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Reducing extracellular pH sensitizes the acinar cell to secretagogue-induced pancreatitis responses in rats

Madhavi Bhoomagoud[1], **Thomas Jung**[1], **Jorunn Atladottir**[4], **Thomas R Kolodecik**[1], **Christine Shugrue**[1], **Anamika Chaudhuri**[1], **Edwin C Thrower**[1], and **Fred S Gorelick**[1,2,3]

[1]Section of Digestive Diseases, Department of Internal Medicine, Yale University School of Medicine, New Haven, Connecticut

[2]Department of Veterans Affairs Connecticut Healthcare, West Haven, Connecticut

[3]Department of Cell Biology, Yale University School of Medicine, New Haven, Connecticut

[4]Department of Surgery, Yale University School of Medicine, New Haven, Connecticut

Abstract

Background and Aims—Protease activation within the pancreatic acinar cell is a key early event in acute pancreatitis and may require low pH intracellular compartments. Clinical studies suggest that acidosis may affect the risk for developing pancreatitis. We hypothesized that exposure to an acid load might sensitize the acinar cell to secretagogue-induced pancreatitis.

Methods—Secretagogues (cerulein, carbachol and bombesin) can induce protease activation in acinar cells at high (100 nM, 1 mM and 10 μM respectively) but not at physiologically relevant concentrations. The effects of decreasing extracellular pH (pHe) in early secretagogue-induced pancreatitis (zymogen activation and injury) were examined in rats: 1) *in vitro* with isolated acini; 2) *in vivo* with an acid challenge.

Results—In acini, lowering pHe from 7.6 to 6.8 enhanced secretagogue-induced zymogen activation and injury, but did not affect secretion. For cerulein, this sensitization was seen over a range of concentrations (0.01-100 nM). However, reduced pHe alone had no effect on zymogen activation, amylase secretion or cell injury. We have reported that zymogen activation is mediated by the vacuolar ATPase (vATPase), a proton transporter. vATPase inhibition, using concanamycin (100 nM), blocked the low pHe effects on zymogen activation. An acute acid load given *in vivo* enhanced cerulein (50 μg/kg) induced trypsinogen activation and pancreatic edema.

Conclusion—These studies suggest that acid challenge sensitizes the pancreatic acinar cell to secretagogue-induced zymogen activation and injury and may increase the risk for the development and severity of acute pancreatitis.

Corresponding Author: Fred Gorelick, MD, Corresponding Author's Address and Email: Room # D 226, Bldg.5 VA Medical Center 950 Campbell Avenue West Haven, CT 06516. Fred.gorelick@yale.edu, Phone: 203-932-5711 extension 3680; Fax: 203-937-3852, Corresponding Author's Institution: West Haven VA Medical Center.

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Introduction

Premature activation of digestive zymogens, particularly proteases, within the pancreatic acinar cell is a key step in initiating acute pancreatitis, but the mechanisms of activation remain unclear. Cholecystokinin (CCK) or its orthologue cerulein, at concentrations 10-100 fold greater than that generated by the physiologic response to a meal, cause zymogen activation in the acinar cell and are used to generate experimental pancreatitis both *in vivo* and *in vitro*.[1, 2]

Experimental and clinical observations that link low pH environments to acute pancreatitis include: 1) Acidic vacuoles are generated within the pancreatic acinar cell in both the CCK hyperstimulation and the choline-deficient, methionine-supplemented diet models of acute pancreatitis;[3] 2) The two major proposed mechanisms for pathologic trypsinogen activation, activation by cathepsin B and trypsinogen autoactivation, have an acidic pH optimum;[4] 3) Chloroquine, an agent that raises intracellular pH, ameliorates experimental pancreatitis, [5, 6] and also blocks zymogen activation in acini;[7] 4) A proton pump, the vacuolar ATPase (vATPase), regulates zymogen activation in the acinar cell;[7] 5) Clinical conditions causing acidosis such as diabetic ketoacidosis, organic acidemias and HIV therapy-induced lactic acidosis are linked to acute pancreatitis. Based on these observations, we hypothesized that an acid load might promote the development of acute pancreatitis.

In the present study, the effects of low pH on zymogen activation, amylase secretion and cell injury were examined in dispersed pancreatic acini and *in vivo* in rats. We demonstrate that lowering extracellular pH (pHe) alone has no effect, but reduced pHe enhanced secretagogue-induced zymogen activation and injury in acinar cells. The effects of reduced pHe on zymogen activation in acini were mediated by vATPase. Similarly, an acute acid load *in vivo* sensitized rats to the development of early cerulein-induced pancreatitis. These findings suggest that low pH environments might play an important role in the pathogenesis of acute pancreatitis.

Materials and methods

Preparation of isolated pancreatic acini

All experiments and procedures using animals were performed in accordance with a protocol approved by the VA IACUC, West Haven, CT. Pancreatic acini were isolated as previously described.[8] Briefly, fasted male Sprague-Dawley rats 75-150 g (Charles River Laboratories, Wilmington, MA) were euthanized by CO_2. The pancreas was collected in buffer A: 40 mM Tris (pH 7.4), 95 mM NaCl, 4.7 mM KCl, 0.6 mM $MgCl_2$, 1 mM NaH_2PO_4, 10 mM glucose, 2 mM glutamine, plus 0.1% BSA, 1× MEM-amino acids (GIBCO-BRL, San Jose, CA). The pancreas was minced in 5 ml of buffer A, washed, then suspended in 12 ml of buffer A supplemented with 1.3 mM $CaCl_2$ and 50 units/ml of type-4 collagenase (Worthington, Freehold, NJ) and then incubated for 1 hour at 37°C with shaking (120 rpm) under constant O_2. The digest was filtered through a 300-400 μm mesh (Sefar American, Depew, NY) and the resulting acini (groups of 20-100 acinar cells) were distributed in a 24-well Falcon tissue culture plate in buffer B (buffer A supplemented with 1.3 mM $CaCl_2$). All reagents were purchased from Sigma-Aldrich, St. Louis, MO unless otherwise noted.

Acinar cell stimulation

Tissue culture plates with acini were incubated for 1 hour at 37°C under constant O2 with shaking (90 rpm). After a media exchange and additional 1 hour incubation, acini were switched to different pHs by 3×0.5 ml media changes using incubation media of the appropriate pH (pH range 6.8-8.0). Acini were allowed to equilibrate to the new pH for 15 minutes followed by stimulation with cerulein (0.01-100 nM), JMV-180 (1-1000 nM) (Research Plus, Barnegat,

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NJ), bombesin (0.01-10 μM) or carbachol (1 mM). When concanamycin (100 nM) was used, the inhibitor was added to the acini 30 minutes prior to changing media pH and was present during all subsequent media changes.

Adjustment of pH for studies in pancreatic acini

For most studies extracellular pH was adjusted by adding HCl or NaOH to buffer B at 37°C. For studies with lactic acid, the pH of buffer B was adjusted to 6.8, 7.0 and 7.4 with lactic acid giving a final concentration of 36 mM, 30 mM and 25 mM lactic acid respectively. For propionate studies, sodium propionate (25 mM and 50 mM) was added in buffer B. As a control for the addition of sodium, equivalent concentrations of NaCl were added in buffer B to the wells without propionate. For these studies, acini were stimulated with cerulein (100 nM) for 15 minutes before propionate-induced cytosolic acidification to initiate zymogen activation and then the media was exchanged with buffer B either with or without propionate. Cerulein was then re-added to acini for additional 45 minute incubation.

Enzymatic activity assays in acinar cells

Samples were frozen at -80°C, then thawed in ice, and homogenized. Protease activity assays were performed using fluorogenic substrates as described.[9] Briefly, enzyme substrate (40 mM) (chymotrypsin; Calbiochem, San Diego, CA and trypsin; Peptides International, Louisville, KY) was added to each sample in assay buffer (50 mM Tris pH 8.1, 150 mM NaCl, 1 mM $CaCl_2$) and read using a fluorometric plate reader (HTS 7000; Perkin-Elmer Analytical Instruments, Shelton, CT). The slope of the resulting line, which represents enzyme activity, was normalized to total amylase activity.

Amylase assay

Samples were thawed on ice and a cell free supernatant was assayed for secreted amylase as described and calculated as percent secretion.[9]

Lactate dehydrogenase (LDH) and Trypan blue retention assays

Dispersed acini were equilibrated to the appropriate pHe (7.4 or 7.0) for 15 minutes prior to treatment with or without cerulein (0.1 or 100 nM) for 2 hours and processed for either LDH or trypan blue assays as previously described. [9]

Preparation of acini for light microscopy

Dispersed acini were prepared as above, stimulated with cerulein (0.1 nM and 100 nM) at pHe 7.0 and 7.4 and then fixed in 2% glutaraldehyde and 0.1 M sodium cacodylate buffer at pH 7.2, postfixed in 1.0% osmium tetroxide (Polysciences Inc Warrington, PA), dehydrated in ethanol in propylene oxide, embedded in 100% Spurr's resin, and sectioned (500 nm) using an ultramicrotome.

In vivo studies with cerulein and acid load

Sprague-Dawley rats (Charles River Laboratories) between 250-275 grams were fasted overnight. The animals were anesthetized by intraperitoneal injection of Inactin (thiobutabarbital sodium) 150 mg/kg. Tracheostomy was performed and 2 cm PE-240 tubing was inserted into the trachea. The external jugular vein was cannulated using PE-50 tubing. All bolus treatments and constant infusions were performed using a Harvard pump (Harvard Bioscience Co, Holliston, MA). Body temperature was maintained by placing animals on an isothermal heating pad. Animals were divided into four groups: 1) Group A, control: Animals received a bolus of normal [0.9N] saline (NS) in a volume equal to 1% body weight over 2 minutes, followed by a constant infusion of NS at 4 ml/hour; 2) Group B, acid infusion: Animals

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were given a bolus of 0.1 N HCl in NS in a volume equal to 1% of body weight over 2 minutes, followed by a constant infusion of 0.1 N HCl in NS at 4 ml/hour as described;[10] 3) Group C, cerulein with NS infusion: Animals received a bolus of NS as described above, followed by a cerulein (50 μg/kg or 0.1 μg/kg) bolus given in 0.25 ml of NS over 2 minutes and then a constant infusion of NS at 4 ml/hour; 4) Group D, cerulein with acid infusion: Animals received boluses of acid, followed by cerulein as described above and then a constant infusion of 0.1 N HCL in NS at 4 ml/hour. Animals were assessed for the level of anesthesia every 15 minutes by foot-pad pinch and were euthanized at the end of one hour or four hours. The pancreas was harvested and processed for trypsinogen activation assays, light microscopy (Supplement: Materials and Methods, Histology) and edema as described.

Measurement of edema

Harvested pancreatic tissue was blotted and its wet weight determined. Then tissues were dried at 60°C for 72 hours and re-weighed. Edema was expressed as % wet weight (wet weight-dry weight/wet weight × 100).[11]

Measurement of trypsin activity in whole tissue

Harvested pancreatic tissue was frozen in liquid nitrogen and stored at -80°C. Thawed pancreatic tissue was homogenized in buffer (5 ml buffer/gm tissue) containing 5 mM MOPS (pH 7.0), 1 mM Magnesium sulfate and 250 mM sucrose. Trypsin activity was measured using a fluorometric assays as described.[12] Results were expressed as activity per μg protein, which was measured using the Bradford assay(Bio-Rad Laboratories, Hercules, CA).

Histology of pancreatic tissue

Pancreatic tissue from *in vivo* studies (1 mm pieces) was immersion fixed in 4% formalin, dehydrated, embedded in paraffin, sectioned at 5 μm and then stained with haematoxolin and eosin.

Statistical Analysis

Data represent the mean + SE of at least 3 individual experiments, with each experiment performed in at least duplicate. Statistical significance was determined by one-way ANOVA followed by a Newman-Keuls post hoc test for *in vitro* data and the Mann-Whitney test for *in vivo* data. P values <0.05 were assigned significance.

Results

Reducing extracellular pH (pHe) sensitizes acini to protease activation

Changes in intracellular pH (pHi) have been shown to correspond to pHe except in the range of pHe ~7.3-7.5 where effective intracellular buffering is observed.[13] To determine the effects of medium acidification on zymogen activation, dispersed pancreatic acini were incubated in medium buffered at pHe 6.8-7.6 and hyperstimulated with cerulein (100 nM). Trypsin and chymotrypsin activities were measured as markers for zymogen activation. Medium acidification alone had no effect on zymogen activation or amylase secretion, but in the presence of 100 nM cerulein, trypsin and chymotrypsin activity were enhanced. Reducing pHe to 7.0 from 7.4 increased the levels of cerulein-induced trypsin and chymotrypsin activity by 2.5 and 5 fold respectively. Increasing pHe to 7.6 from 7.4 blocked the cerulein-induced zymogen activation (Figure 1A and B). When a HEPES buffer was substituted for Tris, similar effects of reducing pHe on activation were observed (data not shown). Cerulein stimulated amylase secretion was slightly, but not significantly, reduced at pHe 7.0 compared to pH 7.4 (Figure 1C and 2C). Similarly, zymogen activation and amylase secretion responses were observed when acini were treated with other G-protein coupled receptor agonists (bombesin

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and carbachol; Figure S1 and Figure S2). Stimulation of zymogen activation requires activation of the low-affinity form of the CCK_A receptor.[11] JMV-180 is an agonist of the CCK_A high-affinity receptor state and an antagonist at the low-affinity CCK_A receptor state in rat pancreatic acini. Low pHe had no effect on JMV-180 mediated protease activation and secretory responses in acini (Figure S3). When cells were simultaneously treated with cerulein and JMV-180, there was an inhibition of the effects of acid on zymogen activation (Figure S4). Thus, the sensitizing effects of low pHe on CCK stimulated require activation of the low-affinity state of the CCK receptor.

To determine if reducing pHe sensitized acinar cells to physiologically relevant concentrations of cerulein, a range of cerulein concentrations were examined at pHe 7.4 and 7.0. Although there was a tendency for low pHe to cause enhanced activation at physiologic levels of cerulein (0.1 and 1 nM), significant effects were observed only at higher concentrations (10 and 100 nM). Trypsin and chymotrypsin activities were enhanced 2.0 and 4.0 fold, respectively, when comparing pH 7.4 to pH 7.0 at 10 or 100nM cerulein (Figure 2A and B). Secretion of amylase from acinar cells in response to increasing concentrations of cerulein is biphasic.[14, 15] Reducing pHe from 7.4 to 7.0 did not change the pattern of this cerulein response and had little effect on overall secretion (Figure 2C).

To examine whether low pHe might cause trypsinogen activation after its secretion, extracellular medium was collected after physiologic cerulein stimulation and then incubated at various pHs. Although trypsinogen was present in the media, very little was active. Further the amount of active trypsin did not increase with time and was not affected by changing the pH of the media (Figure S5).

pHe effects are reversible

To examine the reversibility of the low pHe effects, acini were incubated in different pHe and then hyperstimulated with cerulein. As shown in Figure 3, when acini incubated pHe of 7.0 for 15 minutes and then returned to pHe 7.4 (Group 3), the sensitizing effects of transiently lowering pHe on zymogen activation were eliminated. Further, shifting the pHe had no effect on the secretory response to cerulein (data not shown). These observations suggest that the sensitizing effects of low pHe on cerulein stimulation are reversible.

Reducing pHe with lactate sensitizes acini

There are two major forms of metabolic acidosis, hyperchloremic and lactic acidosis. Having shown the sensitizing effects of low pHe with medium containing HCl (a hyperchloremic acid load), we next examined the effect of a reducing pHe with lactic acid[16]. Medium buffered to pH 6.8 and 7.0 with lactate caused sensitization to cerulein-dependent zymogen activation (Figure 4A and B). Similar to HCl, lactate did not significantly affect cerulein-dependent amylase secretion (Figure 4C). These studies suggest that reducing pHe by either HCl or lactate can sensitize acini to zymogen activation.

Reducing intracellular pH (pHi) with sodium propionate sensitizes acini

Extracellular or intracellular targets could mediate the sensitizing effects of reducing pHe. For example, the activity of G-protein coupled cell surface receptors is sensitive to changes in pHe independent of pHi.[17] To determine whether changes in pHi alone could cause sensitization, acini were treated with sodium propionate, a short-chain fatty acid which enters cells and causes transient cytosolic acidification without affecting pHe.[18] To monitor the effects of propionate on pHi, acini were loaded with the fluorescent pH sensor, BCECF. We observed that treatment with buffer containing 50mM sodium propionate rapidly reduced pHi from 7.20 to 6.0 (Figure S6). Similar to the effects observed with reducing pHe, sodium propionate increased cerulein-induced zymogen activation in a concentration dependent manner (Figure 5). Sodium

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propionate (50 mM) alone did not affect zymogen activation, but it enhanced cerulein-mediated trypsin and chymotrypsin activation 2.2 and 3.0 fold, respectively. Sodium propionate alone caused a slight increase in amylase secretion compared to control acini and did not significantly effect cerulein-induced amylase secretion (Figure 5C). These findings suggest that reducing pHi may be sufficient for sensitization to cerulein.

Reducing pHe enhances cerulein-induced injury

The effects of low pH on cerulein-induced cellular injury were evaluated by assaying the release of the cytosolic protein lactate dehydrogenase (LDH) into the medium (Figure 6A), trypan blue retention (Figure 6B) and light microscopy (Figure 6C). To perform these experiments, acini were treated for two hours with 100 nM cerulein for hyperstimulation or 0.1 nM for physiologic stimulation. Previous studies showed the most prominent injury occurs after protease activation in the acinar cell.[9] When acini were hyperstimulated at pHe 7.4 there was a 4-fold increase in LDH release compared to control at pHe 7.4; this effect was further enhanced 7.9 fold at pHe 7.0 compared to control at pHe 7.0 (Figure 6A). Low pHe alone did not affect LDH release in unstimulated acini. Treatment of acini with cerulein (0.1nM) caused a 1.7 fold increase in LDH release at pHe 7.4 that increased to 3.2 fold when cells were incubated at pHe of 7.0. A similar trend was observed when trypan blue retention was used as an indicator of cell injury (Figure 6B). The morphologic appearance of acinar cells also suggested that reducing pHe enhanced cerulein-induced injury. Thus, basolateral membrane blebbing and large cytoplasmic vacuoles, two early acinar responses in acute pancreatitis, were evident with cerulein hyperstimulation at pH 7.4 (Figure 6C panel 5). Neither pHe of 7.0 alone nor physiologic cerulein stimulation at pHe 7.4 (Figure 6C panel 2 and 3) affected morphology. However, when pHe 7.0 was combined with physiologic concentrations of cerulein (Figure 6, panel 4) the blebbing and vacuolization that appeared were similar to that noted with hyperstimulation (Figure 6C, panel 5). These biochemical and morphologic studies demonstrate that reducing pHe from 7.4 to 7.0 sensitizes the acinar cell to cerulein-induced cell injury.

The sensitizing effects of reducing pHe are mediated by a vacuolar ATPase

Concanamycin A specifically inhibits vATPases at nanomolar concentrations. Pretreatment of acini with concanamycin A (100 nM) blocked the effects of lowering pHe on cerulein-induced trypsin and chymotrypsin activation. Cerulein-induced zymogen activation was also blocked by raising pHe to 8.0 (Figure 7). Amylase secretion was not affected by concanamycin (data not shown). Similar effects were seen with bafilomycin, another vATPase inhibitor (data not shown)

In vivo acid infusion sensitizes rats to the development of early cerulein-induced pancreatitis responses

The effects of an acute acid load on cerulein-induced acute pancreatitis were examined after one hour *in vivo* in rats. Animals in Group B (acid infusion alone) had no significant change in trypsin activity or edema compared to Group A (normal saline (NS) control). However, animals in Group C (cerulein hyperstimulation with NS infusion) had a 7 fold increase in trypsin activity (Figure 8A) and a 16.7% increase in edema compared to Group A (Figure 8B). Animals in Group D (cerulein hyperstimulation and acid infusion) had a 20 fold increase in trypsin activity (Figure 8A) and a 23% increase in edema (Figure 8B) compared to animals in Group A. These findings indicate that an acid load increases the severity of pancreatitis. To determine whether an acid load could sensitize the pancreas *in vivo*, a physiologic concentration of cerulein (0.1 μg/kg) was used. This treatment significantly enhanced pancreatic trypsin activation by 3.1 fold over physiological cerulein treatment alone (Figure S8), but had no effect on pancreatic edema at 4 hours (Data not shown). These *in vivo* studies confirm our *in vitro*

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observations and demonstrated that an acid load worsens cerulein-induced pancreatitis and may sensitize the pancreas to physiologic concentrations of CCK.

Pancreatic histology from cerulein hyperstimulation in vivo

Morphologic markers of pancreatitis including cytoplasmic vacuoles and lobular separation, an indicator of pancreatic edema, were evaluated. The controls, Group A and Group B (Groups are as described above), did not show evidence of pancreatitis (Figure S7 Panel 1 and 2). These markers were prominent in tissues from Group C and D (Figure S7 Panel 3 and 4). However, the vacuoles observed in Group D tended to be smaller than Group C. Rarely, pyknotic cells and basolateral blebbing were observed in groups C and D. No changes in inflammation were observed after these one hour treatments.

Discussion

Clinical and experimental observations suggest that pancreatic sensitization to potentially injurious stimuli has an important role in the pathogenesis of acute pancreatitis.[8, 19, 20] The present study demonstrates that in both cellular and *in vivo* models of acute pancreatitis, an extracellular acid load sensitizes the acinar cell to secretagogue-induced zymogen activation and injury. Notably, reducing pHe alone has no effect. These findings support previous clinical and experimental observations suggesting that an acute acid load may affect the development of acute pancreatitis.

In a prospective study of diabetic ketoacidosis an 11% incidence of pancreatitis was observed. Multivariant analysis identified admission arterial pH as the most significant risk factor for developing pancreatitis.[21] Syndromes such as organic acidemias, mutations in mitochondrial tRNA, and drugs such as nucleoside reverse transcriptase inhibitors have also been associated with mitochondrial dysfunction that cause lactic acidosis and are also linked to acute pancreatitis.[22, 23] In addition to generalized metabolic acidosis, local factors such as reduced blood flow, may selectively reduce pancreatic pHe.[24] Clinically relevant conditions that would decrease the pancreatic pH have also been described in animal models. In the cat pancreas, the basal parenchymal pH is about 7.35 and decreases to less than 7.25 after inducing chronic pancreatitis.[25] Moreover, acute ethanol exposure in the cat can rapidly decrease pancreatic blood flow by 60% and can cause a drop in pancreatic tissue pHe to less than 7.1. Metabolic acidosis can result from an HCl or lactate load and these can have both overlapping and distinct effects.[26] We observed that reducing pHe with either acid sensitized the acinar cell to cerulein-induced zymogen activation. These findings suggest that an acute acid load from either HCl or lactate might sensitize the pancreas to zymogen activation and injury.

We found that lowering pHe sensitized the acinar cell to zymogen activation induced by a range of G-protein coupled acinar cell receptors including those for bombesin, CCK and a cholinergic agonist. With respect to CCK, we observed that stimulation of the low-affinity form of the CCK_A receptor was required for sensitization. Notably, lowering pHe had little effect on amylase secretion. These findings are consistent with a previous report demonstrating that distinct acinar cell signals regulate secretion and zymogen activations.[14]

The sensitizing effects of reducing pHe could result from extracellular effects such as stimulation of plasma membrane proton sensors or by reducing pHi.[13] However, when pHi was reduced with sodium propionate and pHe was buffered to 7.4, cerulein sensitization was still observed.[18] This finding suggests that an intracellular proton load alone can support acinar cell sensitization. Though many intracellular signaling pathways can be affected by changes in cytoplasmic pH, we reported that the proton-transporting vATPase regulates zymogen activation.[7] In this study, the vATPase inhibitor, concanamycin, blocked the sensitizing effects of low pHe on cerulein-induced zymogen activation. vATPase activity is dynamically

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regulated by ATP concentration, its ATP affinity, the proton gradient, and assembly of the soluble V1 complex on the V0 membrane complex.[27] Although it is likely that the sensitizing effects of reducing pHi are mediated by providing a favorable proton gradient for the transporter, other effects on the vATPase are possible. For example, the assembly and disassembly of the vATPase are regulated by specific cellular signals.[28] In this context, signaling molecules including calcium, PyK2, p38 and Erk can sense changes in pHi and need to be evaluated as potential regulators of acinar cell vATPase activity.[29]

Finally, to reproduce the metabolic acidosis seen in various clinical conditions, an acid load was delivered *in vivo*. After a one hour acid infusion, we observed enhanced effects of hyperstimulatory concentrations of cerulein on zymogen activation and markers of injury, such as edema, that were consistent with our *in vitro* studies. Further, acid infusion also significantly increased the levels of intrapancreatic trypsin observed after stimulation with physiologically relevant concentrations of cerulein (Figure S8). These studies confirm that an acute acid load sensitizes the pancreas to the effects of a diverse range of secretagogues on pancreatic zymogen activation and has the potential to convert a physiologic stimulus into a pancreatitis response.

Although this study on the effects of pHe has focused on the zymogen activation responses in acute pancreatitis, reducing pHe may have other relevant effects. *In vitro* studies have shown that low pHe increases epithelial cell paracellular permeability by affecting the tight junctions.[30] *In vivo* studies have shown that metabolic acidosis can affect immune responses including stimulating leukocytes, inducing inflammatory cytokine release from macrophages and activating pH-sensitive neural secretion of pro-inflammatory neuropeptides such as substance P and calcitonin gene-related peptide.[31] Some of these responses, such as substance P release, appear to modulate pancreatic inflammation.[32, 33] Studies of the longer-term effects of an acid load *in vivo* on the progression of pancreatitis and complex elaboration of inflammatory mediators will be examined in future studies.

In summary, our *in vitro* and *in vivo* studies show that acutely reducing pHe sensitizes pancreatic acinar cells to cerulein-induced zymogen activation and injury, key early acute pancreatitis markers. Further, we find that reduced pHe can sensitize acinar cells to three different secretagogues that signal through distinct receptors. It is likely that reducing pHe is sensitizing the cell to secretagogue-dependent zymogen activation by affecting common downstream post-receptor mediated responses that are modulated by cytosolic pH. Although the effects of an acid load on the evolution of acute pancreatitis, including inflammatory responses, needs to be examined in longer-term studies, the present work suggests that factors that acutely reduce pancreatic acinar cell pH, whether systemic or local, might affect the risk for developing or the severity of acute pancreatitis. Since increasing pHe blocked cerulein-dependent zymogen activation *in vitro*, raising pancreatic pHe might provide a therapeutic benefit in specific clinical conditions. In this context, a recent study reported that in an animal model of post-ERCP pancreatitis, disease development was affected by the pH of the contrast media.[34] Thus in acidic media-induced pancreatitis; increasing contrast media pH significantly reduced the disease response. Whether humans will exhibit this response awaits future clinical study.

Supplementary Material

Refer to Web version on PubMed Central for supplementary material.

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Abbreviations

pHe
extracellular pH

pHi
intracellular pH

vATPase
vacuolar ATPase

CCK
cholecystokinin

NS
normal saline

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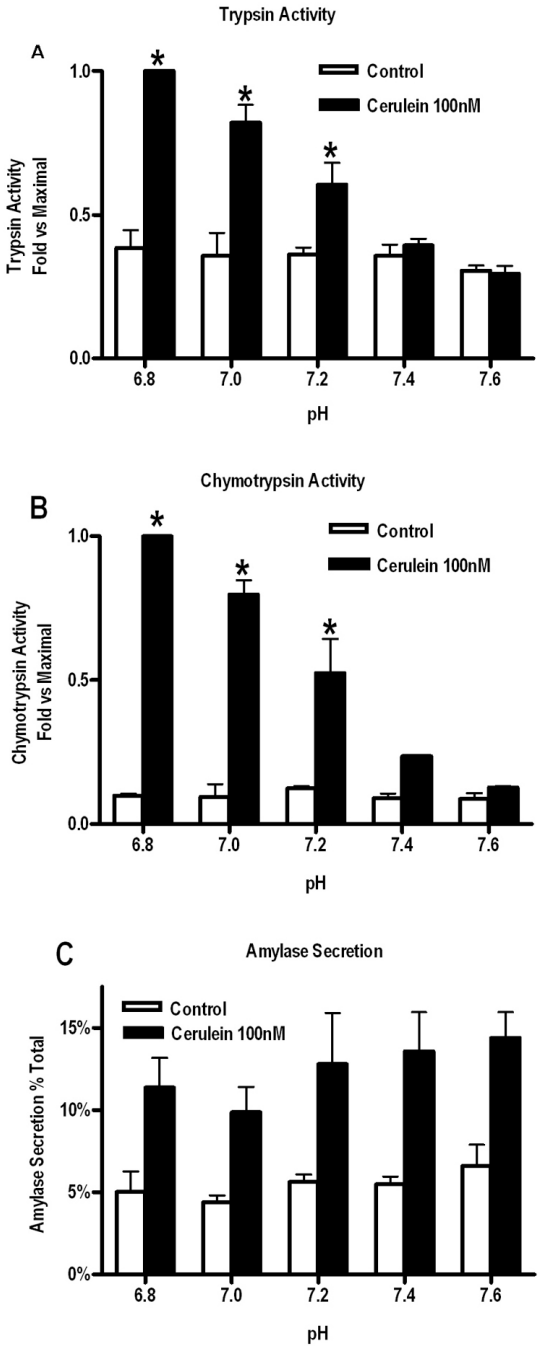

Figure 1.

Low pHe enhances cerulein-induced zymogen activation without significantly affecting amylase secretion. Dispersed acini were isolated at pH 7.4 and then equilibrated into various pH buffers for 15 minutes prior to treatment with or without cerulein (100 nM) for 1 hour. Acini were collected and assayed for (A) trypsin, (B) chymotrypsin activity and (C) amylase secretion. 'Control' indicates no cerulein treatment. Data are the mean +/-SEM of at least 3 individual experiments. *=P<0.05 compared to cerulein 100 nM at pH 7.4

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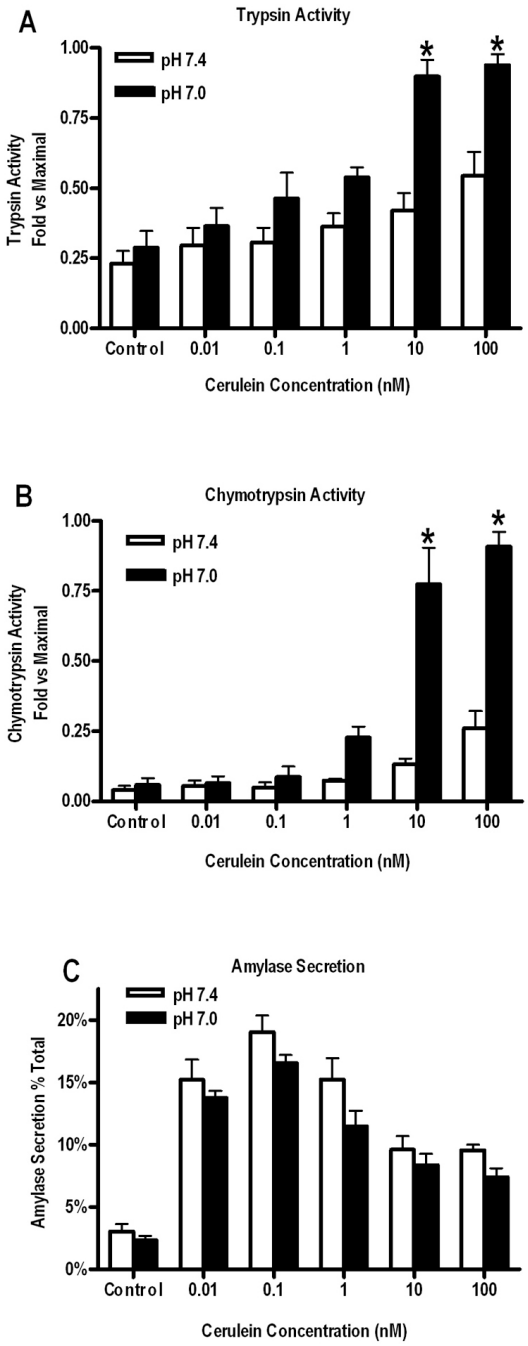

Figure 2.

Low pHe enhances zymogen activation over a range of cerulein concentrations without significantly affecting amylase secretion. Dispersed acini were isolated at pH 7.4 and equilibrated into buffer pH 7.4 or 7.0 for 15 minutes prior to treatment with or without cerulein (0.01 nM-100 nM) for 1 hour. Acini were collected and assayed for (A) trypsin, (B) chymotrypsin activity and (C) amylase secretion. Data are the mean +/-SEM of at least 3 individual experiments. *=P<0.05 compared to analogous cerulein treatment at pHe 7.4.

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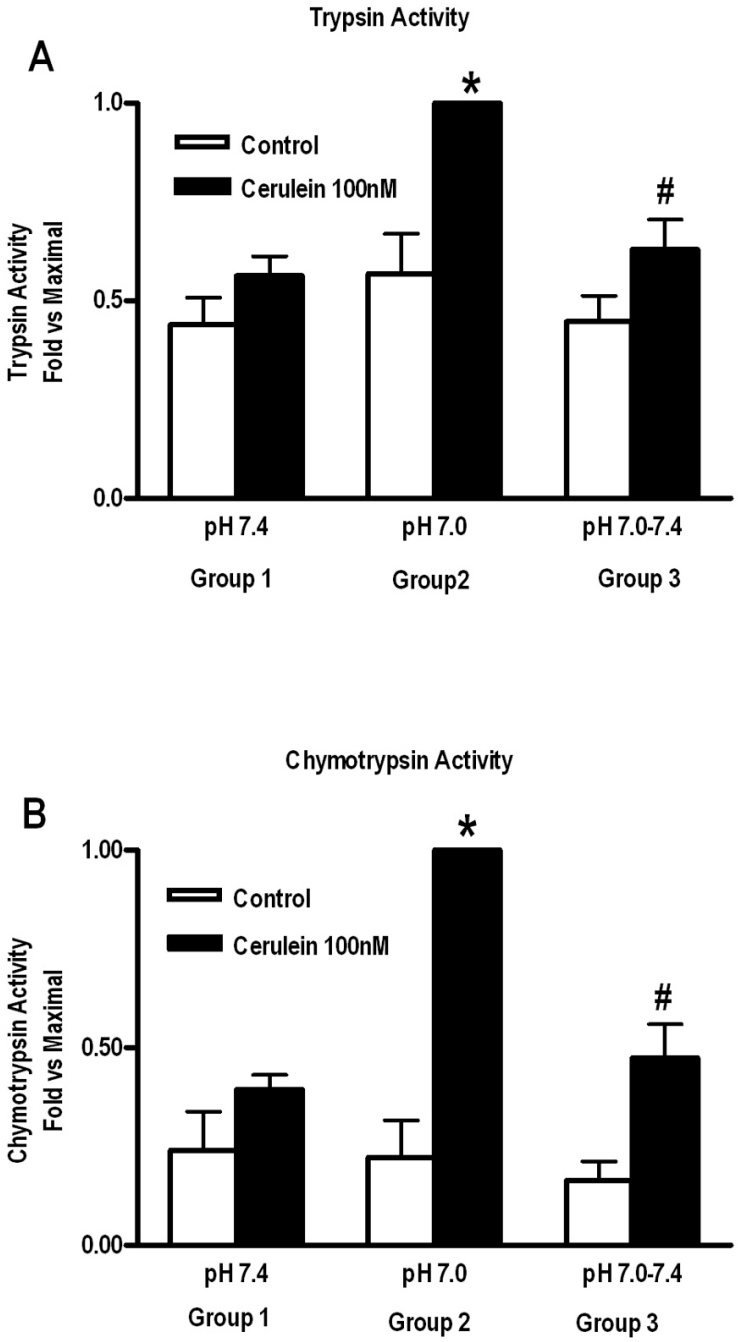

Figure 3.

The effects of low pHe on cerulein-induced zymogen activation are reversible. Acini were exposed to different pHe conditions: Group 1: pHe 7.4; Group 2: pHe 7.0 for 30 minutes prior to cerulein hyperstimulation and maintained at pHe 7.0 for the remainder of the experiment; Group 3: pHe 7.0 for 15 minutes and then pHe was reversed to pH 7.4 and was allowed to equilibrate for another 15 minutes prior to treatment with or without cerulein (100 nM) for 1 hour. Acini were collected and assayed for (A) trypsin, (B) chymotrypsin activity. Data are the mean +/-SEM of at least 4 individual experiments. *=P<0.05 compared to analogous treatment at pHe 7.4, # =P<0.05 compared to analogous treatment at pHe 7.0.

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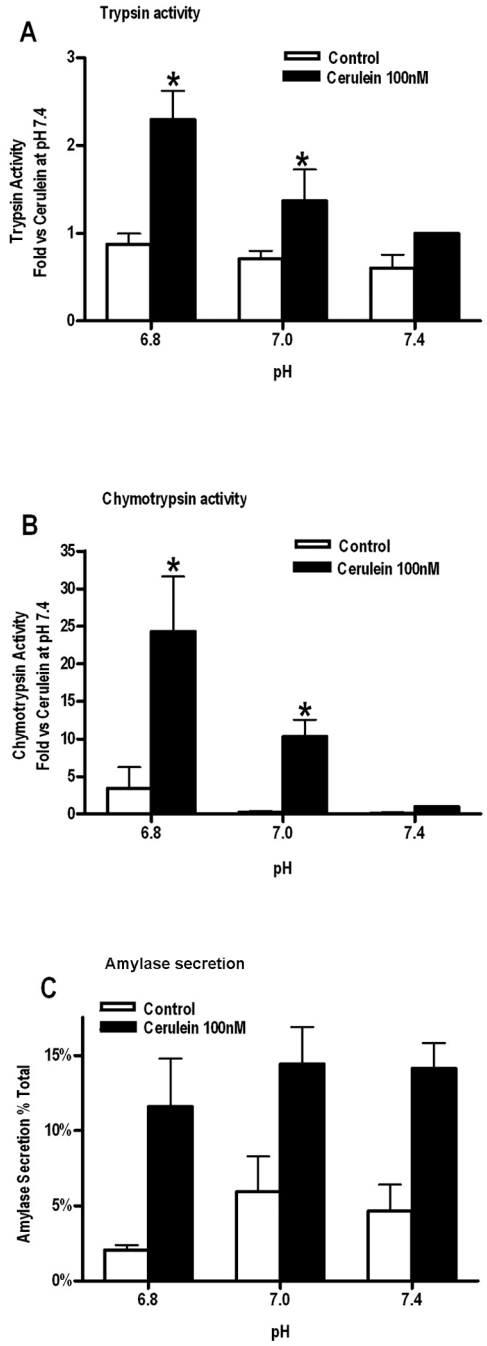

Figure 4.

Lactic acid enhances cerulein stimulated zymogen activation. Dispersed acini were treated with or without cerulein 100 nM for 15 minutes and then incubated in a buffer of pH 6.8, 7.0 and 7.4 with lactic acid concentrations of 36 mM, 30 mM and 25 mM, respectively. Acini were collected and assayed for (A) trypsin, (B) chymotrypsin activity and (C) amylase secretion. Data are the mean +/-SEM of at least 4 individual experiments. *=P<0.05 compared to cerulein 100nM at pHe 7.4.

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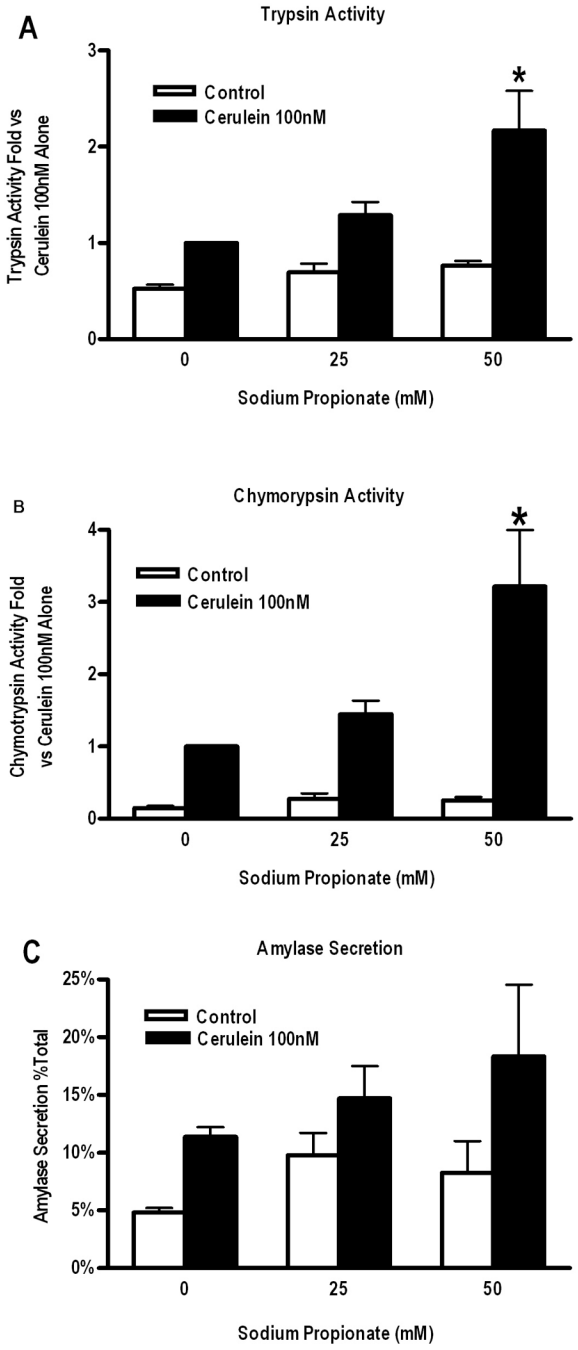

Figure 5.

Sodium propionate enhances cerulein-induced zymogen activation

Dispersed acini were treated with or without cerulein 100 nM for 15 minutes and then incubated in a buffer of pH 7.4 with or without sodium propionate at 25 mM or 50 mM for 45 minutes. Acini were collected and assayed for (A) trypsin and (B) chymotrypsin activity (C) amylase secretion. Data are the mean +/-SEM of at least 4 individual experiments. *=P<0.05 compared to cerulein 100 nM alone.

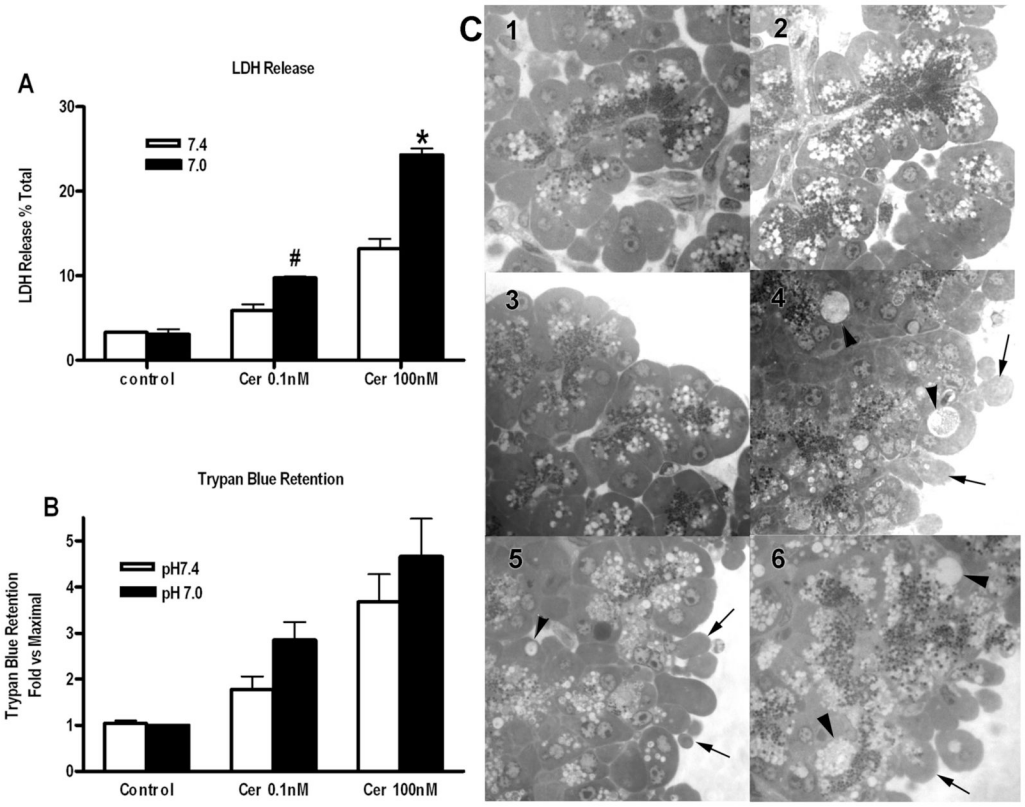

Figure 6.

Low pHe enhances cerulein-induced cell injury. Dispersed acini were equilibrated at either pHe 7.0 or 7.4 and stimulated with or without cerulein (Cer) at 0.1 nM and 100 nM for 2 hrs. The acini were collected and examined for (A) LDH release and (B) trypan blue retention (C) vacuole formation and membrane blebbing under light microscopy. (1) pHe 7.4 control, (2) pHe 7.0 control, (3) pHe 7.4 cerulein 0.1 nM, (4) pHe 7.0 cerulein 0.1 nM, (5) pHe 7.4 cerulein 100 nM and (6) pHe 7.0 cerulein 100 nM. Arrowheads: vacuoles, arrows: blebs. Data are the mean +/-SEM of at least 4 individual experiments. *=P<0.05 compared to cerulein 100 nM at pHe 7.4, #=p<0.05 compared to cerulein 0.1 nM at pH 7.4.

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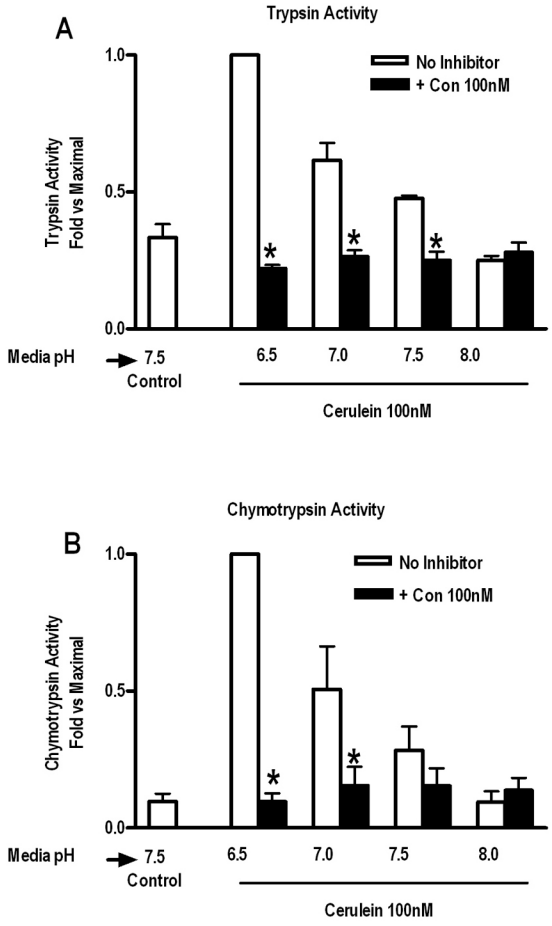

Figure 7.

The vATPase inhibitor concanamycin blocks cerulein-induced zymogen activation over a range of pHs. Dispersed acini were equilibrated with pHs ranging from 6.5-8.0. The acini were preincubated with concanamycin (Con) 100 nM for 30 minutes followed by treatment with or without cerulein 100 nM for 1 hour. Acini were collected and assayed for (A) trypsin, (B) chymotrypsin activity. Data are the mean +/-SEM of at least 4 individual experiments. *=P<0.05 compared to analogous cerulein treatment without concanmycin

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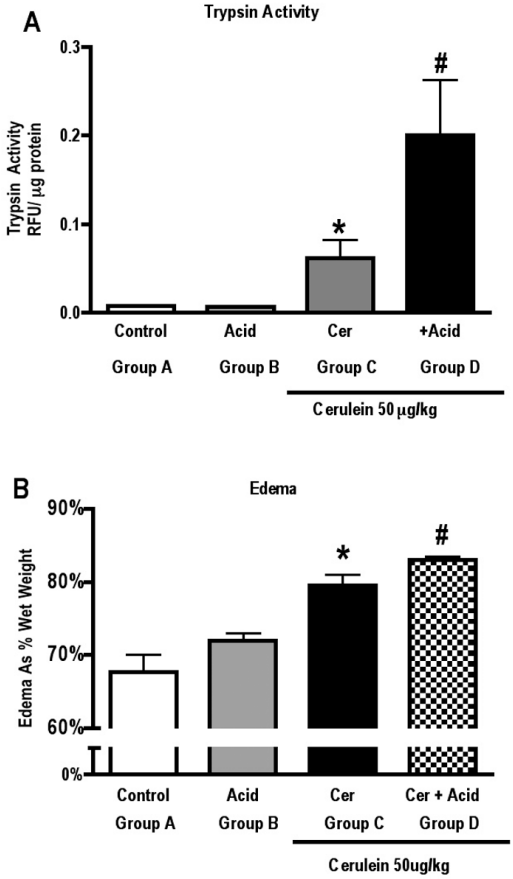

Figure 8.

Short-term acid infusion enhances cerulein hyperstimulation-induced trypsin activation and edema *in vivo*. Animals were treated with or without an intravenous cerulein (Cer) bolus of 50 µg/kg followed by an infusion of either 0.1N HCl in normal saline (NS) or NS alone for one hour. Harvested tissue was assayed for (A) trypsin activity and (B) edema. Group A: NS controls; Group B: HCl infusion; Group C: cerulein bolus + NS infusion and Group D: cerulein bolus + HCl infusion. Data are the mean +/-SEM of at least 4 animals. The *=P<0.05 compared to control. #=P<0.05 compared to cerulein treatment without acid infusion