by enhancing connexin degradation. Specifically, low pH enhances Cx32 degradation through the lysosomal, proteasomal, and autophagic pathways. Further work is needed to determine the mechanisms responsible for these effects and evaluate the potential importance of these responses in other tissues.

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DISCLOSURES

No conflicts of interest, financial or otherwise, are declared by the authors.

AUTHOR CONTRIBUTIONS


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