Cytochemical Quantification of Physiologic Regulation of Oxyntic Cell Carbonic Anhydrase[a]

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INTRODUCTION

Although the primary function of carbonic anhydrase (CA) is to catalyze the hydration of CO_2 to form carbonic acid,[1] the abundance of CA in the gastric oxyntic cell[2-4] has raised the question of the role of CA in acid secretion. In order to evaluate the physiologic regulation of CA in the guinea pig oxyntic cell, we studied the effects of three known acid secretagogues, gastrin, histamine, and carbamylcholine, on the activation of CA using quantitative cytochemistry.

METHODS

CA was measured by the technique utilized by Loveridge[5] in segments and modified for quantitative cytochemical measurement of CA activity in 18 μm sections of guinea pig fundic mucosa.[6-8] Sections were cut from the mounted tissue in a cryostat at −20°C and were mounted on glass slides. Test solutions (100 μl) in 0.025 M HEPES buffer at pH 7.0 were delivered simultaneously to 24 sections and allowed to react at 20°C for 90 sec. The reaction was terminated by the addition of the staining reagents. Fifteen to twenty oxyntic cells in each of the randomized/coded sections were read at 550 nm. Values for the density of the CoS precipitate in each section were read as integrated extinction and were expressed as a percent using the following equation: (mean extinction of oxyntic cells) − (mean extinction of muscularis) × 100/D1, where D is the reading of a standard filter with an optical density of 1.

RESULTS AND DISCUSSION

The oxyntic cells were the most darkly stained. The coefficient of variation of the extinction readings taken on 20 cells in an unstimulated section varied from 4.1 to 7.8% and for cells stimulated by a 10^{-12} M dose of gastrin was 4.6%. Thus upon stimulation, all cells appeared to respond in an equivalent manner.

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Time Study

The response to stimulation was biphasic. The early peak response to gastrin, histamine, and carbamylcholine occurred at 90 sec of exposure and a second at 240 seconds with persistance of the response thereafter. Removal of the incubate and reexposure to the secretagogues again caused an increase in CA activity similar to that found with the initial exposure. However, for these studies all subsequent experiments were carried out using 90 sec of exposure time.

Secretagogue Dose-response Curves

Dose-response curves for CA activation by histamine, gastrin and carbamylcholine are shown in FIGURE 1. Gastrin had the greatest efficacy (100%), histamine 68 ± 4% and carbamylcholine 81 ± 4% of the maximum response to gastrin.

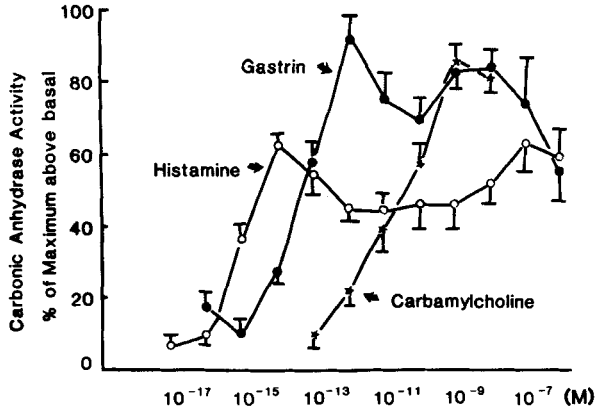

FIGURE 1. A comparison of the effects of histamine, gastrin and carbamylcholine on oxyntic cell carbonic anhydrase activity. The values are expressed as % of the maximum response to gastrin, which was arbitrarily ascribed a value of 100%.

The doses required to produce half maximal effects (D_{50}) were: histamine = 7.0×10^{-16} M, gastrin = 3.0×10^{-14} M and carbamylcholine = 3.0×10^{-11} M.

Inhibition of CA Activity

Addition of the CA inhibitor, acetazolamide (10^{-5} M) did not affect basal CA activity, but abolished the effects of gastrin ($p < 0.025$) and histamine ($p < 0.001$) stimulated activity. Cimetidine (10^{-5} M) reduced by 75% the effects of histamine but that of gastrin by only 25%. Atropine had no effects on the response to gastrin or histamine, but virtually abolished the actions of carbamylcholine (FIG. 2). The stimulation of CA activity by all three secretagogues was inhibited by 10^{-3} M of both NaSCN and H149/94 (FIG. 3).

We have shown that the acid secretagogues activate CA within 90 seconds. The reason for the inordinate sensitivity is not clear, but it contrasts with the effect of direct stimulation of isolated bovine red cell CA, which required larger concentrations (10^{-6} M – 10^{-7} M) of secretagogues[9] than those used here, suggesting that an amplification system within the cell was present. The response is biphasic. It is not clear at present whether the factors regulating the different phases are the same. The selective effects of inhibitors suggest that the activation of CA by the three agonists is mediated by separate receptors for histamine, carbamylcholine, and gastrin.[10]

It is evident that a number of close parallels may be drawn between activation of CA activity in the sections of oxyntic cells and acid secretion. Hersey[11] suggested that the function of CA is to act as a buffer to maintain intracellular pH during acid secretion. Activation of CA by all three gastric acid secretagogues was inhibited by acetazolamide, indicating that they activate CA. H149/94, a substituted benzimidazole inhibits the $H^+ + K^+$-ATPase as well as acid secretion.[12] In our studies, inhibition of secretagogue-stimulated CA activity was achieved by 10^{-3} M – 10^{-5} M H149/94, suggesting that the action of secretagogues on CA may be linked to the $H^+ + K^+$ – ATPase.[12] Although these data do not prove a cause and effect relationship between CA and acid secretion, they suggest that intracellular events initiated by receptor stimulation may modify CA activation in the same manner that they affect acid secretion.

SUMMARY

We have studied the physiologic factors regulating oxyntic cell activity using cytochemical quantification of carbonic anhydrase (CA) activity. Gastrin (10^{-16} to 10^{-12} M), histamine (10^{-17} to 10^{-13} M), and carbamylcholine (10^{-13} to 10^{-8} M) caused a dose-dependent increase in CA in the oxyntic cells in guinea pig gastric fundus, maximal at 90 sec. The stimulation of CA by all three secretagogues was

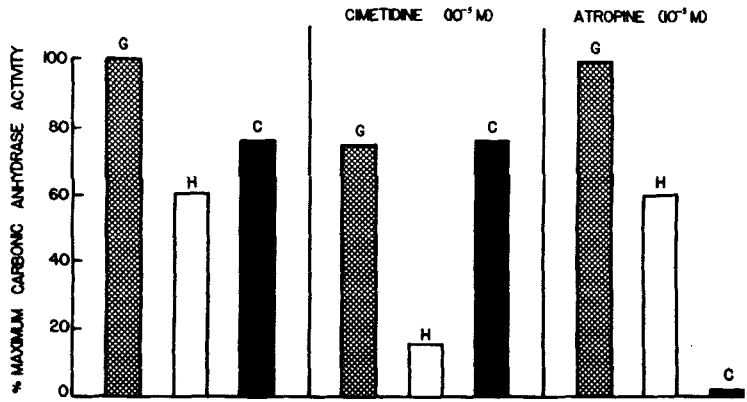

FIGURE 2. Carbonic anhydrase activity of tissues reacted with gastrin (G), histamine (H), or carbamylcholine (C) alone and in the presence of the H_2-blocker cimetidine and the muscarinic cholinergic antagonist atropine.

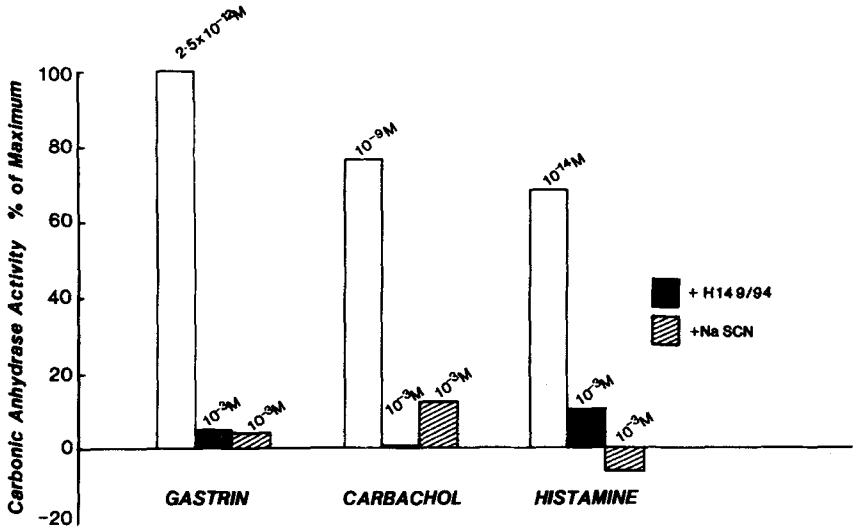

FIGURE 3. Histogram showing mean maximum activation of CA by the secretagogues, gastrin, histamine, and carbamylcholine and the effects of NaSCN (10^{-3} M) and H149/94 (10^{-3} M).

inhibited by the CA inhibitor, acetazolamide. The agonist activities were selectively blocked by respective antagonists. The benzimidazole derivative compound Hassle 149/94 (10^{-3} M) abolished the actions of all agonists.

Thus, histamine, gastrin and carbamylcholine have independent actions on oxyntic cell CA. The inhibition of the activity of all three secretagogues by H149/94 suggests a close link between CA activity and the functioning of the proton pump $H^+ + K^+$-ATPase.

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