Acidosis Inhibits Gallbladder Contraction Mediated by Protein Kinase C Activation

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ABSTRACT—The effects of extracellular acidosis on gallbladder contraction were investigated using gallbladder strips isolated from guinea pigs. In an acidic medium (pH 6.9), gallbladder contraction induced by histamine and prostaglandin E2 was significantly lower than that in a normal medium (pH 7.4). Acidosis affected neither gallbladder contraction induced by histamine in the absence of extracellular Ca2+ nor that induced by KCl. Acidosis significantly inhibited Ca2+-induced contraction in the presence of sodium fluoride and phorbol 12,13-dibutyrate but not that in the presence of KCl. Staurosporine (30 nM) significantly inhibited gallbladder contraction induced by histamine and prostaglandin E2, but not that by KCl. Histamine-induced contraction in the presence of staurosporine was not affected by acidosis. Acidosis significantly inhibited Ca2+-induced contraction in the presence of histamine but not that in the presence of both histamine and staurosporine. These results suggest that extracellular acidosis selectively inhibits gallbladder contraction mediated by protein kinase C activation.

Keywords: Gallbladder contraction, Extracellular pH, Protein kinase C

Acidosis is known to impair smooth muscle contractility in various organs. In vascular, tracheal and detrusor smooth muscles, extracellular acidosis depresses their contractility by reducing transplasmalemmal Ca2+ entry through L-type Ca2+ channels (1–3). A recent report has shown that acidosis inhibited increases in intracellular free-Ca2+ concentration and tension of corpus cavernosum smooth muscle in response to KCl (4). In esophageal, gastric and intestinal smooth muscles, acidosis has also been reported to depress their contractility, although its mechanisms have not yet been clarified (5, 6). There are a few previous reports on the effects of pH on gallbladder contractility in vitro: acidosis inhibited gallbladder contraction induced by histamine and cholecystokinin (7, 8). However, the details of the mechanism have not been determined. There is a possibility that acidosis of gallbladder wall affects bile transport due to hypocontractility of gallbladder smooth muscle. Thus, the present study investigated the effects of acidosis on gallbladder contraction in vitro by contractile stimulants with different mechanisms, using gallbladder strips isolated from guinea pigs.

MATERIALS AND METHODS

Preparation of gallbladder strips

The study protocols regarding treatment of animals were carried out in accordance with Guide for Animal Experimentation, Yamagata University School of Medicine and Japanese Governmental Law (No. 105). Male Hartley guinea pigs weighing 400–500 g were anesthetized with sodium pentobarbital (50 mg/kg) and killed by exanguination. The gallbladder was removed rapidly and rinsed in physiological salt solution with the following composition: 10 mM HEPES/Tris, pH 7.4, containing 135 mM NaCl, 5 mM KCl, 1.5 mM CaCl2, 1 mM MgCl2, 1 mM K2HPO4 and 10 mM glucose at 37°C and oxygenated with 100% O2. The gallbladder was cut into longitudinal strips 4-mm-wide and 7-mm-long.

Measurement of isometric tension

Each strip was connected to a force displacement transducer (Nippon Kohden Kogyo, Tokyo). After 1 h of equilbrium at a resting tension of 1 g, which was reported to
be optimal for the measurement of changes in the tension of gallbladder strips from guinea pig (9), the strips were contracted with 60 mM KCl. After washing out the bath solution, the KCl-induced contraction protocol was repeated once more. The preliminary experiments showed that a constant contraction of the gallbladder strips by KCl was obtained after these two protocols of KCl contraction. Then, each experimental protocol was started. Data are presented as the percent of the KCl (60 mM)-induced contractile tension in each strip in the normal medium with pH 7.4. The mean contractile tension induced by 60 mM KCl was 2.85 ± 0.15 g. EC<sub>50</sub>, the concentration required to induce a half-maximal contractile response was determined graphically after calculating the linear regression of the 20 – 80% region of each log concentration-response curve, and the sensitivity of the contractile response was evaluated using pD<sub>2</sub>, negative log EC<sub>50</sub> (M). Experiments using a Ca<sup>2+</sup>-free medium were performed as follows: the gallbladder strips were washed three times with Ca<sup>2+</sup>-free solution containing 1 mM EGTA (10 mM HEPES/Tris, pH 7.4, containing 135 mM NaCl, 5 mM KCl, 1 mM EGTA, 1 mM MgCl<sub>2</sub>, 1 mM KH<sub>2</sub>PO<sub>4</sub>, and 10 glucose at 37°C), followed by stabilization in Ca<sup>2+</sup>-free solution without EGTA for 5 min. Then, each stimulant was added to the organ bath. In the experiments of Ca<sup>2+</sup>-induced contraction, CaCl<sub>2</sub> was subsequently added to the organ bath in a cumulative manner.

**Induction of acidosis**

Extracellular acidosis was induced by the addition of HEPES to reduce the pH of the medium from 7.4 to 6.9. An adequate volume (1.5% of the volume of the medium in an organ bath) of a concentrated HEPES solution (1 M), which changes the pH of the medium from 7.4 to 6.9, was determined in preliminary experiments. The gallbladder strips were stabilized in the acidic solution for 30 min before contraction was elicited by each contractile stimulant. We used a medium with pH 6.9 as a condition of acidosis in the present study, a condition that has often been used in previous studies on acidosis effects in vitro.

**Chemicals**

The drugs used were phorbol 12,13-dibutyrate (PDBu), prostaglandin E<sub>2</sub> (PGE<sub>2</sub>), histamine dichloride and staurosporine from Sigma-Aldrich Japan K.K. (Tokyo) and sodium fluoride from Wako Pure Chemical Co. (Osaka). Sodium fluoride and histamine were each dissolved in distilled water to make stock solutions of 1 M and 10 mM, respectively, and kept at 4°C. PDBu and PGE<sub>2</sub> were each dissolved in dimethylsulfoxide to make stock solutions of 1 and 10 mM, respectively, and kept at –20°C. Staurosporine was dissolved in dimethylsulfoxide to make a stock solution of 0.1 mM and kept at –20°C. Dimethylsulfoxide at 0.1% did not affect the concentration-response relationship of KCl-induced contraction. The concentrations of sodium fluoride and PDBu used in the present study were those that cause maximum contraction in the guinea pig gallbladder. The concentration of staurosporine used did not affect KCl-induced contraction but completely inhibited PDBu-induced contraction in the guinea pig gallbladder. The concentration of each drug was expressed as the final concentration in the organ bath.

**Statistical analyses**

The data are shown as means ± S.E.M. Statistical analyses were performed by analysis of variance and subsequent Scheffé F-test for the data from concentration-response relationships and Student’s t-test for the data from the experiments using a single dose of histamine. P values less than 0.05 were regarded as significant.

**RESULTS**

The resting tension of gallbladder strips was not affected by induction of acidosis by the addition of HEPES solution (data not shown). Acidosis (pH 6.9) inhibited histamine-induced contraction of gallbladder strips (Fig. 1A). Both maximum contractile force and pD<sub>2</sub> value for histamine-induced contraction were significantly lower in the acidic medium than in the normal medium [pD<sub>2</sub>: 4.93 ± 0.15 (pH 6.9) vs 5.43 ± 0.12 (pH 7.4)]. Histamine (100 µM)-induced contraction in the Ca<sup>2+</sup>-free medium was not affected by acidosis [49.5 ± 7.7% (pH 7.4) vs 46.3 ± 8.9% (pH 6.9) (n = 6)]. Acidosis (pH 6.9) strongly inhibited PGE<sub>2</sub>-induced contraction of the gallbladder strips (Fig. 1B). Both maximum contractile force and pD<sub>2</sub> value for PGE<sub>2</sub>-induced contraction were significantly lower in the acidic medium than in the normal medium [pD<sub>2</sub>: 7.15 ± 0.03 (pH 6.9) vs 7.63 ± 0.11 (pH 7.4)].

Neither KCl-induced contraction nor Ca<sup>2+</sup>-induced contraction in the presence of a high concentration (60 mM) of KCl was affected by acidosis (pH 6.9) (Figs. 1C and 2A). Contractile responses to Ca<sup>2+</sup> in the presence of sodium fluoride (5 mM) and PDBu (1 µM) were significantly inhibited by acidosis (pH 6.9) (Fig. 2: B and C). Staurosporine (30 nM) significantly inhibited contractile responses to histamine and PGE<sub>2</sub> (Fig. 3: A and B), but not that to KCl (Fig. 3C).

Histamine-induced contraction in the presence of staurosporine was not affected by acidosis (Fig. 4A). Acidosis significantly inhibited Ca<sup>2+</sup>-induced contraction in the presence of histamine but not that in the presence of both histamine and staurosporine (Fig. 4B). Histamine contraction of gallbladder strips was also significantly inhibited immediately after acidosis was induced (Fig. 4C).
DISCUSSION

Agonist-induced contraction of gallbladder was inhibited by acidosis, while KCl-induced contraction was not. These results suggest that the common signal transduction pathway after receptor stimulation by histamine and PGE$_2$ is sensitive to acidosis, while the contractile mechanism of gallbladder smooth muscle directly related to the contractile apparatus is not affected by acidosis. Moreover, trans-
Fig. 3. Effects of staurosporine (30 nM) on contractile responses of gallbladder strips to histamine (A), prostaglandin E\(_2\) (B) and KCl (C) in the normal medium with pH 7.4. Asterisks denote significant differences compared to the control pretreated with vehicle (*\(P<0.05\), **\(P<0.01\)), n = 6.

Fig. 4. Effects of acidosis on histamine-induced contraction of gallbladder strips under various conditions. A. Effects of acidosis (pH 6.9) on contractile response to histamine in the presence of staurosporine (30 nM). n = 6. B. Effects of acidosis (pH 6.9) on Ca\(^{2+}\)-induced contractile response of the gallbladder strips stimulated with histamine in the absence and presence of staurosporine (30 nM). The gallbladder strips were washed three times with a Ca\(^{2+}\)-free medium containing EGTA (1 mM) and then contracted with histamine in a Ca\(^{2+}\)-free medium containing EGTA (0.1 mM) in order to deplete stored Ca\(^{2+}\) in response to histamine. After washout with a Ca\(^{2+}\)-free medium, Ca\(^{2+}\) was cumulatively added. Asterisks denote significant differences compared to the responses in the acidic medium (pH 6.9) not containing staurosporine (\(P<0.01\)). n = 6. C. Effects of acute acidosis on contraction induced by histamine. Contractile response to histamine immediately after induction of acidosis (pH 6.9) was observed. Asterisks denote significant differences compared to the responses immediately after addition of a vehicle (pH 7.4) (*\(P<0.05\)), n = 4.
plasmalemmal Ca\(^{2+}\) entry through voltage-dependent Ca\(^{2+}\) channels of gallbladder smooth muscle may not be affected by acidosis. This is different from the results regarding the effects of acidosis reported in other kinds of smooth muscle, e.g., vascular, tracheal and detrusor smooth muscles, of which the plasmalemmal L-type Ca\(^{2+}\) channels were inhibited by lowering extracellular pH (1 – 3). Thus, the sensitivity of smooth muscle L-type Ca\(^{2+}\) channels to acidosis varies according to the type of smooth muscle. On the other hand, contractile response to sodium fluoride, an activator of Gq protein (10), was inhibited by acidosis, suggesting that acidosis acts on the pathway after receptor stimulation. Moreover, extracellular Ca\(^{2+}\)-independent contraction induced by histamine, in which inositol trisphosphate may be involved like cholecystokinin-induced contraction (11), was not affected by acidosis. This implies that acidosis may not directly inhibit phosphoinositide hydrolysis. Therefore, acidosis affects the signal transduction pathway after phosphoinositide hydrolysis. Staurosporine, a protein kinase C inhibitor, at the concentration used in the present study, which did not affect KCl-induced contraction of gallbladder, significantly inhibited the contractile responses to histamine and PGE\(_2\); the degree of inhibition was greater for PGE\(_2\)-induced contraction than for that induced by histamine. This tendency was similar to that of the degree of inhibition by acidosis of contractile responses to histamine and PGE\(_2\). Therefore, a protein kinase C-mediated pathway is involved in agonist-induced contraction, which agrees with a recent report showing that cholecystokinin-induced gallbladder contraction is mediated by protein kinase C activation (11).

Our recent study has shown that PDBu-induced gallbladder contraction is completely dependent on transplasmalemmal Ca\(^{2+}\) entry through voltage-dependent Ca\(^{2+}\) channels (12). In the present study, Ca\(^{2+}\)-induced contraction in PDBu-stimulated gallbladder strips as well as that in histamine or sodium fluoride-stimulated ones was also attenuated by acidosis, while that in KCl-stimulated gallbladder was not. Moreover, histamine-induced contraction in the presence of staurosporine was not affected by acidosis. This implies that acidosis selectively influences the PKC-related pathway of gallbladder contraction. On the other hand, PKC-mediated contraction of gallbladder smooth muscle was completely inhibited by the L-type Ca\(^{2+}\) channel antagonist (12). Therefore, acidosis affects PKC-mediated Ca\(^{2+}\) entry through L-type Ca\(^{2+}\) channels but not other Ca\(^{2+}\) entry pathways, including receptor-operated Ca\(^{2+}\) channels. To our knowledge, this is the first study investigating the mechanism of acidosis effects on gallbladder contraction in relation to signal transduction mechanisms after receptor stimulation. Acidosis affected neither extracellular Ca\(^{2+}\)-dependent contraction due to KCl depolarization nor Ca\(^{2+}\)-induced contraction in the presence of both histamine and staurosporine. Thus, it is unlikely that acidosis decreases Ca\(^{2+}\) sensitivity of gallbladder contraction.

Acidosis has been reported to activate protein kinase C in brain and renal brush border (13, 14). This is regarded as a protective mechanism against intracellular acidosis by stimulation of the Na\(^+-\)H\(^+\) antiporter. Moreover, a recent report has shown that cardiac acidosis was attenuated by preceding stimulation of the protein kinase C-activated Na\(^+-\)H\(^+\) antiporter system (15). Thus, a possible explanation for the present finding is that acidosis acutely activates protein kinase C, and the following down-regulation of protein kinase C results in attenuation of gallbladder contraction mediated by the protein kinase C pathway. However, in the present study histamine contraction of gallbladder strips was also significantly inhibited immediately after acidosis was induced. Therefore, down-regulation of protein kinase C may not be involved in the inhibitory effects of acidosis on gallbladder contraction. Further studies are necessary to clarify the molecular mechanism of the inhibition by acidosis, including pH effects on protein kinase C activity and translocation in gallbladder smooth muscle.

In conclusion, extracellular acidosis inhibits gallbladder contraction mediated by protein kinase C activation. pH dependency of gallbladder motility is possibly involved in the pathophysiology of gallbladder dysfunction.

REFERENCES

1 Klöckner U and Isenberg G: Calcium channel current of vascular smooth muscle cells: extracellular protons modulate gating and single channel conductance. J Gen Physiol 103, 665 – 678 (1994)
2 Lindeman KS, Croxton TL, Lande B and Hirshman CA: Hypocapnia-induced contraction of porcine airway smooth muscle. Eur Respir J 12, 1046 – 1052 (1998)
3 Wu C and Fry CH: The effects of extracellular and intracellular pH on intracellular Ca\(^{2+}\) regulation in guinea-pig detrusor smooth muscle. J Physiol (Lond) 508, 131 – 143 (1998)
4 Saenz de Tejada I, Kim NN, Daley JT, Royai R, Hypeolite J, Broderick GA, Garcia-Diaz F and Levin R: Acidosis impairs rabbit trabecular smooth muscle contractility. J Urol 157, 722 – 726 (1997)
5 Lofqvist J and Nilsson E: Influence of acid-base changes on carbachol- and potassium-induced contractions of taenia coli of the rabbit. Acta Physiol Scand 111, 59 – 68 (1981)
6 Schulze-Dörfl K and Lepsien G: Depression of mechanical and electrical activity in muscle strips of opossum stomach and esophagus by acidosis. Gastroenterology 82, 720 – 724 (1982)
7 La Morte WW, Hingston SJ and Wise WE: pH-dependent activity of H\(_2\)- and H\(_3\)-histamine receptors in guinea-pig gallbladder. J Pharmacol Exp Ther 217, 638 – 644 (1981)
8 La Morte WW, Hingston S, Matolo N, Birkett DH and Williams LF Jr: Effects of pH on in vitro gallbladder responses to chole-
cystokinin octapeptide. Dig Dis Sci 27, 615 – 623 (1982)
9 Moummi C, Gullikson GW and Gaginella TS: Monochloramine induces contraction of guinea pig gallbladder via two different pathways. Am J Physiol 260, G881 – G886 (1991)
10 Cockcroft S and Taylor JA: Fluoroaluminates mimic guanosine 5'-[γ-thio]triphosphate in activating the polyphosphoinositide phosphodiesterase of hepatocyte membranes. Role for the guanine nucleotide regulatory protein Gp in signal transduction. Biochem J 241, 409 – 414 (1987)
11 Yu P, Chen Q, Xiao Z, Harnett K, Biancani P and Behar J: Signal transduction pathways mediating CCK-induced gallbladder muscle contraction. Am J Physiol 275, G203 – G211 (1998)
12 Masui H and Wakabayashi I: Extracellular Ca²⁺-dependent contractile action of phorbol 12,13-dibutyrate on gall bladder from guinea pig. Life Sci 60, PL311 – PL316 (1997)
13 Pahlavan P, Wang LJ, Sack E and Arruda JA: Role of protein kinase C in the adaptive increase in Na-H antiporter in respiratory acidosis. J Am Soc Nephrol 4, 1079 – 1086 (1993)
14 Katsura K, Kurihara J, Siesjo BK and Wieloch T: Acidosis enhances translocation of protein kinase C but not Ca²⁺/calmodulin-dependent protein kinase II to cell membranes during complete cerebral ischemia. Brain Res 849, 119 – 127 (1999)
15 Rehring TF, Shapiro JI, Cain BS, Meldrum DR, Cleveland JC, Harken AH and Banerjee A: Mechanisms of pH preservation during global ischemia in preconditioned rat heart: roles for PKC and NHE. Am J Physiol 275, H805 – H813 (1998)