Peptic Activity and Gastroduodenal Mucosal Damage

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This contribution reviews briefly the history of the discovery and characterization of peptic activity; secretory models and current concepts regarding the regulation of pepsinogen secretion; and evidence that pepsin is a necessary co-factor for gastroduodenal mucosal injury. Several animal studies indicate that peptic activity is required for acid- and nonsteroidal anti-inflammatory drug-induced gastroduodenal ulceration. A more vigorous approach to the development of anti-peptic drugs for the treatment of peptic ulcer disease is encouraged.

INTRODUCTION

The contribution of pepsin to the pathogenesis of gastroduodenal mucosal damage is implicit in the term “peptic” ulcer disease. Nonetheless, for many years continuing into the present era, elucidation of the physiology and pathophysiology of pepsinogen secretion has taken a back seat to the study of gastric acid secretion. Likewise, medical therapy of gastrointestinal mucosal damage has focused on agents that neutralize or inhibit the secretion of gastric acid. This, despite the importance of gastric acid in preventing infection and promoting the absorption of various nutrients. Surprisingly, little effort has been directed to develop therapies that might inactivate pepsin or prevent the secretion of pepsinogen, maneuvers that are less likely to disrupt gastric homeostasis and result in bacterial overgrowth, gastrin hypersecretion and other side-effects of acid suppression therapy.

In this paper, the author will review briefly the history of the discovery and characterization of peptic activity; secretory models and current concepts regarding the regulation of pepsinogen secretion; and evidence that pepsin is a necessary co-factor in the development of gastroduodenal mucosal damage.

THE DISCOVERY AND CHARACTERIZATION OF PEPTIC ACTIVITY IN GASTRIC JUICE

The story of Dr. William Beaumont's experimentation on the Canadian fur trapper Alexis St. Martin is known to anyone with an interest in gastric physiology. In a series of experiments performed during the 1820s and 1830s, Beaumont took advantage of St. Martin's gastro-cutaneous fistula to study aspects of gastric function. Among many significant observations, these studies confirmed that the human stomach secretes hydrochloric acid. Nonetheless, it is not generally appreciated that Beaumont anticipated the discovery of pepsin. In a simple, but brilliant, experiment, Beaumont prepared a mixture of dilute hydrochloric acid, water and saliva that was adjusted (by taste!) to mimic gastric juice. Identical pieces of meat were suspended in equal volumes of this mixture and gastric juice that had been removed from St. Martin's stomach.

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*Abbreviations: IP₃, inositol triphosphate; DAG, diacylglycerol; NSAID, non-steroidal anti-inflammatory drugs
Into 3 drachms of this mixture immersed XX grs. lean broiled beefsteak unmasticated, but chopped fine with a knife, & for a comparison put the same qty. and kind of meat into 3 drachms gastric juice, and placed them on the bath and treated them together for 6 hours. When the meat in the gastric juice was taken out and filtered dry, weighed 2 grs. only; that in the artificial menstrum was not diminished in weight at all, but had lost its fibrous form and became a jelly-like mass, thick and tremulous, like half dissolved glue-not converted into anything like chyme, nor bore much resemblance to the contents of the gastric juice [1].

From this experiment, Beaumont concluded that, in addition to acid, gastric juice contained a constituent that promoted the digestion of meat. In 1836, acknowledging Beaumont's contribution, Theodor Schwann coined the name pepsin for a proteolytic constituent of gastric juice that was activated by acid and inactivated by alkali [2]. Subsequent investigations from the laboratories of Wasmann, and particularly Langley during the late 1800s, resulted in the discovery that pepsin is secreted as a proenzyme, designated pepsinogen [3-5]. Pepsin (mol. wt., 35.5 kDa) and pepsinogen (mol. wt., 42.5 kDa) were crystallized by Northrop [6] and Herriot [7], respectively.

Herriot established that at acidic pH (< 6.0) pepsinogen undergoes autocatalysis whereby a 3.1-kDa inhibitory piece is cleaved from the N-terminal portion of the molecule and digested by the resulting pepsin as the pH drops below 4.0 [8]. The pH optimum for pepsin, depending on the substrate, ranges between 1.0 and 3.0 [9, 10]. Commonly, pepsin activity is determined by examining the acid hydrolysis of native or radiolabeled hemoglobin or albumin (measuring the release into the medium of tyrosine or radiolabel, respectively).

**PHYSIOLOGICAL MODELS USED TO STUDY PEPSONGEN SECRETION**

As in Beaumont's experiments, models examining secretion in intact, innervated tomachs provide the most physiological approximation of pepsinogen secretion in humans and other species. Nevertheless, because gastric juice is comprised of components secreted by a variety of cells, dissecting the relative contribution of neural and hormonal stimuli to the control of pepsinogen secretion may be quite difficult. "Wash-out" of preformed pepsinogen from gastric glands along with fluid and electrolytes may result in overestimation of secretion. Hence, investigators have developed peptic models, like mucosal cell cultures, gastric glands and dispersed chief cells, which obviate some of these difficulties. The major disadvantage of these models, including the use of dispersed peptic cells from human stomach [11], is that physiological cell-cell interactions may be lost.

**REGULATION OF PEPSONGEN SECRETION**

The following represents a brief description of the regulation of pepsinogen secretion. For a more detailed analysis of signal transduction pathways in gastric chief cells the reader may consult a recent review [12].

In general, there are two major signal transduction pathways that mediate pepsinogen secretion (Figure 1). In one pathway, the interaction of agents like cholecystokinin, gastrin and cholinergic agonists with specific cell membrane receptors results in the activation of phospholipase C, phospholipid turnover, and the production of inositol trisphosphate (IP$_3$) and diacylglycerol (DAG). In turn, IP$_3$ stimulates the release of calcium from intracellular stores thereby activating kinases, like calcium/calmodulin kinase-II [13], and phosphatases, like protein phosphatase-2B (calcineurin) [14]. DAG directly activates different isoforms of protein kinase C [15].

In the other pathway, the interaction of agents like vasoactive intestinal peptide, pituitary adenyl cyclase activating peptide, secretin, glucagon-like peptide-1, prostaglandins
Figure 1. Cartoon illustrating cellular regulation of pepsinogen secretion from dispersed chief cells from guinea pig stomach. On left, colors indicate three sets of agents: those that activate phospholipase C and phospholipid turnover, those that activate adenyl cyclase and cause a rise in cellular cAMP, and those that inhibit agonist-induced stimulation of adenyl cyclase. Increases in cellular calcium, cAMP and DAG activate kinases and phosphatases that mediate pepsinogen secretion. Broken arrow indicates interaction between the phospholipase C and adenyl cyclase pathways. Question mark indicates distal components of signalling pathways that remain to be elucidated. Gq, Gs, Gi = guanine nucleotide binding proteins; IP3 = inositol trisphosphate; DAG = diacylglycerol.

and cholera toxin with specific cell-membrane receptors results in the activation of adenyl cyclase and the production of cAMP. cAMP directly activates protein kinase A. Although some evidence exists that somatostatin, peptide YY and neuropeptide Y may negatively modulate adenyl cyclase in dispersed chief cells, the physiological importance of this observation is not clear [16-18].

Besides the initiation of cascades that result in activation of protein kinases and/or phosphatases, similarities between these signal transduction pathways include the involvement of heterotrimeric guanine nucleotide binding proteins, like Gq and Gs, in mediating the steps following ligand-receptor interaction. Moreover, there is evidence of interaction between the phospholipase C and adenyl cyclase pathways, so-called “cross-talk,” which results in augmentation of cellular levels of cAMP and potentiation of pepsinogen secretion (indicated by the broken arrow in Figure 1) [19, 20].

The current focus of activity in our laboratory is identification and characterization of the substrates for activated chief cell kinases and phosphatases (note the question mark in Figure 1). For example, recent work indicates that agonist-induced increases in chief cell calcium concentration results in activation of protein phosphatase-2B and a consequent reduction in the phosphorylation of 18- and 55-kDa cytoskeletal proteins [14].
Identification and characterization of this protein may be very helpful in elucidating the distal steps that mediate pepsinogen secretion.

**ROLE OF PEPSIN IN THE DEVELOPMENT OF GASTRODUODENAL MUCOSAL DAMAGE**

The precise mechanism(s) leading to gastroduodenal mucosal ulceration remains enigmatic. Despite recent interest in the association of *Helicobacter pylori* infection with ulceration, it is not yet clear how the presence of this organism facilitates mucosal injury. Nevertheless, it is apparent that factors such as an increase in the luminal concentration of hydrochloric acid, gastric mucosal ischemia and drugs, such as non-steroidal anti-inflammatory drugs (NSAIDs), that break down the mucosal barrier predispose to ulceration. Many of these events are associated with a drop in intramucosal pH, which leads to activation of pepsinogen to pepsin, and approaches the pH optimum for this acid protease.

Several observations indicate that pepsin is a necessary co-factor for gastric mucosal ulceration. Early studies indicated that the addition of pepsin to acid perfusates of canine or feline stomach or intestine, respectively, increased the likelihood and severity of mucosal ulceration [21-23]. More recent studies indicate not only that pepsin potentiates acid injury to the gastrointestinal tract, but that, in the absence of pepsin, acid may be relatively innocuous. In rats, Alphin et al. [24] found that inhibition of peptic activity prevented gastric ulceration even when the pH of the perfusate was as low as 1.3. Ford et al. [25] demonstrated that acid proteinase activity in rat gastric mucosa increased following ischemia and that this was associated with mucosal erosion. Treatment with luminal inhibitors of peptic activity reduced tissue proteinase activity and the number of erosions [25]. Similar findings were observed by Joffe et al. [26] when rat duodenal were perfused in the absence or presence of pepsin.

In a recent study, Gaw et al. [27] treated rats with subcutaneous indomethacin after they had received a pepsin inhibitor (pepstatin-A) or vehicle orally. Treatment with the pepsin inhibitor caused a dose-dependent decrease in indomethacin-induced gastric antral mucosal ulceration. Moreover, ulceration under these conditions was prevented even if acidified methylcellulose ([HCl] = 100 mM) was given orally [27]. In contrast, treatment with a histamine-2 receptor blocker decreased indomethacin-induced ulceration but did not prevent the lesions caused by the addition of acid methylcellulose. Hence, these studies indicate that pepsin is an obligatory co-factor for acid- and indomethacin-induced mucosal damage.

**CONCLUSIONS**

For nearly a century, peptic ulcer therapy focused on neutralization of gastric acid with antacids, or the suppression of acid secretion with anticholinergics, vagotomy, H2-blockers or, more recently, proton pump inhibitors. As reviewed above, it is conceivable that the major benefit derived from anti-acid therapy is the maintenance of a luminal pH > 4.0 that decreases conversion of pepsinogen to pepsin and creates an environment that is suboptimal for the proteolytic actions of the acid protease. Despite the recent revolution in therapy caused by recognition of the role of *H. pylori* in ulcer disease, current treatment recommendations for acute ulceration regard antibiotics as adjuncts to measures that reduce gastric acid. Moreover, several studies have found that *H. pylori* plays no role in the pathogenesis of an increasingly important fraction of the ulcer population, those caused by NSAID use. Hence, given the data presented in this review, it appears that ulcer therapy directed at inhibiting peptic activity may be the only approach that is likely to prevent or heal ulcers caused by gastric acid, *H. pylori* infection or the use of NSAIDs without the potential side effects associated with antisecretory drugs, antibiotics and
prostaglandins. More research directed at developing clinically useful anti-peptic therapy is warranted.

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