The Pathways Regulating Acid Secretion: 
The View from the Isolated Cell

Che-Nan Chuang, Monica C.Y. Chen and Andrew H. Soll a

VA Wadsworth Hospital Center and the School of Medicine, 
University of California at Los Angeles, Los Angeles, California

(Received February 25, 1994; accepted December 12, 1994)

Although many aspects of the regulation of acid secretion at the cellular level among different species remains controversial, certain concepts have emerged that span the differences between species, model systems and investigators. The paracrine, endocrine, neural and autocrine pathways mediate acid secretion by acting both directly on the parietal cell and indirectly via modulation of mucosal paracrine cell function. Studies with cells isolated from the acid secreting canine oxyntic mucosa indicate that gastrin and cholinergic receptors are present on parietal cells, somatostatin cells, and the histamine-enterochromaffin-like cell (ECL). Subtypes of these receptors are clearly important; the gastrin receptor on the ECL cell and parietal cell are "B" type CCK/gastrin receptors, whereas the receptor on the somatostatin cell is an A type CCK receptor. From the vantage point of studies in the canine oxyntic mucosa, the challenge is no longer to determine whether parietal, histamine or somatostatin cells have gastrin or muscarinic receptors but to establish the physiologic relevance of the specific actions (secretory, trophic or differentiative) of these receptor subtypes. Furthermore, the mechanisms integrating these paracrine, exocrine and neural elements require elucidation.

INTRODUCTION

Although controversy has surrounded the cellular mechanisms and receptors accounting for the actions and interactions on secretagogues on acid secretion over the last several decades, considerable agreement has begun to emerge regarding the basic elements of this regulation. The most controversial element has been the localization and mechanisms of action of gastrin and acetylcholine on acid secretion. Code hypothesized that histamine was the final common chemostimulator of the parietal cell [1] for the actions of acetylcholine and gastrin. He postulated that receptors for these chemostimulators resided on gastric histamine-containing cells, not on parietal cells. Although this model accounted for the ability of histamine H2-receptor antagonists to block the acid-secretory response to all stimuli, it did not explain inhibition by anticholinergic agents of the response to gastrin. Grossman proposed an alternate hypothesis [2] that the parietal cell possessed separate and specific receptors for histamine, gastrin and acetylcholine, with potentiating interactions among the secretagogues at the parietal cell itself. The controversy centered about whether gastrin and acetylcholine acted in parallel with histamine on the parietal cell or acted in series, with the final effector at the parietal cell being histamine. After many years

aTo whom all correspondence should be addressed: Dr. Andrew H. Soll, VA Wadsworth Hospital Center, Bldg. 115, Rm. 215 (691/151H), Los Angeles, California 90073. Tel: (310) 312-9479; Fax: (310) 824-6623 or (310) 824-6752.

bAbbreviations: CCK, cholecystokinin; ECL, enterochromaffin-like cell.
of controversy, it is now clear that both answers are correct, but the physiological relevance of each of the redundant receptors present on several cell types remains to be determined.

**COMPLEXITIES OF THE REGULATORY PATHWAYS**

Several elements have contributed to the difficulty encountered in resolving these questions. These regulatory mechanisms involve stimulatory and inhibitory pathways. Furthermore, regulatory transmitters for several of these elements are in the vicinity or delivered to the parietal and paracrine cells in amounts sufficient to exert control over function in the basal state. In vivo, specific receptor antagonists, or occasionally monoclonal antibodies, have held the key to revealing the physiologic importance of specific receptors, such as the $H_2$ histamine receptor or the type A cholecystokinin (CCK)$^b$ receptor. The role of histamine in the regulation of acid secretion was debated until the $H_2$ receptor antagonists were shown to block all forms of acid secretion. Specific CCK type A receptor antagonists enhance the acid secretory response to CCK, indicating the importance of inhibitory pathways mediated by these receptors. Definition of the specific pathways and receptor localization for neural input have been difficult to quantify and define because these pathways impinge on numerous specific cell types, because transmitter output cannot be measured and because specific pharmacologic tools are not yet available to unravel these overlapping and redundant receptors subtypes.

**THE IN VITRO APPROACH**

Removal of the parietal and paracrine cells from the influences of these endogenous stimulants represents a major advantage of in vitro studies. With isolated cells or glands, cell function can be directly monitored and stimulants added alone or in combination to assess the specificity of receptors and potential mechanisms for secretagogue interdependence. Another major advantage of isolated cell studies is the potential for cell separation. Gastric mucosa represents a complex mixture of cell types so that enrichment of various subpopulations becomes essential for certain studies, such as localization of receptors or characterization of cell activation mechanisms.

**A MODEL FOR REGULATORY CONTROL OF PARACRINE AND EXOCRINE ELEMENTS**

Isolated cell systems have proven useful for studying the receptors mediating secretion because they allow the functional response on individual elements to be assessed in the absence of intrinsic regulatory input. A model for the major pathways and cell types mediating secretory function in the oxyntic mucosa is depicted in Figure 1. This model illustrates the major stimulatory pathways, with parietal cells receiving paracrine (histamine), neural (acetylcholine) and endocrine (gastrin) input; although the existence of these receptors is clear, only the $H_2$ histamine receptor stands unchallenged as the receptor mediating the acid secretory response to histamine. Histamine-ECL cells receive endocrine (gastrin) and neural (acetylcholine) input [3]. The somatostatin cell is the major mediator of paracrine inhibition in the oxyntic mucosa, receiving endocrine input primarily via CCK/A receptors. Neural input to the somatostatin cell mediated by acetylcholine acting at muscarinic receptors serves to inhibit somatostatin release, thereby enhancing the acid secretory response. Somatostatin, in turn, appears to primarily exert paracrine down-regulation of histamine release from ECL cells, although other targets for somatostatin action may be important. The pathways regulating acid secretion, therefore, converge at several sites, including parietal, histamine-ECL and somatostatin cells. Of uncertain physiologic importance are the oxyntic mucosal mast cells, which contain sufficient histamine in close proximity to parietal cells to markedly impact secretory function; the
Figure 1. A model for regulation of acid secretory function. The pathways regulating acid secretion provide input both to the parietal cell and to stimulatory (histamine-ECL) and inhibitor (somatostatin) paracrine cells. Neural input provides critical integration.

physiology of histamine release from oxyntic mucosal mast cells is unknown. Many other cell types in the lamina propria, such as macrophages, may also exert control of mucosal cell function, but details remain unknown.

THE INTERACTIONS BETWEEN PATHWAYS

Mirroring the redundancy of the pathways regulating acid secretion, interactions between pathways occur via several mechanisms. The histamine dependency of cholinergic and gastrin action in part reflects cholinergic and gastrin induction of histamine release from ECL cells. However, this mechanism would not account for the enhancement of gastrin or cholinergic action by added histamine, which can be demonstrated in vivo and in vitro studies with parietal cells. Potentiating interactions at the parietal cell itself are also hypothesized to account for a component of the interdependency between secretagogues. Other interactions, such as cholinergic-gastrin interdependency, may reflect the summation of actions on different cell types: cholinergic input enhances release of histamine, stimulates the parietal cell and inhibits release of the paracrine mediator somatostatin; therefore, each of these actions would serve to enhance gastrin stimulation of acid secretion. A primary element in this interaction may be reduced somatostatin inhibition of gastrin-induced histamine release. In contrast, with the administration of antimuscarinic agents, stimulation by gastrin might be reduced by withdrawal of the additive effects of muscarinic input at parietal and histamine cells and by removal of cholinergic inhibition of somatostatin release. However, the physiological importance of the integration of paracrine input by neural mechanisms remains speculative and has not been tested in vivo.

THE PHYSIOLOGIC RELEVANCE OF RECEPTORS ON PARIENTAL AND ECL CELLS

Studies utilizing isolated oxyntic mucosal cells clearly indicate that gastrin and acetylcholine receptors are present on several cell types, including parietal cells, somatostatin cells, and histamine-containing endocrine cells. However, the contribution to control of secretion, cell replication and differentiation of each of these different receptor sites remains uncertain.
ECL cell receptors

The ability of gastrin to induce histamine release from gastric mucosal endocrine cells has been established [4-8]. In rabbit and rat gastric mucosa, the magnitude of histamine release by gastrin appears to be sufficient to stimulate parietal cell function, supporting the physiologic importance of this indirect limb of gastrin action for acid secretion regulation [7, 9]. Histamine-ECL cells from the oxyntic mucosa from several species respond well to gastrin in vitro, in contrast to the definite, but weak and variable, responses of the parietal cell to gastrin. Therefore, it appears likely that gastrin receptors on histamine-ECL cells mediate gastrin action on acid secretion.

Parietal cell receptors

There are, however, several lines of evidence indicating that functional gastrin receptors are also present on parietal cells [3]. The potentiating interactions observed between gastrin and histamine or post-receptors activators of the cyclic AMP pathway cannot be explained without hypothesizing a parietal cell gastrin receptor. Furthermore, in vivo, gastrin receptors appear to exert effects on acid secretion that are independent of histamine H₂ receptors. For example, Michelangeli and Ruiz found that, in the presence of cimetidine, dibutryl-cAMP enhanced gastrin stimulation of acid secretion [10], arguing for direct gastrin action of the parietal cell, with interactions via post-receptor cyclic AMP mechanisms. Black has also found direct interactions between dibutyryl cyclic AMP and cholinergic stimulation at parietal cell receptors [11]. In neonatal rats, Ackerman [12] found a dissociation between gastrin- and histamine-stimulated acid secretion; the acid secretory response to exogenous pentagastrin was detectable at 14 days of age, whereas response to exogenous histamine did not become detectable until day 19 to 22. Furthermore, the response to gastrin at 14 days of age was not blocked by H₂ blockers, suggesting an direct component of gastrin action in vivo independent of histamine. Taken together, the available data strongly indicate that functional gastrin receptors exist on parietal cells; however, data do not indicate the proportion of the acid secretory response to gastrin that reflects interaction with these parietal cell gastrin receptors.

It is possible that gastrin stimulation of the parietal cell and induction of histamine release are both physiologically relevant effects, but other functions may be served by these receptors in addition to the acute regulation of acid secretory mechanisms. The gastrin effect on either of these cell types may reflect trophic actions and/or induction and maintenance of cell differentiation. For example, gastrin has been reported to induce expression of the H⁺/K⁺-ATPase messenger RNA in parietal cells [13] and to induce histamine decarboxylase activity in the rat oxyntic ECL [14]. Approaches are needed to determine the physiologic importance of these differentiative effects in comparison to the effects of gastrin that stimulate acid secretion via either induction of histamine release or direct activation of the parietal cell.

INHIBITORY MODULATORS

Several inhibitory elements are also responsive to input from paracrine, endocrine and neural input. Using immunoneutralization, somatostatin has been shown to exert paracrine inhibitory effects in vivo, ex vivo and in tissue culture systems, leaving no question that the inhibitory effects of somatostatin are physiologically relevant. Studies with ex vivo preparations provided evidence that oxyntic somatostatin plays a role in acid feedback inhibition of acid secretion [15]. Although somatostatin inhibits gastrin and parietal cells, inhibition of oxyntic mucosal histamine release is a sensitive and dramatic effect of somatostatin. The major effect of somatostatin action may be inhibition of histamine release; this conclusion is consistent with the in vivo findings that somatostatin more effectively inhibits gastrin than histamine stimulation.
The physiologic importance of the inhibitory paracrine circuit mediated by somatostatin has been supported by studies involving gastrin/CCK receptor family subtypes. CCK type A receptors on the oxyntic mucosal somatostatin cell exert inhibitory effects on acid secretion. In the dog, the poor efficacy of CCK stimulating acid secretion, despite an efficacy equal to gastrin at both parietal and histamine-ECL cell receptors, appears to reflect greater activation of the inhibitory circuit by CCK, since selective blockade of CCK type A receptors markedly enhances the acid secretory response and attenuates release of somatostatin in response to CCK. It appears that gastrin may also induce somatostatin release via type B receptors on somatostatin cells, but the physiological relevance of these receptors remains uncertain.

INTEGRATION OF INHIBITORY AND STIMULATORY PATHWAYS

With the opposing stimulatory and inhibitory actions of regulators such as gastrin and CCK, integrating mechanisms are critical, but not yet well understood. As noted above, muscarinic agents inhibit somatostatin release from somatostatin cells from canine oxyntic mucosa. Cholinergic input, therefore, exerts acid stimulatory effects via direct stimulation of the parietal cell, induction of histamine release and suppression of the inhibitory circuit mediated by somatostatin.

RECEPTORS ON IMMUNE CELLS

A recent report from Mezey and Palkovits [16] localizes mRNA for histamine, gastrin, muscarinic and dopamine receptors by in situ hybridization to immunocytes in gastric lamina propria, but not to mucosal cells. Although provocative, it remains to be determined whether the probes interacting with lamina propria cells are hybridizing with authentic mRNA for these receptors, whether these receptors are expressed on and regulate paracrine function of these immunocytes and whether any released transmitters in fact modulate mucosal paracrine or exocrine cell function. Further studies are needed to determine the physiologic significance of these positive findings. However, there is little question that the negative findings (the lack of gastrin, cholinergic and histamine receptors on exocrine and endocrine mucosal cells) are not true negatives, but rather reflect the relative insensitivity of the in situ hybridization methods as utilized in these studies [16]. The concept that the receptors regulating acid secretion are present on multiple cell types is consistent with data from numerous investigators indicating that the regulation of acid secretion reflects a complex paracrine/exocrine interaction involving several mucosal cells. It is also quite possible that lamina propria cells modulate mucosal cell function; one example is that macrophages are a major source of mucosal prostaglandin production [17]. It is likely that lamina propria cells are involved in the crosstalk regulating secretory function; however, the absence of mucosal paracrine and exocrine cells in this conversation seem quite unlikely.

THE RATIONALE FOR REDUNDANCY

Why does such redundancy exist in the pathways regulating acid secretion? Most likely the consequences of acid hypersecretion or hyposecretion from a standpoint of phylogeny are sufficiently deleterious to dictate overlapping circuits to maximize the reliability of the physiologic regulation. We surmise that the multiple gastrin/CCK and muscarinic receptors on parietal cells, histamine-ECL cells and somatostatin cells will each demonstrate effects of physiologic or pathophysiologic importance. Critical processes such as the regulation of acid secretion and the maintenance of mucosal integrity apparently require redundant control circuits. A challenge confronting future investigation will be to sort out the physiologic or pathophysiologic settings in which each of these receptors impacts regulatory processes.
Sorting out the relevance of these redundant receptors mediating stimulatory and inhibitory pathways involved in secretory regulation will be challenging, particularly since these receptors may produce opposing effects on cell function. Examples include prostaglandins, adenosine and cholinergic agents acting on somatostatin and G cells [3]. It likely that some of these responses are down- or up-regulated in vivo, but the mechanisms and physiology of such regulation remains to be defined. At this point, these opposing actions simply create confusion as one attempts to extrapolate from in vitro findings to in vivo physiology and pathophysiology.

The models and approaches used to unravel the mysteries of acid secretory regulation will continue to evolve, but the concept that the pathways mediating acid secretion both converge in parallel at the parietal cell and act in series, by modulating the release of stimulatory (histamine) and inhibitory (somatostatin) paracrine transmitters, remains an attractive hypothesis.

Acknowledgement: This work was supported by NIDDK grants DK 19984 and DK 30444 and by the Medical and Research Services of the Department of Veterans Affairs.

REFERENCES

1. Code, C.F. Histamine and gastric secretion: a later look, 1955-1965. Fed. Proc. 24:1311-1321, 1965.
2. Grossman, M. and Konturek, S.J. Inhibition of acid secretion in dog by metiamide, a histamine antagonist acting on H₂ receptors. Gastroenterology 66:517-521, 1974.
3. Soll, A.H. and Berglindh, T. Receptors regulating acid secretory function. In: Johnson L.R., Christensen, J., Jackson, M.J., Jacobson, E.D., Walsh, J.W., eds., 3rd ed. New York: Raven Press, 1994.
4. Prinz, C., Kajimura, M., Scott, D.R., Mercier, F., Helander, H.F., and Sachs, G. Histamine secretion from rat enterochromaffin-like cells. Gastroenterology 105:1-13, 1993.
5. Chuang, C.N., Tanner, M., and Soll, A.H. Gastrin induction of histamine release from canine fundic mucosal cells in primary culture. Am. J. Physiol. 263:G460-G465, 1992.
6. Chuang, C.N., Tanner, M., Lloyd, K.K.C., Wong, H., and Soll, A. Inhibition by endogenous somatostatin of histamine release from canine oxyntic mucosal cells in primary culture. Am. J. Physiol. (in press), 1993.
7. Sandvik, A.K., Waldum, H.L., Kleveland, P.M., and Schulze-Sognen, B. Gastrin produces an immediate and dose-dependent histamine release preceding acid secretion in the totally isolated, vascularly perfused rat stomach. Scand. J. Gastroenterol. 22:803-808, 1987.
8. Nylander, O., Bergqvist, E., and Obrink, K.J. Dual inhibitory actions of somatostatin on isolated gastric glands. Acta Physiol. Scand. 125:111-119, 1985.
9. Bergqvist, E. and Obrink, K.J. Gastrin-histamine as a normal sequence in gastric acid stimulation in the rabbit. Uppsala J. Med. Sci. 84:145-154, 1979.
10. Ruiz, M.C. and Michelangeli, F. Stimulation of oxyntic and histaminergic cells in gastric mucosa by gastrin C-terminal tetrapeptide. Am. J. Physiol. 251:G529-G537, 1986.
11. Black, J.W. Neurochemical control of oxyntic cell secretion. Hepatogastroenterology 37(Suppl 1):31-35, 1990.
12. Ackerman, S.H. Ontogeny of gastric acid secretion in the rat: evidence for multiple receptors regulating acid secretion response systems. Science 217:75-77, 1982.
13. Campbell, V.W. and Yamada, T. Acid secretagogue-induced stimulation of gastric parietal cell gene expression. J. Biol. Chem. 264:11381-11386, 1989.
14. Dimaline, R. and Sandvik, A.K. Histidine decarboxylase gene expression in rat fundus is regulated by gastrin. FEBS Lett. 281:20-22, 1991.
15. Schubert, M.L., Hightower, J., and Makhlouf, G.M. Linkage between somatostatin and acid secretion: evidence from use of pertussis toxin. Am. J. Physiol. 256:G418-G422, 1989.
16. Mezey, E. and Palkovits, M. Localization of targets for anti-ulcer drugs in cells of the immune system. Science 258:1662-1665, 1992.
17. Chen, M.C., Sanders, M.J., Amirian, D.A., Thomas, L.P., Kauffman, G., Soll, A.H. Prostaglandin E₂ production by dispersed canine fundic mucosal cells: contribution of macrophages and endothelial cells as major sources. J. Clin. Invest. 84:1536-1549, 1989.