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Role of the Cholinergic Nervous System in Acid Secretion

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Abstract. Stimulation of acid secretion by muscarinic agents involves receptors with a higher apparent affinity to the M1-antagonists, pirenzepine and telenzepine, than those regulating heart rate and salivary secretion. However, the localization of the proposed M1-receptors regulating acid secretion remains unclear. Studies with parietal cells isolated from several species indicate that parietal cells have a muscarinic receptor with low affinity for the M1-antagonists. Our studies with somatostatin cells isolated from canine fundic mucosa indicates that the muscarinic receptor inhibiting somatostatin release also is of low affinity for M1-antagonists. We have found no evidence for regulation of histamine release from canine fundic mast cells, whereas there is evidence that acetylcholine induces histamine release from the enterochromaffin-like cells of the rat and rabbit fundic mucosa. Further studies will be necessary to determine which of the muscarinic receptors potentially involved in the regulation of acid secretion is responsible for the M1-behavior of this pathway.

Vagal stimulation and exogenous cholinomimetics stimulate gastric acid secretion indirectly by releasing gastrin and by direct action on the parietal cell. Acid secretion may also be enhanced by inhibition of somatostatin release by carbachol or by stimulation of histamine release. Muscarinic cholinergic receptors have been identified by functional studies on the parietal cell. Recently, subtypes of muscarinic receptors have been described. Some controversy exists as to the subtype of muscarinic receptor-regulating acid secretion. In vivo studies indicated the involvement of M1 receptors [1] but the specific locus of these receptors has not been established by in vitro studies [2, 3].

Using cells isolated from canine gastric fundus and antrum we have studied the characteristics of muscarinic receptors on enriched cell populations and demonstrate functional and/or binding evidence for muscarinic receptors on parietal, chief, somatostatin and gastrin cells. These studies have demonstrated the presence of multiple cholinergic muscarinic receptor populations with characteristics intermediate between the classic M1- in neural tissue and M2-subtypes in heart. We postulate that either the reported M1 receptor exists on another cell population such as neurons in the intramural plexus, or that the additive effects of multiple receptor populations alters the apparent

receptor affinity in vivo. We have employed a 'reductionist' approach in the study of gastric muscarinic receptors, utilizing cell separation and culture techniques as described in detail elsewhere [4–8]. The protocols used for binding and functional experiments have also been reported elsewhere [10–13].

The presence of cholinergic receptors on gastric mucosal cells has been established by functional and radioligand studies with the properties indicative of a muscarinic receptor site. In isolated canine fundic cells binding sites for [^{3}H]-QNB were present in all of the elutriator-separated fractions [10], consistent with distribution of muscarinic receptors on mucous, endocrine/mast and chief cells in addition to parietal cells.

Recent binding studies using [^{3}H]-NMS confirm that the receptor present on the parietal cell is of a muscarinic type, with a high affinity for NMS and atropine. Comparable binding studies with a crude membrane fraction and with intact, enriched canine parietal cells indicated that [^{3}H]-QNB binding was rapid, reversible and saturable [10]. The presence of binding sites for muscarinic agonists has been confirmed by functional studies demonstrating atropine inhibition of aminopyrine accumulation stimulated by carbachol [11].

We examined the possibility that the muscarinic receptor on the canine parietal cell possessed a high affinity for pirenzepine or telenzepine and therefore could be of the M1-subtype. The binding studies with [^{3}H]-NMS showed that the affinity for pirenzepine of the parietal cell muscarinic receptor was nearly 100-fold less than for atropine. Functionally, pirenzepine was 100-fold and telenzepine 8-fold less potent than atropine as an inhibitor of carbachol-stimulated AP accumulation. These data are consistent with other studies showing 5- to 25-fold greater potency of telenzepine compared with pirenzepine [14, 15] and 5- to 10-fold greater potency of atropine compared with telenzepine [14]. These findings of a low to intermediate affinity of pirenzepine and telenzepine for parietal cell receptors are consistent with the binding [16] and functional studies [14, 17–19] of others, indicating clearly that parietal cells do not possess a muscarinic receptor of an M1-subtype.

We have also investigated the presence of muscarinic receptors on other cells of the gastric mucosa. Specific QNB binding was present in the small cell elutriator fraction containing endocrine cells (somatostatin and glucagon) and histamine-producing mast cells. In a linear gradient separation of this fraction, we found specific QNB binding sites in all the fractions of intermediate and light density, the fractions containing the markers for glucagon and somatostatin [1]. Nonparietal cell muscarinic receptors could mediate the release of paracrine or endocrine transmitters that in turn modulate the acid secretory response. In functional studies, cholinergic agents inhibited somatostatin release from cultured cells, an action blocked by nanomolar concentrations of atropine with an apparent dissociation constant of 0.4 nmol/l [12], consistent with the properties of muscarinic receptors. These findings indicate that muscarinic agents directly inhibit the release of somatostatin, an effect that might serve to enhance the overall acid secretory response. Pirenzepine and telenzepine also competitively inhibited this receptor, but with affinities 100- and 10-fold lower, respectively, than that found for atropine [9]. Thus, the somatostatin cell does not seem a likely cell to possess a high affinity pirenzepine receptor [20].

Cholinergic agents may also mediate acid secretion indirectly by actions on histamine release from mast cells. To date, we have found no evidence for cholinergic binding or functional activity on canine mast cells. Linear gradient separation of the small cell elutriator fraction resulted in an inverse correlation between QNB binding and histamine content, with no detectable binding present in high-density fractions maximally enriched in mast cells [7]. Functionally, cholinergic agents alone, or in combination with gastrin, did not induce histamine release from canine fundic mast cells in primary culture [7]. These findings may not be taken as generalized phenomena in all species, however, since cholinergic mechanisms stimulate histamine release from rabbit gastric glands [21] and frog gastric mucosa [22].

In rat and rabbit, the predominant stores of fundic mucosal histamine are contained in enterochromaffin-like (ECL) cells, rather than in mast cells, as is the case in dog and probably human fundic mucosa. Therefore, it can be anticipated that the regulation of histamine release would differ among these two cell types.

Cholinomimetics directly stimulate gastrin release from cultured antral canine G-cells [23, 24], likely at a muscarinic site. Studies in the rat indicated that gastrin release stimulated by carbachol was 100-fold less sensitive to pirenzepine than to atropine [20], suggesting that gastric M1 receptors are not present on the gastrin cell.

Cholinergic agents stimulate pepsinogen secretion [6, 25], providing evidence for muscarinic receptors on chief cells. Recently, Sakamoto et al. [26] demonstrated greater affinity for atropine than pirenzepine of these receptors, suggesting that they also are not of the M1-subtype.

The failure to detect M1 receptors on parietal and other epithelial cells of the canine stomach is consistent with the hypothesis that M1 receptors involved in acid secretion may exist on neurons in the enteric nervous system. Two subtypes of muscarinic receptors have been localized on intramural neurons of the guinea pig. M1 receptors appeared to be likely candidates to mediate postsynaptic muscarinic events whereas M2 receptors were involved in presynaptic inhibitory modulation [27]. For example, acetylcholine has been reported to stimulate bombesin release from enteric neurons [28] which could therefore influence gastric acid secretion by modulation of gastrin release. The specific pathways and receptors mediating muscarinic modulation of acid secretion in the fundic mucosa remain to be defined.

Binding and functional studies have demonstrated the presence of muscarinic receptors on the parietal cell, as well as on somatostatin, gastrin and chief cells. The summated effects of cholinergic agonists on multiple cell types result in stimulation of acid secretion; however, the relative physiologic importance of each specific receptor population cannot be assessed by the reductionist approach. The receptors on isolated parietal, chief and somatostatin cells are less sensitive to pirenzepine or telenzepine than to atropine, suggesting that canine gastric mucosal cells do not possess a definitive M1 receptor. Further investigation is needed to clarify whether a gastric M1 receptor exists, for example on intramural neurons, or whether the apparent affinity of muscarinic antagonists in vivo reflects interaction of effects between muscarinic receptors on several cell types or mechanisms such as altered delivery to the receptor site [19]. The presence of muscarinic receptors with a specificity dis-

tinct from either M1- or M2-subtypes may represent a third muscarinic subtype of pharmacological interest.

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Discussion

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A major point of discussion was that several cells in the gastric mucosa have muscarinic receptors, in addition to the parietal cell. However, the physiological importance of the other receptor sites in the regulation of acid secretion remains unclear.

One particularly interesting question is the mechanism explaining the inhibition of gastrin stimulation of acid secretion by antimuscarinic agents. Prof. Soll noted that muscarinic agonists inhibit somatostatin secretion and stimulate parietal cell function. Why such a complicated regulatory system with multiple receptors and interactions exists for such a simple event as acid secretion remains unclear. Prof. Soll suggested that some of the receptors that have been found in the fundic mucosa may not have a physiologically important role. On the other hand, interaction between receptors and between cell types may provide a mechanism for fine tuning and modulation of the acid secretory response. For example, according to Prof. Soll, gastrin may stimulate the parietal cell while also promoting negative feedback due to stimulation of somatostatin release. In contrast, muscarinic agonists serve to inhibit somatostatin release while stimulating the parietal cell. Therefore, vagal or muscarinic stimulation may serve to enhance the gastrin response by attenuating somatostatin release and complementing stimulation at the parietal cell. Elimination of cholinergic input, on the other hand, may inhibit the acid secretory response to gastrin in part because of removing muscarinic inhibition of somatostatin release. This suggested mechanism for cholinergic-gastrin interaction remains hypothetical.

Prof. Sewing believed that, in analogy to the data obtained by Prof. Soll in dog parietal cells, cholinergic and histaminergic receptor systems interact in parietal cells of guinea pig. The mechanism of this interaction, however, is not known. Prof. Soll pointed out that, in canine parietal cells, no interaction between cholinergic agonists and gastrin was demonstrated, but such interaction had in fact been observed between gastrin and histamine, and cholinergic agonists and histamine. Interactions between muscarinic receptors and gastrin receptors in dog should, therefore, be localized proximal to the parietal cell.