THE YALE JOURNAL OF BIOLOGY AND MEDICINE 65 (1992), 753–759

Regulation of Histamine Release from Oxyntic Mucosa

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Received November 30, 1992

The regulation of histamine release from oxyntic mucosa is complex because of two potential sources of histamine: mast cells and enterochromaffin-like (ECL) cells. A gastrin-responsive histamine pool was identified in the rat oxyntic mucosa two decades ago, but these ECL cells from the rat have not yet been isolated or characterized *in vitro*. *In vivo* studies in canine and human mucosa have been more difficult because of the high content of histamine in mast cells. Using enzyme-dispersed canine oxyntic mucosal cells, we have studied regulation of histamine release from a mast cell-depleted fraction prepared by sequential elutriation and density gradient. Histamine-like immunoreactivity was demonstrated, using peroxidase-anti-peroxidase immunohistochemistry. After short-term culture, histamine was released in response to gastrin, cholecystokinin, carbachol, and forskolin. Somatostatin potently and effectively inhibited the response to gastrin. The cultures used for these studies also contained somatostatin cells, and, furthermore, the response to gastrin was enhanced by incubation with monoclonal antibodies to somatostatin. The latter findings suggested that somatostatin was acting in these cultures by a paracrine route. This pattern contrasts with that obtained in previous studies of canine oxyntic mucosal mast cells.

HISTAMINE RELEASE FROM OXYNTIC MUCOSAL STORES

The discovery of the dramatic effect of H_2 blockers on gastrin-stimulated acid secretion [1] left no question that the delivery of histamine from oxyntic mucosal stores to the parietal cell was central to the physiologic control of acid secretion. Controversy has surrounded the regulation of histamine cell function, however, and one major reason for divergent views and confusion is that the oxyntic mucosal histamine is stored in two cell types [2].

Oxyntic Mucosal Mast Cells

In some species, such as dog and man, the oxyntic mucosa contains a large number of mast cells, and a correlation exists between histamine content and the content of mast cells [3,4], suggesting that mast cells account for a large component of mucosal histamine.

In our studies, we found that dispersed canine oxyntic cells contained considerable histamine, and the histamine content was highest in the small-cell fractions of the elutriator separation [5]. Subsequent density gradients performed on the small-cell

Abbreviations: CCK: cholecystokinin Con A: concanavalin A ECL: enterochromaffin-like (cells) HDC: histidine decarboxylase MEN-I: multiple endocrine neoplasia, type I PAP: peroxidase-anti-peroxidase ZE: Zollinger-Ellison (syndrome)

Supported by NIADDK grants DK 19984, DK 30444 and by the Medical and Research Services of the Department of Veterans Affairs

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elutriator fractions revealed a close correlation between the distribution of histamine and of content of mast cells. There was no obvious enrichment of histamine in fractions that were enriched in endocrine or endocrine-like cells [5,6]. These findings supported the view that mast cells account for a major portion of the histamine present in the canine oxyntic mucosa.

In our studies of the factors regulating histamine release from mast cell-enriched fractions of oxyntic mucosal cells, these fractions were placed in suspension culture overnight [7]. The responsiveness of mast cells under these conditions was established by demonstrating stimulation of histamine release by the plant lectin concanavalin A (Con A) and in response to cross-linking IgE receptors using passive sensitization and an antibody to IgE or ragweed antigen. Histamine release was also induced with the calcium ionophore A23187 and the phorbol ester β-TPA. Despite manipulations of culture conditions, gastrin and carbachol failed to stimulate histamine release. Furthermore, in studies combining elutriation with density gradient separation of the small-cell fraction, ^{125}I-[Leu^{15}]-G17 binding correlated negatively with the number of mast cells [6]. Thus both functional and radioligand studies argued against the presence of gastrin receptors on oxyntic mast cells. No other agonists of physiologic interest have been identified. It is possible that mast cells, which lie in close proximity to mucosal nerves, release histamine in response to neuronal neurotransmitters; however, such release mechanisms have thus far not been defined. Although oxyntic mast cells can participate in responses to allergens presented to gastric mucosa [8], whether the high number of mast cells in the oxyntic mucosa reflects a role in inflammatory responses or in the regulation of acid secretion remains to be established.

Histamine ECL Cells in the Rat

Studies with rat oxyntic mucosa have most clearly demonstrated the presence of histamine in non-mast cell stores. In this species, gastrin stimulates the formation of histamine by inducing the activity of histidine decarboxylase [9,10]. In addition, studying a perfused rat stomach model, Sandvik, Waldum, and co-workers have demonstrated that gastrin induces histamine release in parallel with increases in acid secretory function [11]. Also in the rat, a dramatic trophic effect of gastrin on the histamine-containing cells in rat oxyntic mucosa is evident in studies utilizing very high doses of potent antisecretory agents, such as omeprazole or ranitidine [12]. When given in high doses for 20 days, these agents induce achlorhydria, hypergastrinemia, and hyperplasia of oxyntic mucosal enterochromaffin-like (ECL) cells; antrectomy reversed the hypergastrinemia and ECL-cell hyperplasia [12].

There is little question that these oxyntic ECL cells in the rat mucosa contain immunoreactivity for both histamine and histidine decarboxylase (HDC). Furthermore, in response to prolonged treatment of rats with omeprazole, parallel increases are found in the number of cells staining with histamine-like and HDC-like immunoreactivity, in histamine content, and in the number of cells with the silver-staining properties of ECL cells [12].

Although the histamine-containing cells of the rabbit gastric mucosa have received less detailed study, histamine is also present in an endocrine cell [13]. Furthermore, gastrin has been demonstrated to induce histamine release from rabbit gastric glands *in vitro* [14,15].

Histamine ECL Cells in the Human and Canine Oxyntic Mucosa

There has been considerable controversy regarding the presence of histamine-ECL cells in the human and canine fundic mucosa, because of both the high content of mast cells and the lower number of ECL cells; however, Hakanson et al. demonstrated histamine-like immunoreactivity in endocrine cells in both species [13]. Furthermore, the early hypotheses that histamine stores in canine and human are released in response to gastrin have been confirmed by recent studies, using a very sensitive assay and carefully quantifying degradation products [16].

Gastrin receptors also appear to exert a trophic effect on ECL cells in humans. The sustained hypergastrinemia that occurs in the Zollinger-Ellison (ZE) syndrome and in atrophic gastritis [17–19] is associated with ECL-cell hyperplasia. Of interest is the fact that formation of ECL-cell carcinoids occurs with sustained hypergastrinemia in the setting of ZE syndrome, but only in the subset of patients with MEN-I (multiple endocrine neoplasia, type I). In contrast, ECL-cell hyperplasia without dysplasia or carcinoid formation occurs in sporadic ZE syndrome [19,20]. In a similar fashion, among experimental models of sustained hypergastrinemia, carcinoid formation uniquely occurs in the rat, but only at the end of the two-year life span and at an age when other endocrine cell neoplasia also occurs [19,20]. Therefore, it appears that a carcinoid requires interaction between hypergastrinemia and the altered regulation of endocrine cell proliferation linked to the defect on chromosome 11 in MEN-I [21] or found in aged rats [19].

Isolation and Characterization of Canine Oxyntic Mucosal Histamine-ECL Cells

In studies using a similar approach to those mentioned above for canine mucosal cells, we also investigated cells isolated from rat oxyntic mucosa [22] and found the presence of histamine, histidine decarboxylase, and DOPA decarboxylase activity in cells of light density. In the canine oxyntic mucosa, markers for enterochromaffin cells (DOPA-decarboxylase and serotonin) and endocrine cells (somatostatin and glucagon) are present in cells of light and intermediate density, respectively [6,23]. In the dog, however, histamine content in this region of the density gradient is low. These contrasts between rat and canine oxyntic cells supported the notion that dramatic differences existed in the cells storing histamine in the oxyntic mucosa of different species. It is now clear, however, that these differences are of a quantitative, rather than a qualitative, nature.

The findings that endocrine cells containing histamine are present in the canine gastric mucosa [13] and that gastrin induced histamine release *in vivo* in the dog [16] prompted our further efforts to develop approaches for identifying and characterizing gastrin-responsive histamine cells in the canine cell preparations [24]. For these studies aimed at non-mast cell histamine stores, we adapted methods originally used for studying somatostatin cells in short-term culture [25]. In contrast to our initial report [25], however, we recognized that mast cells from the small-cell elutriator fraction were also adherent to the culture substrate under these conditions. Therefore, we used step density gradients of albumin to prepare fractions largely depleted of mast cells (< 0.6 percent). In these studies, cellular histamine was demonstrated, using peroxidase-anti-peroxidase (PAP) immunohistochemistry with rabbit antibody 8431, kindly provided by Hakanson, Sundler, and co-workers [13]. Although there were occasional mast cells in our cultures after density step separation, mast cells

accounted for a small proportion of the cells positive for histamine-like immunoreactivity. Unfortunately, with the low initial content of non-mast cell histamine cells, even after density step separation and culture, the non-mast cell histamine cells accounted for only about 3 percent of the adherent cells.

We focused efforts initially on developing methods for the sensitive measurement of histamine and on adapting culture methods to allow for the reduction of basal histamine release. The latter was accomplished by using type IV collagen (Matrigel™) as a substrate [24], and initially culturing cells in 2 percent, rather than 10 percent, serum, followed by a final three-hour incubation in serum-free medium.

Using canine cells prepared, separated, and cultured in this fashion, gastrin and cholinergic agents increased histamine release in a time-dependent fashion. A response to gastrin and cholinergic agents was evident at two minutes [24]. Gastrin stimulated histamine release in a dose-dependent fashion, with an apparent ED_{50} of about 0.2 nM. In contrast to our findings with mast cells, forskolin stimulated histamine release from the mast cell-deleted fraction [24]. As an important control, we compared data from these mast cell-depleted cultures with the pattern of histamine release obtained from mast cell-containing preparations cultured in suspension [7,26] or cultured on Matrigel under conditions identical to the mast cell-depleted preparation [24]. Consistently, the mast cell preparations lacked detectable responses to gastrin or cholinergic agents.

The Gastrin/CCK Receptor Modulating Histamine Release

Gastrin and the octapeptide of cholecystokinin (CCK) were of equal efficacy in stimulating histamine release from our preparation of non-mast cells from canine oxyntic mucosa. These findings thus suggested a receptor that was comparable to the gastrin receptor on the canine parietal cell [27,28]. In our studies, the receptor antagonist L365,260 has a low potency for the canine receptor on both histamine-ECL and parietal cells [unpublished observations], a finding similar to that reported for the cloned receptor expressed in COS cells [28]. In contrast, in rabbit gastric glands, L365,260 was about 50 times more potent than L364,718 in inhibiting gastrin-stimulated histamine release [29]. Recently, the gastrin receptor on the ECL-carcinoid cells has been cloned [30] and found to be comparable to the B-type gastrin receptor originally cloned from the canine parietal cell. Thus, present data indicate that the ECL cell and the parietal cell possess B-type gastrin receptors. For as yet unexplained reasons, species variability exists in the relative affinities of subtype-specific antagonists.

Inhibition of Oxyntic Mucosal Histamine Release

We found that the somatostatin analog SMS 201-995 very effectively inhibited gastrin-stimulated histamine release from the mast cell-depleted fractions, with the ID_{50} (dose producing 50 percent inhibition) being 3×10^{-10} M. The response to gastrin was significantly enhanced by treatment with the monoclonal antibody S6 to somatostatin. These cultures also contain somatostatin cells, and this result suggested that somatostatin in these cultures was producing "paracrine" inhibition of histamine release. In contrast to this very effective inhibition of histamine release by somatostatin, much higher concentrations of somatostatin were required to produce less efficacious inhibition of canine parietal cell function [31]. The high potency for somatostatin inhibition of histamine release, the magnitude of this inhibitory re-

sponse, and the demonstration of paracrine inhibition in our cultures all point to the importance of inhibition of histamine release as a component of the *in vivo* antisecretory action of somatostatin. Previous findings with rat stomach *in vivo* and rabbit gastric glands have indicated dramatic inhibition by somatostatin of oxyntic mucosal histamine release [32,33].

Thus, data obtained studying canine gastric mucosa also indicate a population of histamine-containing non-mast cells that release histamine in response to gastrin and cholinergic agents, with somatostatin producing inhibition of probably physiological importance.

GASTRIN RECEPTORS: WHAT IS PHYSIOLOGIC?

From the vantage point of the studies utilizing isolated canine oxyntic mucosal cells, it is difficult to avoid the conclusion that gastrin receptors are present on several cell types, including parietal cells, somatostatin cells, and histamine-containing endocrine cells. It is possible that gastrin actions at these different sites are related in variable degrees to enhancement of secretory function, a trophic action, or a differentiative effect. The important question regarding the relative physiological importance of these different actions of gastrin cannot be determined with present data. Several lines of evidence indicate, however, that gastrin receptors on the parietal cell are functional in intact mucosa, at least under certain circumstances. For example, Ruiz and Michelangeli have found that, in the presence of cimetidine, gastrin enhances the response to dbcAMP [34]. In neonatal rats, Ackerman [35] found a dissociation between gastrin- and histamine-stimulated acid secretion; the acid secretory response to exogenous pentagastrin was detectable at an age of 14 days, whereas response to exogenous histamine did not become detectable until age 19 to 22 days. Furthermore, the response to gastrin at 14 days of age was not blocked by H_2 blockers, providing further evidence that a component of gastrin action *in vivo* was independent of histamine, and, therefore, presumably mediated by gastrin receptors on the parietal cell itself. We confidently conclude that a functional gastrin receptor exists on parietal cells, but present data do not indicate the component of the acid secretory effects of gastrin that reflects interaction with these parietal cell receptors.

It is possible that the ability of gastrin to stimulate the parietal cell and to induce histamine release are both physiologically relevant effects, but that other functions may be activated by these receptors instead of or 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 maintenance of cell differentiation. For example, gastrin has been reported to induce expression of the H^+/K^+-ATPase messenger RNA in parietal cells [36] and to induce histamine decarboxylase activity in the rat oxyntic ECL [37]. 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 parietal cell receptors.

THE THEME: REDUNDANT CONTROL MECHANISMS REGULATE THE ACID SECRETORY RESPONSE

The physiologic importance of the inhibitory paracrine circuit mediated by oxyntic mucosal somatostatin on parietal and histamine ECL cells requires further elucida-

tion. Studies with *in vitro* preparations have provided evidence that oxyntic somatostatin plays a role in acid feedback inhibition of acid secretion [38]. Activators of this inhibitory paracrine circuit remain to be established, but CCK is a leading candidate [39,40]. Present data suggest the importance of somatostatin inhibition of ECL-cell histamine release.

An emerging recurrent theme is that considerable redundancy exists in the pathways regulating acid secretion. Most likely the consequences of acid hypersecretion or acid hyposecretion, from a standpoint of phylogeny, must be sufficient to dictate overlapping control circuits that serve to increase the probability that control will be maintained. We surmise that the multiple receptors for gastrin 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 require redundant control circuits. A challenge confronting future investigation in this field will be to sort out the physiologic or pathophysiologic settings in which each of these receptors affects regulatory processes.

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