Digestive Diseases and Sciences, Vol. 37, No. 7 (July 1992), pp. 1045–1050

Role of Prostaglandin E_2 in Cholinergic-Mediated Glycoprotein Synthesis in Canine Antrum

KENJI YOSHIMURA, MD, EUGENE R. KRAUS, MS, SHUYA SHIMAKURA, MD, JAMES M. SCHEIMAN, MD, and C. RICHARD BOLAND, MD

We studied the mechanism of cholinergic stimulation of mucin synthesis in canine antral explants, including the role of PGE_2 as an intermediate messenger. Isolated antral mucosa was incubated with 10^{-5} M carbachol (Cb), 10^{-5} M indomethacin (IND), 10^{-5} M pirenzepine (PZ), 10^{-5} M Cb + 10^{-5} M PZ, 10^{-5} M Cb + 10^{-5} M IND, and 10^{-5} M IND + PGE_2 (10^{-8}, 10^{-7} and 10^{-6} M) in the presence or absence of [^{3}H]glucosamine. After 24 hr, total glycoprotein synthesis was quantitated by Sepharose-4B chromatography and by 10% TCA/1%PTA precipitation with lipid extraction. PGE_2 released into the media was measured by radioimmunoassay (RIA). Cb significantly increased total glycoprotein synthesis and produced a significant increase in PGE_2 release. The increase in glycoprotein synthesis and the release of PGE_2 was blocked by the addition of muscarinic antagonist PZ. The addition of IND significantly inhibited glycoprotein synthesis and almost entirely suppressed PGE_2 secretion. IND also inhibited the effect of Cb on glycoprotein synthesis and PGE_2 release. Moreover, PGE_2 (10^{-6} and 10^{-7} M) significantly increased the glycoprotein synthesis in the canine stomach. This suggests the coordinate participation of PGE_2-releasing cell population in modulation of glycoprotein synthesis in gastric mucosa.

KEY WORDS: acetylcholine; muscarinic receptors; gastric mucin; prostaglandin E_2.

Acetylcholine, an important mediator of gastric acid secretion (1, 2), has been shown by many laboratories to stimulate mucin secretion in the stomach (3–6). Coceani et al (7) have shown that cholinergic stimulation also significantly increased the release of prostaglandins (PGs), and that scopolamine completely suppressed the release of PGs induced by parasympathetic nerve stimulation. Seidel et al (8) have reported that intact vagal innervation is necessary for a mild irritant such as 30% ethanol to mediate the formation of mucosal PGs in the rat stomach. Moreover, Bickel and Kauffman (9) have demonstrated that PGE_2 increases gel mucus thickness in the stomach, and Seidler and coworkers (5, 10) have reported that both endogenous and exogenous prostaglandins stimulate glycoprotein secretion. However, the mechanism by which cholinergic agonists stimulate mucin secretion and their role in mucin synthesis, if any,

Manuscript received March 22, 1991; revised manuscript received January 15, 1992; accepted January 15, 1992.

From the Department of Internal Medicine, VA Medical Center; and University of Michigan School of Medicine, Ann Arbor, Michigan.

Supported in part by the Research Service of the Veterans Administration and grant R01-DK37989 and Michigan Gastrointestinal Peptide Research Center, 5P30-DK34933 awarded by the PHS, DHHS.

Presented in part at the annual meeting of American Gastroenterological Association in 1990 (*Gastroenterology* 98:A153).

Address for reprint requests: Dr. C. Richard Boland, GI Section (111D), VA Medical Center, 2215 Fuller Road, Ann Arbor, Michigan 48105.

0163-2116/92/0700-1045$06.50/0

remains unclear. In the present study, we examined mucin synthesis induced by a cholinergic agonist in canine antral explants, focusing on the role of PGE_2 as a possible intermediate messenger.

MATERIALS AND METHODS

Reagents. Surital (thiamylal sodium) was obtained from Parke-Davis (Morris Plains, New Jersey). Gentamicin was obtained from Flow Laboratories, Inc. (McLean, Virginia). DME (Dulbecco's modified Eagle's medium)–F-12 (1:1) tissue culture medium with L-glutamine and 15 mM HEPES (*N*-2-hydroxyethylpiperazine-*N'*-2-ethanesulfonic acid), sodium bicarbonate, carbachol (carbamylcholine chloride), pirenzepine dihydrochloride, indomethacin (1-[*p*-chlorobenzoyl]-5-methoxy-2-methylindole-3-acetic acid) crystalline, prostaglandin E_2 ([5*Z*,11α,13*E*,15*S*]-11,15-dihydroxy-9-oxoprosta-5,13-dien-1-oic acid) and phosphotungstic acid (PTA) were purchased from Sigma Chemical Company (St. Louis, Missouri); trichloroacetic acid (TCA) from Fisher Scientific (Livonia, Michigan); D-[6-^{3}H]glucosamine hydrochloride from Amersham Corporation (Arlington Heights, Illinois); diethyl-ether from Mallinckrodt Inc. (Paris, Kentucky); 100% ethyl alcohol from Midwest Grain Products Co. (Weston, Missouri); Safety-Solve from Research Products International Corp. (Mount Prospect, Illinois).

Tissue Culture. The stomachs were excised immediately after conditioned mongrel dogs were anesthetized with 5 ml of 2% Surital. The muscular layer was dissected from the antrum, and the mucosal layer was cut into equal 2 × 2-mm pieces using razor blades mounted at 2-mm intervals on a cutting frame. Preliminary studies revealed that this method provided mucosal explants of uniform mass and geometry for culture. Ten tissue pieces were placed in each plastic culture well (diameter 35 mm) with 2 ml of medium consisting of DME/F12 (1:1), L-glutamine, 15 mM HEPES, 7.5% fetal bovine serum (FBS), 7.5% heat-inactivated dog serum, and 0.05 mg/ml gentamicin (11). We added 10^{-5} M carbachol (Cb), 10^{-5} M pirenzepine (PZ), 10^{-5} M indomethacin (IND), 10^{-5} M Cb + 10^{-5} M PZ, 10^{-5} M Cb + 10^{-5} M IND + PGE_2 (10^{-8}–10^{-6} M) to individual wells, and used phosphate-buffered saline (PBS) as a control additive. The doses of each agent were selected based upon prior experiments that indicated a maximal increase in mucin synthesis by isolated canine mucosal cells in response to 10^{-5} M carbachol (JM Scheiman, ER Kraus, and CR Boland, unpublished data). The explants were incubated in the presence of 5 μCi/well D-[1,6-^{3}H]/glucosamine at 37° C under 5% CO_2–95% Air for 24 hr. Wells were also maintained in the absence of [^{3}H]glucosamine for the measurement of PGE_2.

Assessment of Glycoprotein Synthesis. At the end of the period of incubation, media and tissue fragments were harvested together. After homogenization and sonication, they were centrifuged at 100,000*g* at 4° C for 1 hr to remove cellular debris. The supernatant was collected, and the glycoprotein was quantitated as follows: Two methods were used in an attempt to sensitively and specifically measure mucin. Newly synthesized mucin was metabolically labeled with [^{3}H]glucosamine; one method relied upon the high molecular weight of mucin, and the other relied upon the ability to acid precipitate all proteins and glycoproteins. Our laboratory has used column chromatography extensively to purify gastric mucins; however, this does not remove noncovalently associated lipids that migrate with the mucins (11). Acid precipitation permits the isolation of proteins and glycoproteins, and, moreover, permits the extraction of noncovalently bound lipids. Unfortunately, this technique may also measure nonmucin glycoproteins, which make up a small percentage of the glycoproteins produced by these cells. For method I, 1 ml of each supernatant was applied to a Sepharose-CL-4B column (1.5 × 50 cm) equilibrated in PBS and eluted at 20 ml/hr. The void volume was pooled, and ^{3}H radioactivity was counted by liquid scintillation counting (12). For method II, the supernatant was precipitated with 10% TCA and 1% PTA at 4° C overnight. Then, the samples were centrifuged at 500*g* for 25 min, and the pellets were resuspended by sonication. Each pellet was washed three times with 10% TCA/1% PTA and twice with ether–ethanol (3:1). The pellets were dissolved in 2 ml of 0.6 M KOH and ^{3}H radioactivity was measured (5).

Measurement of Prostaglandin E_2. Fifty microliters of media was collected from each unlabeled well before (ie, immediately after placement in culture) and 24 hr after the administration of test agent and immediately frozen at −70° C. PGE_2 in media was quantitated by radioimmunoassay (RIA) in the University of Michigan Diabetes Center, Ligand Core Laboratory (13). The PGE_2 antibody was purchased commercially from Cayman Chemical Company (Ann Arbor, Michigan). Cross-reactivities with other prostaglandins are as follows: 15-keto-PGE_2—9.2%, and PGE_1—5%; cross-reactivities were less than 0.2% for the following prostaglandins-PGA_1, PGB_1, PGB_2, PGD_2, $PGF_{1\alpha}$, 6-keto $PGF_{1\alpha}$ $PGF_{2\alpha}$, 13,14-dihydro-15-keto-$PGF_{2\alpha}$, and thromboxane B_2 (manufacturer's specifications).

Statistical Analysis. The results are expressed as means ± SE. *N* is the number of experiments performed on different dogs, although multiple measurements were carried out on each individual canine stomach. Statistical analysis was carried out with the Student's *t* test. *P* values of less than 0.05 were considered to be significant.

RESULTS

The results of each assay are expressed as a percentage of control wells in Figures 1, 3, and 4, and as a percentage of Cb-treated wells in Figures 2 and 5.

Glycoprotein Synthesis. Glycoprotein synthesis measured by method I was termed V_0 (for void volume glycoprotein) and that by method II was termed Ppt (for acid-precipitated glycoprotein). The radioactivity in the 6 ml void volume peak (V_0) under basal conditions of culture was 4335 ± 605 cpm. Using the Ppt method in which the total glycoprotein faction was measured, 21,944 ± 1,345

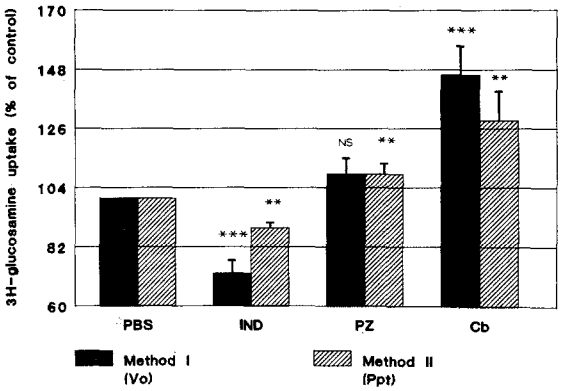

Fig 1. Mucin synthesis in response to 10^{-5} M indomethacin (IND), 10^{-5} M pirenzepine (PZ), and 10^{-5} M carbachol (Cb) measured by Sepharose-4B columns (method I—V_0) and glycoprotein precipitation (method II—Ppt) using 10% TCA/1%PTA and lipid extraction. Note the scale and zero line on the *y* axis. The values are expressed as a percentage of control wells. (N = 7; NS = not significant, $^{**}P < 0.01$, $^{***}P < 0.001$, compared with control.)

cpm were obtained under basal conditions of culture.

As shown in Figure 1, 10^{-5} M Cb significantly increased glycoprotein synthesis to 146 ± 11% of control ($P < 0.001$) as V_0, and 129 ± 11% ($P < 0.01$) as Ppt. The addition of 10^{-5} M IND significantly inhibited basal glycoprotein synthesis to 72 ± 5% as V_0 ($P < 0.001$) and 89 ± 25 as Ppt ($P < 0.01$). PZ, 10^{-5} M, produced a slight stimulation in glycoprotein synthesis as Ppt (109 ± 4%, $P < 0.01$, but not as V_0.

As shown in Figure 2, IND significantly reduced the effect of Cb on glycoprotein synthesis (V_0 = 74 ± 9%, $P < 0.05$, Ppt = 80 ± 8%, $P < 0.05$). In like

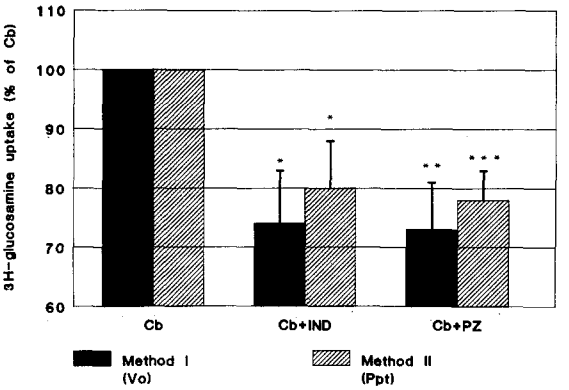

Fig 2. The effect of 10^{-5} M IND and 10^{-5} M PZ on 10^{-5} M carbachol-induced mucin synthesis measured by method I (Vo) and method II (Ppt). Note the scale and zero line on the *y* axis. The values are expressed as a percentage of carbachol-treated wells. (N = 7; $^{*}P < 0.05$, $^{**}P < 0.01$, $^{***}P < 0.001$, compared with the carbachol-treated wells.)

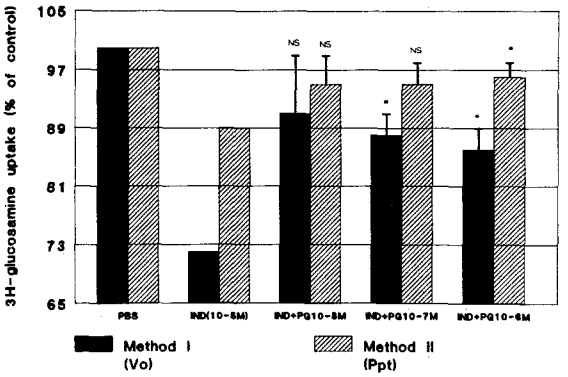

Fig 3. The effect of PGE_2 (10^{-8} M to 10^{-6} M) on 10^{-5} M IND-treated mucin synthesis measured by method I (V_0) and method II (Ppt). Note the scale and the zero line on the *y* axis. The values are expressed as a percentage of control wells. (N = 4; NS = not significant, $^{*}P < 0.05$, compared with the IND-treated wells.)

manner, PZ also decreased the effect of Cb on glycoprotein synthesis to 73 ± 8% ($P < 0.01$) as V_0 and to 78 ± 5% ($P < 0.001$) as Ppt.

As shown in Figure 3, 10^{-6} M PGE_2 significantly restored glycoprotein synthesis in the presence of an inhibitory concentration of IND (10^{-5} M), when measured as either V_0 ($P < 0.05$, compared with the IND-treated wells) or Ppt ($P < 0.05$). PGE_2, 10^{-7} M, significantly restored glycoprotein synthesis compared to IND alone, but only when measured as V_0 ($P < 0.05$).

Prostaglandin E_2 Release. The basal concentration of PGE_2 after 24 hr in culture was 43,178 ± 6113 pg/ml of media. As shown in Figure 4, 10^{-5} M Cb significantly increased PGE_2 release to 229 ± 18% of control wells ($P < 0.001$). IND, 10^{-5} M, caused a near-complete suppression of PGE_2 re-

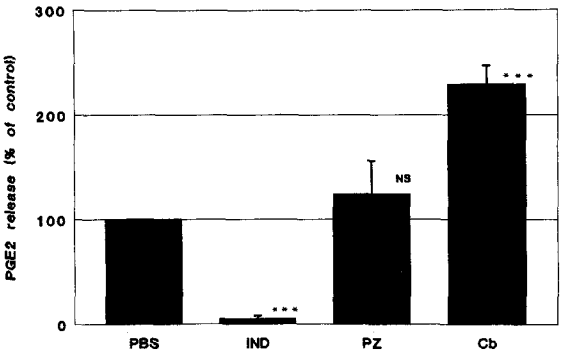

Fig 4. PGE_2 release in response to 10^{-5} M IND, 10^{-5} M PZ, and 10^{-5} M Cb measured by radioimmunoassay (RIA). Note that the scale and zero line are different from those in Figures 1–3. The values are expressed as a percentage of control wells. (N = 6; NS = not significant, $^{***}P < 0.001$, compared with PBS control.)

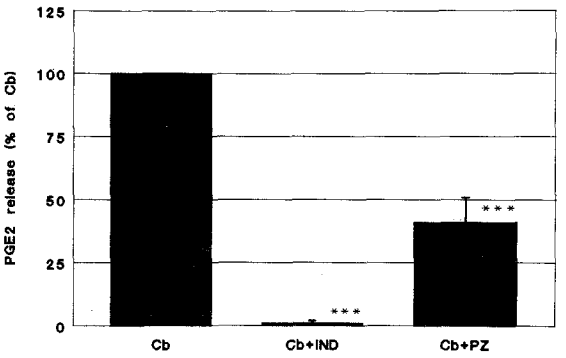

Fig 5. The effect of 10^{-5} M IND and 10^{-5} M PZ on 10^{-5} M Cb-induced PGE_2 release by the explants of canine gastric antrum measured by radioimmunoassay (RIA). The values are expressed as a percentage of 10^{-5} M carbachol-treated wells. ($N = 7$; $^{***}P < 0.001$, compared with the Cb-treated wells.)

lease to 5 ± 3% of control ($P < 0.001$). PZ, 10^{-5} M, failed to induce a significant change in PGE_2 release.

As shown in Figure 5, the addition of 10^{-5} M IND suppressed the effect of 10^{-5} M Cb on PGE_2 release to 1 ± 1% ($P < 0.001$) of Cb-treated wells. PZ, 10^{-5} M, also significantly inhibited the PGE_2 release stimulated by Cb to 41 ± 10% ($P < 0.001$) of that observed in Cb-treated wells.

DISCUSSION

The stimulant effect of the cholinergic agents on mucin synthesis or secretion has been reported from several laboratories. Gerard et al (4) have used histochemistry and incorporation of ^{35}S into gastric epithelium to show that urecholine increased the apparent synthesis and secretion of mucus by fundic crypt cells and antral pyloric glands *in vivo*. Vagne and Perret (6) have demonstrated that the secretion of fucose- and galactose-containing gastric juice glycoconjugates was stimulated by carbachol. In the present study, [^{3}H]glucosamine incorporation into antral tissues and new glycoprotein synthesis were significantly stimulated by the addition of carbachol to cultured explants, which is similar to the findings of Seidler and Sewing on glycoprotein secretion (5). The dose of carbachol used to stimulate mucin synthesis (10^{-5} M) may be pharmacological rather than physiological. However, it is very difficult to know what constitutes a physiological concentration of a neurocrine or paracrine substance, since unexpectedly high concentrations may be achieved at the target cell membranes. We have shown maximal stimulation of mucin synthesis by cultured canine gastric mucous cells by carbachol 10^{-6} M to 10^{-5} M, which is inhibitable by 10^{-4} M atropine (unpublished data, manuscript submitted).

Various results have been reported for the effects of indomethacin on gastric mucous cell function. Some authors have reported that indomethacin decreased apparent gastric mucus secretion and the amount of carbohydrate in secreted "mucosubstances" (10, 14) and that it reduced the viability of mucosal cells (15). In contrast, others have reported that the mucus content of antral mucosa showed no change during indomethacin therapy (16) and that indomethacin did not affect the viability of isolated gastric cells (17). Our present study has shown that indomethacin significantly decreased glycoprotein synthesis, and the effect was reversed by exogenous PGE_2 replacement. These discrepancies might be due to differences in experimental conditions but appear to reflect that the influence of indomethacin on mucous cells is not direct.

Prostaglandins (PGs) produced by the stomach have been shown to stimulate all recognized mucosal defense factors, including gastric and duodenal bicarbonate secretion, the production and release of mucus, surfactant lipid production and release with mucin (11), and they may have trophic effects on the gastric mucosa (18). It has been reported that both endogenous and exogenous PGE_2 stimulated glycoprotein secretion (5, 10) and that PGE_2 caused a significant increase in mucous gel thickness (9, 19). Offerhaus et al (20) have found that the increase in mucosal thickness after treatment with PGE_2 did not seem to be caused by enhanced proliferative activity, but rather by retarded senescence, especially of the foveolar cell layer. It has been shown that PGE_2 had a direct cellular "cytoprotective" action on gastric mucosal cells (15, 17). It has been recently reported that gastric mucosa had specific receptors for PGE_2 and that receptor binding was an important initial step in PGE_2-mediated cellular responses in the stomach (21).

Wollin et al (22) have reported that the adenylate cyclase activity was stimulated by both PGs and histamine in fundic mucosa, but only by PGs in antral mucosa, and that histamine and PGs stimulated two different adenylate cyclase systems. Soll, Wollin, and their colleagues (23, 24) have shown that PGE_2 stimulated the production of cyclic AMP by nonparietal cells. Heim et al (25) have recently reported that PGE_2 stimulated glycoprotein synthesis by pig gastric mucous cells. Our present study has shown that PGE_2 significantly increased the

glycoprotein synthesis suppressed by 10^{-5} M indomethacin at a dose of 10^{-6} M and 10^{-7} M. However, exogenous PGE_2 restored glycoprotein synthesis to a level slightly less than measured in control tissues. This might indicate that other PGs coordinately regulate glycoprotein synthesis or that indomethacin reduces the viability of mucous cells.

The present work demonstrates that the glycoprotein synthesis stimulated by carbachol was significantly reduced by both indomethacin and pirenzepine. This indicates that both PGs and muscarinic receptors participate in the stimulus–response mechanism of Cb. However, as we and other authors (26, 27) have shown, the M1 antagonist pirenzepine alone does not suppress glycoprotein synthesis. Nakamura et al (28, 29) have used [^{3}H]pirenzepine and autoradiography to show that pirenzepine-binding sites are localized predominantly on parietal cells, chief cells, and capillary endothelium and that surface mucous and mucous neck cells had fewer pirenzepine binding sites. They have also suggested that parietal and chief cells possessed low-affinity M1-muscarinic receptors, while the perivascular plexuses have high-affinity receptors. On the other hand, Lundell (30) has shown that acetylcholine stimulated histamine formation through muscarinic receptors on mast cells in a dose-dependent manner. Soll (2) has demonstrated that parietal cells account for most of the increase in oxygen uptake produced by exposure to gastric secretagogues in fundic isolated cell fractions. Thus, it is not likely that mucous and mucous neck cells have the same muscarinic receptors as parietal cells (1). Pirenzepine at a dose of 10^{-5} M may have a nonspecific muscarinic effect (ie, may not be M1 selective), so the precise definition of the receptor subtype remains to be proven.

It has been shown that cholinergic nerve stimulation and carbachol stimulate the release of PGE_2 in the rat stomach (7) and that vagotomy prevents adaptive cytoprotection to the gastric mucosal injury induced by ethanol (8). Postius et al (31) have suggested that more PGE_2 is produced by parietal than by mucosal or chief cells; moreover, Arakawa et al (17) have shown that surface epithelial cells predominantly produce 6-keto-prostaglandin $F_{1\alpha}$ and thromboxane B_2, rather than PGE_2. In the present work, carbachol increased PGE_2 release to a value more than twice the control value and significantly stimulated glycoprotein synthesis. The stimulation of PGE_2 release by carbachol was suppressed almost completely by the addition of indomethacin and was reduced to less than half by the addition of pirenzepine. This indicates the obligatory role of muscarinic receptors as an intermediate PGE_2-releasing cell and suggests the participation of an additional cellular species in the regulation of mucus production in the intact stomach.

Seidler and Sewing (5) have demonstrated that cholinergic stimulation of mucus secretion is accompanied by an increase in intracellular Ca^{2+}, but that forskolin and PGE_2 stimulate mucus secretion by independent mechanisms. In light of this, it may be suggested that there are at least two mechanisms by which mucin synthesis is induced by acetylcholine in the stomach. One requires PGE_2-releasing cells that have muscarinic receptors, which could include capillary endothelial cells or mast cells. In fact, Chen et al (32) have demonstrated that macrophages and capillary endothelial cells are the major producers of PGE_2 in the canine fundus. PGE_2 may activate adenylate cyclase and stimulate cAMP production in mucous cells through their specific receptors, stimulating mucin synthesis. In addition, nonspecific, low-affinity muscarinic receptors on mucus cells may function through a second mechanism to increase intracellular calcium and independently stimulate mucin synthesis and the secretion of mucus. The role of multiple agonists utilizing independent second messengers has been well demonstrated in the regulation of acid secretion by parietal cells (2, 23). Although PGs inhibit secretory function in parietal cells through an interaction with the G(i) subunit of the transmembrane signal transduction mechanism (33), the intracellular mechanism of mucous cell response to PGs is not well understood.

In summary, we have demonstrated that the stimulation of glycoprotein synthesis by a cholinergic agonist may be inhibited by either a muscarinic antagonist (possibly M1) or by inhibiting PG production. This suggests that PGs serve as obligatory intermediate messengers in the mediation of cholinergic stimulation of mucous cell function. The intracellular events that mediate the post-PG response remain to be elucidated.

REFERENCES

1. Soll AH: Potentiating interaction of gastric stimulants on aminopyrine accumulation by isolated canine parietal cells. Gastroenterology 83:216–223, 1982
2. Soll AH: The actions of secretagogues on oxygen uptake by isolated mammalian parietal cells. J Clin Invest 61:370, 1978
3. Boland CR, Kraus ER, Dobbins WO: Modulation of gastric

mucin synthesis by gastrin and histamine in a new gastric mucous cell culture system. Gastroenterology 90:1349, 1986 (abstract).
4. Gerard A, Lev R, Glass GBJ: Histochemical study of the mucosubstances in the canine stomach. Lab Invest 19:29–39, 1968
5. Seidler U, Sewing KF: Ca^{2+}-dependent and -independent secretagogue action on gastric mucus secretion in rabbit mucosal explants. Am J Physiol 256:G739–G746, 1989
6. Vagne M, Perret G: Regulation of gastric mucus secretion. Scand J Gastroenterol 11(suppl 42):63–74, 1976
7. Coceani F, Pace-Asciak C, Volta F, Wolfe LS: Effect of nerve stimulation on prostaglandin formation and release from the rat stomach. Am J Physiol 213(4):1056–1064, 1967
8. Seidel ER, Henagan JM, Miller TA: Vagotomy prevents adaptive cytoprotection to ethanol-induced gastric mucosal injury. Gastroenterology 82:1175, 1982
9. Bickel M, Kauffman GL: Gastric gel mucus thickness: Effect of distention 16,16-dimethyl prostaglandin E_2 and carbenoxolone. Gastroenterology 80:770–775, 1981
10. Seidler U, Knafla K, Kownatzki R, Sewing K-Fr: Effects of endogenous and exogenous prostaglandins on glycoprotein synthesis and secretion in isolated rabbit gastric mucosa. Gastroenterology 95:945–951, 1988
11. Scheiman JM, Kraus ER, Bonnville LA, Weinhold PA, Boland CR: Gastric surfactant production in isolated gastric mucosal cells. Evidence for a mucin glycoprotein phospholipid complex and regulation by PGE_2. Gastroenterology 100:1232–1240, 1991
12. Goso K, Ishihara K, Ohara S, Kakei M, Hotta K: Characterization of sulfated glycoconjugates in human gastric mucosa. Digestion 31:205–212, 1985
13. Peters-Golden M, Thebert P: Inhibition by methylprednisolone of zymosan-induced leukotriene synthesis in alveolar macrophages. Am Rev Respir Dis 135:1020–1026, 1987
14. Menguy R, Desbaillets L: Role of inhibition of gastric mucous secretion in the phenomenon of gastric mucosal injury by indomethacin. Am J Dig Dis 12:862–866, 1967
15. Tarnawski A, Brzozowski T, Sarfeh IJ, Krause WJ, Ulich TR: Prostaglandin protection of human isolated gastric glands against indomethacin and ethanol injury. J Clin Invest 81:1081–1089, 1988
16. Nicoloff DM: Indomethacin. Arch Surg 97:809–815, 1968
17. Arakawa T, Nakamura A, Fukuda T, Satoh H, Kobayashi K: Protection of isolated rate gastric cells by prostaglandins from damage caused by ethanol. J Clin Gastroenterol 10(suppl 1):S28–S34, 1988
18. Johansson C, Bergstrom S: Prostaglandins and protection of the gastroduodenal mucosa. Scand J Gastroenterol 77(suppl):21–37, 1982
19. Takeuchi K, Magee D, Critchlow J, Matthews J, Silen W: Studies of the pH gradient and thickness of frog gastric mucus gel. Gastroenterology 84:331–340, 1983
20. Offerhaus GJA, Tytgat GNJ, Samson G, Weinstein WM: Cell proliferation kinetics of the gastric mucosa after oral 15(*R*)-15-methyl prostaglandin E_2 administration. Gastroenterology 86:1199, 1984 (abstract)
21. Wu-Wang CY, Wang SL, Slomiany A, Slomiany BL: Characterization of gastric mucosal prostaglandin E_2 receptor. FASEB J 4:A475, 1990 (abstract)
22. Wollin A, Code CF, Dousa TP: Interaction of prostaglandins and histamine with enzymes of cyclic AMP metabolism from guinea pig gastric mucosa. J Clin Invest 57:1548–1553, 1976
23. Soll AH, Wollin A: The effects of histamine, prostaglandin E_2, and secretin on cyclic AMP in separated canine fundic mucosal cells. Gastroenterology 72:1166, 1977 (abstract)
24. Wollin A, Soll AH, Samloff IM: Actions of histamine, secretin, and PGE_2 on cyclic AMP production by isolated canine fundic mucosal cells. Am J Physiol 237(5):E437–E443, 1979
25. Heim H-K, Oestmann A, Sewing K-Fr: E-type prostaglandins stimulate protein-, glycoprotein- and mRNA-production in the mucous glycoprotein release from isolated pig gastric mucosal cells. Gastroenterology 98:A57, 1990 (abstract)
26. Chierichetti SM, Gaetani M, Petrin G: Pirenzepine in peptic ulcer. Scand J Gastroenterol 14(suppl 57):7–9, 1979
27. Guslandi M, Testoni PA, Fesce E, Ballarin E, Tittobello A: Behaviour of gastric mucus during pirenzepine treatment: A double-blind controlled study versus cimetidine. Curr Ther Res 27:714–718, 1980
28. Nakamura M, Oda M, Yonei Y, Tsukada N, Komatsu H, Kaneko K, Tsuchiya M: Muscarinic acetylcholine receptors in rat gastric mucosa. Histochemistry 83:479–487, 1985
29. Nakamura M, Oda M, Yonei Y, Tsukada N, Watanabe N, Komatsu H, Tsuchiya M: Demonstration of the localization of muscarinic acetylcholine receptors in the gastric mucosa. Acta Histochem Cytochem 17:297–307, 1984
30. Lundell L: The role of cholinergic excitation in the formation of histamine within the rat gastric mucosa. Acta Physiol Scand 97:145–149, 1976
31. Postius S, Ruoff HJ, Szelenyi I: Prostaglandin formation by isolated gastric parietal and nonparietal cells of the rat. Br J Pharmacol 84:871–877, 1985
32. Chen MC, Sanders MJ, Amirian DA, Thomas LP, Kauffman G, Soll AH: Prostaglandin E_2 production by dispersed canine fundic mucosal cells. J Clin Invest 84:1536–1549, 1989
33. Soll AH, Berglindh HT: Physiology of isolated gastric glands and parietal cells: Receptors and affectors regulating function. *In* Physiology of the Gastrointestinal Tract, LR Johnson, J Christensen, MJ Jackson, ED Jacobson, JW Walsh (eds.). New York, Raven Press, 1987, pp 883–911