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Research Article

The role of calcium in stimulation of the oxyntic cell by histamine and carbamylcholine was studied using a sensitive quantitative cytochemical staining technique that measures oxyntic cell hydroxyl ion production (HIP) as an index of acid secretion. Histamine (10(-17)-10(-14) M), carbamylcholine (10(-12)-10(-9) M), and extracellular calcium (10(-7)-10(-3) M) caused a linear, dose-dependent stimulation of the oxyntic cell. EGTA (10(-6) M) inhibited carbamylcholine by 50% but not histamine-stimulated activity. Lanthanum chloride (10(-6) M) caused 100% inhibition of carbamylcholine-induced activity but did not affect histamine-stimulated activity. A maximally effective dose of calcium (10(-4) M) caused additive effects on HIP at low doses of carbamylcholine without alteration of the maximal effect of carbamylcholine. Calcium (10(-4) M) did not enhance the effects of histamine. The calmodulin antagonists, trifluoperazine (10(-5) M), pimozide (10(-5) M), and a naphthalenesulfonamide (W-7), inhibited the integrated response to histamine by 54, 56, and 53%, and that of carbamylcholine by 65, 64, and 99%, respectively. Thus, extracellular calcium per se, stimulates the oxyntic cell. The action of carbamylcholine is completely dependent upon calcium/calmodulin mediation, supporting the concept that cholinergic actions are mediated via calcium-calmodulin events. Although histamine does not require extracellular or membrane calcium events to stimulate the oxyntic cell, calmodulin appears to participate in histamine action.

Role of Calcium and Calmodulin in Activation of the Oxyntic Cell by Histamine and Carbamylcholine in the Guinea Pig

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ABSTRACT The role of calcium in stimulation of the oxyntic cell by histamine and carbamylcholine was studied using a sensitive quantitative cytochemical staining technique that measures oxyntic cell hydroxyl ion production (HIP) as an index of acid secretion. Histamine (10^{-17}–10^{-14} M), carbamylcholine (10^{-12}–10^{-9} M), and extracellular calcium (10^{-7}–10^{-3} M) caused a linear, dose-dependent stimulation of the oxyntic cell. EGTA (10^{-6} M) inhibited carbamylcholine by 50% but not histamine-stimulated activity. Lanthanum chloride (10^{-6} M) caused 100% inhibition of carbamylcholine-induced activity but did not affect histamine-stimulated activity. A maximally effective dose of calcium (10^{-4} M) caused additive effects on HIP at low doses of carbamylcholine without alteration of the maximal effect of carbamylcholine. Calcium (10^{-4} M) did not enhance the effects of histamine. The calmodulin antagonists, trifluoperazine (10^{-5} M), pimozide (10^{-5} M), and a naphthalenesulfonamide (W-7), inhibited the integrated response to histamine by 54, 56, and 53%, and that of carbamylcholine by 65, 64, and 99%, respectively.

Thus, extracellular calcium per se, stimulates the oxyntic cell. The action of carbamylcholine is completely dependent upon calcium/calmodulin mediation, supporting the concept that cholinergic actions are mediated via calcium-calmodulin events. Although histamine does not require extracellular or membrane calcium events to stimulate the oxyntic cell, calmodulin appears to participate in histamine action.

INTRODUCTION

The importance of the calcium ion in the mediation of cellular activity has been established in a number of secretory cells, including those of the adrenal medulla (1) and pancreatic beta cells (2). Transduction of calcium changes within the cell to a physiologic response is thought to involve calmodulin. Calmodulin is an intracellular, calcium-binding protein, which has been shown to regulate a wide variety of enzyme systems including phosphodiesterases, phosphorylase kinases, adenylate cyclase, protein kinases, and others (3, 4). Calmodulin has also been shown to mediate processes for hormone secretion (5) and hormone action (6). Calmodulin must bind calcium to exert its actions, and the increase in intracellular calcium associated with cell stimulation is thought to provide the cell calcium (3).

Carbonic anhydrase (CA)[1] is an enzyme found in high concentrations in the gastric oxyntic cell (7). Its major function appears to be the maintenance of intracellular pH either by hydrating CO_2 to provide hydrogen ions to neutralize hydroxyl ions produced during acid secretion (7, 8), or by directly catalyzing the reaction of hydroxyl ions with CO_2 to form bicarbonate (9). The exact relationship of CA to the proton pump is not known (9), but histochemical staining studies show the enzyme to be associated with microvilli of oxyntic cell secretory canaliculi in close proximity to the presumed proton pump (10). We have

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[1] *Abbreviations used in this paper:* C, carbamylcholine; CA, carbonic anhydrase; H, histamine; HIP, hydroxyl ion production; PMZ, pimozide; TFP, trifluoperazine; W-7, *N*-(6-aminohexyl)-5-chloro-1-naphthalene-sulfonamide.

developed a technique that quantitates hydroxyl ion production (HIP) and presumably reflects hydrogen ion production (11). Using this technique, we have shown that histamine (H) and carbamylcholine (C) activate the oxyntic cell (11). We have used this technique to study the role of calcium in the modulation of oxyntic cell function.

In these studies we show that exogenous calcium stimulates oxyntic cell HIP. The action of C is partially dependent upon extracellular calcium but totally dependent upon membrane calcium, while H-stimulated activity requires neither. C appears in addition to activate the oxyntic cell by a calmodulin-dependent pathway.

METHODS

Quantitative cytochemistry of oxyntic cell function

After an overnight fast, female guinea pigs weighing 450–500 g were killed by asphyxiation in an atmosphere of nitrogen and the stomach rapidly removed. A 3–4-cm strip of gastric fundus was excised from a standard area just below the esophagogastric junction. The tissue was rinsed in 0.025 M Hepes (Sigma Chemical Co., St. Louis, MO) buffer, (pH 7.0, with 10 M KOH solution) and divided into 3–5-mm pieces. These were snap-frozen in a beaker immersed in an *N*-hexane, solid CO_2, absolute alcohol, freezing mixture. The frozen portions of tissue were used within 72 h of freezing.

Sections (18 μm) were cut from the mounted tissue in a −20°C cryostat. Sections were mounted on glass slides using a template to reliably position the sections in a predetermined area and stored for no longer than 6 h in the cryostat chamber before use in the assay.

The slides were placed flat, section upwards, in a slide tray, and allowed to equilibrate to room temperature for 10 min. The test solutions were diluted to desired concentrations in 0.025 M Hepes buffer at pH 7.0. 100 μl of each test solution was delivered by a dispensing apparatus designed to deliver precise volumes of test solution directly and simultaneously to 24 sections and allowed to react with the sections at room temperature (20°C) for 90 s. The reaction was terminated by the addition of the staining reagents. Within each experiment, each test solution was reacted with tissue sections in triplicate.

HIP was quantified by a modification of Hansson's method (12) using 10.5 mM $CoSO_4$, 53 mM H_2SO_4, 157 mM $NaHCO_3$, 1.17 mM KH_2PO_4 in 0.1 M Hepes buffer at pH 7.4 with 0.001% gum tragacanth. This solution was applied so as to just cover the sections. The staining reaction was allowed to proceed with gentle agitation for 2 min at 20°C and then the sections were washed twice with cold water after which they were exposed to a saturated solution of H_2S in water for 90 s, again washed in water and mounted in Farrants' medium (Biomedical Specialties, Santa Monica, CA). The staining procedure led to the formation of visible brown precipitates of CoS within the sections; the most deeply stained cells were the oxyntic cells. The amount of precipitate formed is a reflection of the number of hydroxyl ions trapped (12) and was quantitated by means of an M85 scanning and integrating microdensitometer (13). This staining is based on the reaction between Co^{3+} and OH^- ions produced during stimulation of the oxyntic cell. Whether these hydroxyl ions are produced by CA or are produced by the proton pump is unknown. It has been suggested that CA catalyzes directly the reaction between the hydroxyl ion produced by the proton pump and CO_2 resulting in the formation of a bicarbonate ion (9). This process requires fewer reactions than that proposed by Maren (7) and fits with observed events just as well. Thus, we believe, the staining actually quantifies hydroxyl ions produced by the proton pump. This would provide a direct measurement of hydrogen ion secretion by the oxyntic cell and would reflect a direct mole for mole parallel with hydrogen ion production.

The cells selected for each reading were those that completely filled a mask which, when used with the ×25 objective lens, has a field diameter of 20 μm. 15–20 oxyntic cells in each of the randomized coded sections were read at 550 nm. The same number of measurements was made from the muscularis mucosa in each section. Readings from the muscularis reflect nonspecific absorption of CoS to tissue and were therefore subtracted from those of the oxyntic cells. To correct for possible instrument variation, the densitometer was calibrated by taking readings of a standard filter of known optical density before and after taking readings from each section. 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. The mean integrated extinction thus calculated, allowed comparisons between sections having the same basal HIP. To allow comparisons between sections with different basal HIP, the basal values are subtracted to obtain a mean integrated extinction × 100.

Experimental design

Dose-response curves for H and C. Dose-response curves for H (10^{-17}–10^{-13} M) and C (10^{-12}–10^{-8} M) were initially established. The mean integrated extinction coefficient could then be plotted against the molar concentration of H or C. The concentrations chosen were those previously shown to cause a linear increase in HIP over the range tested (11).

Dose-response curve for calcium. The effects of increasing the Ca^{2+} concentration from 2.1×10^{-7} M–2.1×10^{-3} M on HIP were determined. The Hepes buffer used contains 2.1×10^{-7} M $CaCl_2$. Concentrations of ionized Ca^{2+} were determined by an atomic absorption spectrophotometer (model 475, Varian Associates, Inc., Palo Alto, CA) with acetylene flame.

Calcium dependence of H and C. The role of extracellular calcium in HIP by H and C was investigated by adding EGTA (10^{-6} M) to each concentration of the H (10^{-16}–10^{-11} M) and C (10^{-12}–10^{-8} M) dose-response curves. The effectiveness of EGTA in lowering extracellular Ca^{2+} was measured by adding EGTA (10^{-6} M) to Hepes alone and Hepes with Ca^{2+} (10^{-6}–10^{-3} M $CaCl_2$). The role of membrane-bound Ca^{2+} and membrane Ca^{2+} channels in HIP was investigated by adding $LaCl_3$ (10^{-6} M) in combination with H (10^{-17}–10^{-14} M) and C (10^{-12}–10^{-9} M). The effect of increased extracellular Ca^{2+} on C- and H-stimulated HIP was studied by adding 10^{-4} M Ca^{2+} to sections treated with H (10^{-17}–10^{-14} M) and C (10^{-12}–10^{-9} M).

Calmodulin dependence of H and C stimulation. To determine calmodulin-mediated HIP, the calmodulin antagonists, trifluoperazine (TFP) (10^{-5} M) and pimozide (PMZ) (10^{-5} M) were added individually to each concentration of

H (10^{-17}–10^{-13} M) and C (10^{-12}–10^{-8} M) and HIP measured. Because TFP and PMZ are highly lipid soluble and may therefore have nonspecific effects on the cell membrane unrelated to calmodulin, an additional calmodulin antagonist of a different chemical nature was also tested. *N*-(6-Aminohexyl)-5-chloro-1-naphthalene-sulfonamide (W-7) (10^{-5} M) was added to each concentration of H (10^{-17}–10^{-13} M) and C (10^{-12}–10^{-8} M) and HIP measured.

Reagents

Test solutions were diluted in 0.025 M Hepes buffer (pH 7.0) (Sigma Chemical Co.). Histamine hydrochloride, carbamylcholine chloride, EGTA, calcium chloride, and lanthanum chloride (Sigma Chemical Co.) were diluted directly in Hepes buffer. TFP (Smith, Kline & French Laboratories, Philadelphia, PA) and PMZ (McNeil Laboratories, Philadelphia, PA) and W-7 (Caabco, Inc., Houston, TX) were dissolved in a small amount of ethanol and then diluted further in 0.025 M Hepes buffer.

Statistics

The significance of the effects of the various agents on HIP was determined using Student's *t* test by comparing the mean HIP each concentration of the agent induced, to the mean basal HIP. The significance of effects of the antagonists (EGTA, $LaCl_3$, TFP, PMZ, and W-7) were determined using Student's *t* test by comparing the mean HIP of H and C alone and the mean HIP of H and C plus the antagonist or calcium. The difference between HIP induced by H or C alone and HIP following treatment with an antagonist was also determined by calculating the difference between the mean integrated area under the curves for the different doses of H or C alone and H or C plus antagonist. This allowed calculation of the percentage of inhibition of the mean integrated response. All results are given as mean±SE.

RESULTS

H and C dose-response curves. The dose-response curves for H and C are shown in Fig. 1. Both H and C caused a dose-dependent increase in HIP compared with the base line. H caused significant stimulation ($P < 0.001$) of HIP at 10^{-16}–10^{-13} M. C caused significant stimulation ($P < 0.001$) of HIP at 10^{-11}–10^{-8} M.

Calcium dose-response curve. The dose-response curve for Ca^{2+} is shown in Fig. 2. The addition of $CaCl_2$ to provide concentrations of Ca^{2+} from 2.1×10^{-5}–2.1×10^{-3} M caused significant stimulation of HIP when compared with HIP found at basal conditions ($Ca^{2+} = 2.1 \times 10^{-7}$ M).

Calcium dependence of H and C. The results of adding EGTA (10 μM) to Hepes and Hepes plus Ca^{2+} (10^{-6}–10^{-3} M) are shown in Table I.

Addition of EGTA (10^{-6} M) and $LaCl_3$ (10^{-6} M) alone to the section had no effect on basal HIP.

Removal of extracellular Ca^{2+} by EGTA (10^{-6} M) had no effect on H-induced HIP but significantly reduced HIP stimulated by C at all concentrations of C (Fig. 3). There was a 50% reduction in the area of the response.

Blockade of membrane calcium channels by $LaCl_3$ (10^{-6} M) had no effect on H-induced HIP but totally abolished (100% inhibition) HIP induced by C in the dose range of 10^{-11} to 10^{-9} M (Fig. 4).

Addition of exogenous Ca^{2+} (10^{-4} M, D_{max} for Ca^{2+}) to the dose-response curve for H (10^{-17}–10^{-13} M) resulted in a significant increase in stimulated HIP only at low doses of H 10^{-17} and 10^{-16} M ($P < 0.025$). This

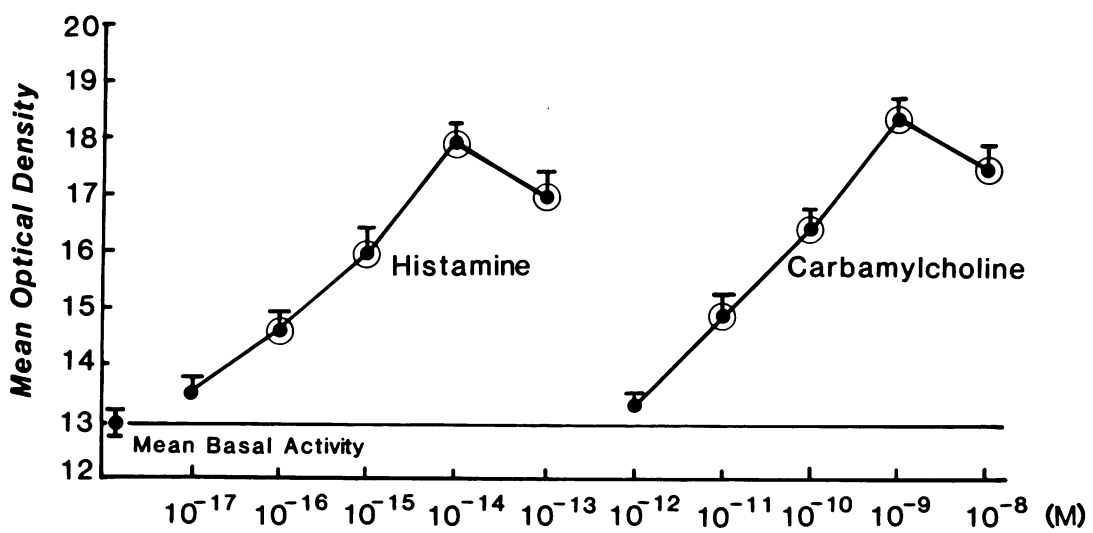

FIGURE 1 Activation of HIP by H and C. Integrated extinction is a reflection of HIP. O = significant ($P < 0.001$) increase above base line.

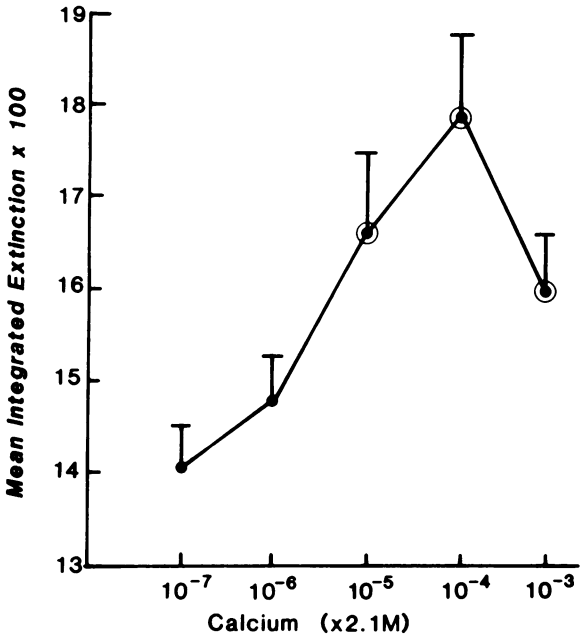

FIGURE 2 Effects of calcium on acid secretion. The effect of increasing concentrations of Ca^{2+} on guinea pig oxyntic cell HIP measured as integrated extinction. O = significant increase above base line.

increase was not significantly different from HIP stimulated by 10^{-4} M Ca^{2+} alone (Fig. 5).

When a D_{max} dose of Ca^{2+} (10^{-4} M) was added to the dose-response curve for C (10^{-12}–10^{-8} M), significant enhancement occurred at 10^{-12}–10^{-10} M of C ($P < 0.01$). Addition of Ca^{2+} to low doses of C caused additive effects that were greater than those with C alone, but not different from HIP induced by 10^{-4} M Ca^{2+} alone (Fig. 6). There was no significant increase in the maximal response.

Calmodulin dependence of H and C and inherent activity of TFP, PMZ, and W-7. When TFP (10^{-5} M), PMZ (10^{-5} M), and W-7 (10^{-5} M) were each added alone to the sections, no effect on basal HIP occurred. However, when TFP (10^{-5} M) was added to H, a significant reduction in H-induced HIP occurred at 10^{-15}–10^{-13} M concentrations of H ($P < 0.05$). PMZ also caused a significant reduction on H-induced HIP at high concentrations of H, 10^{-14}–10^{-13} M ($P < 0.05$) (Fig. 7). When the integrated areas of the responses were measured and compared before and after treatment with TFP and PMZ, TFP caused 54 and PMZ 56% inhibition of the action of H.

C-induced HIP was reduced significantly ($P < 0.05$) by the addition of TFP (10^{-5} M) and PMZ (10^{-5} M) (Fig. 8). TFP caused 65 and PMZ 64% inhibition of the response to C when measured as the integrated response.

When W-7 was added to H, a significant ($P < 0.05$) reduction of H-induced HIP occurred only at H concentrations of 10^{-14}–10^{-15} M (Fig. 9). When measured as an integrated response, however, there was a 52.9% inhibition of H-induced HIP by W-7 (10^{-5} M). The dose-response values for different concentrations of W-7 (10^{-10}–10^{-5} M) added to the D_{max} of H (10^{-14} M) gave a significant reduction from H alone with 10^{-7} and 10^{-5} M W-7 (Table II).

When W-7 (10^{-5} M) was added to C (10^{-12}–10^{-8} M), a significant reduction of HIP occurred with 10^{-11}–10^{-8} M of C (Fig. 10). The integrated response to the maximally effective dose of C (10^{-9} M) was significantly inhibited by every dose of W-7 (10^{-10}–10^{-5} M) (Table II).

TABLE I
Effects of EGTA on the Ca^{2+} Concentration in Hepes Buffer

Hepes (0.025 M)	Ca^{2+}
	M
Alone	2.1×10^{-7}
+1 mM $CaCl_2$	3.5×10^{-4}
+1 mM $CaCl_2$, 10 μM EGTA	3.5×10^{-4}
+0.1 mM $CaCl_2$, 10 μM EGTA	3.9×10^{-5}
+10 μM $CaCl_2$, 10 μM EGTA	6.0×10^{-6}
+1 μM $CaCl_2$, 10 μM EGTA	2.0×10^{-8}
+10 μM EGTA	2.0×10^{-8}

Ca^{2+} was measured by atomic absorption spectrophotometry.

DISCUSSION

Cytochemical staining of oxyntic cells has been shown to provide a quantitative measurement of HIP (14). We (15) and Loveridge et al. (16) have used this method to quantify activation of guinea pig fundic oxyntic cells by gastrin at levels comparable to those used for H and C in our studies (17). These findings are also in agreement with previous studies in our laboratory using this system to characterize the oxyntic cell response to H, C, and gastrin (11, 15, 17). CA is thought to catalyze the reaction that consumes a hydroxyl ion and results in generation of a bicarbonate ion (18), and acid secretion halts if CA is inhibited (18–20). Although cytochemical measurement of HIP by the oxyntic cell cannot be said unequivocally to represent a direct measurement of acid secretion by the oxyntic cell, all models for proton secretion by the cell require the concomitant release of a hydroxyl ion within the cell and measurement of HIP may well reflect acid secretion. The doses of H and C are much lower than those used in other systems. The reasons for this sensitivity are not known. Direct application

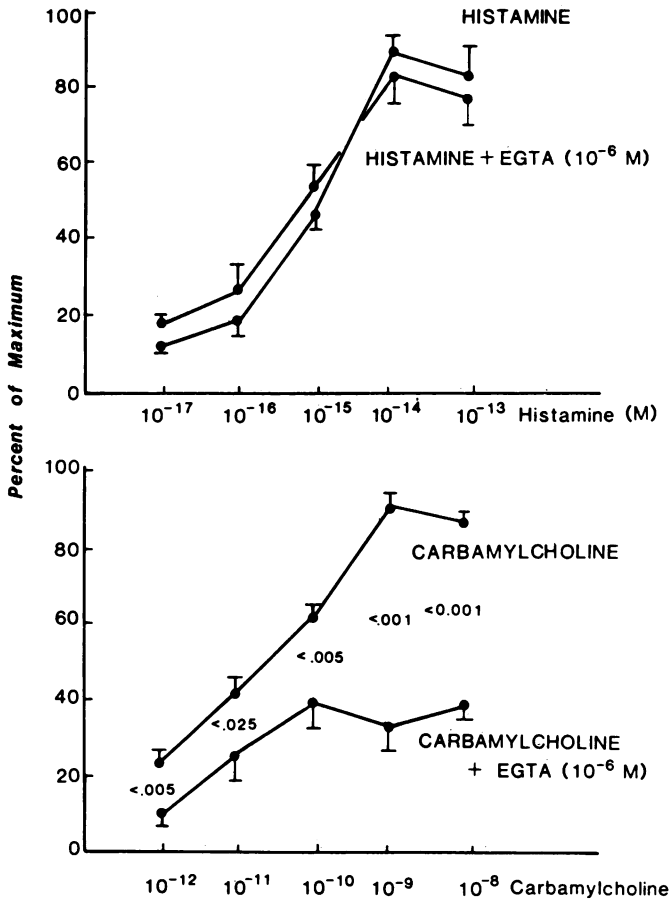

FIGURE 3 The effects of EGTA on H- and C-stimulated HIP. The most efficacious stimulus is gastrin (10^{-12} M) for which the D_{max} has arbitrarily been ascribed the value of 100% and the relative activities of H and C are given as fractions thereof. Significant differences with EGTA treatment are shown.

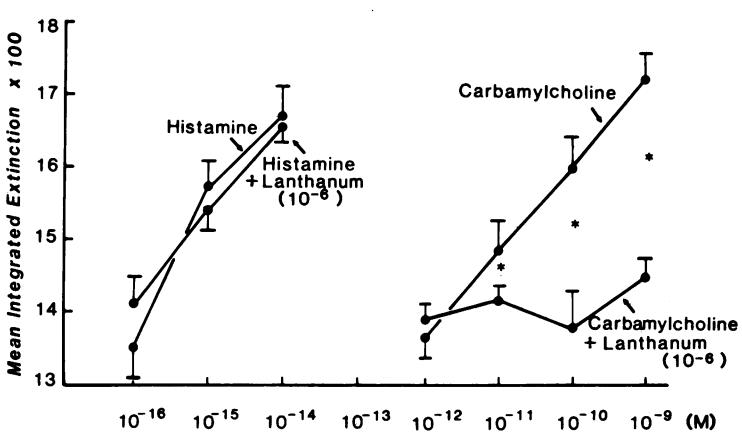

FIGURE 4 The effects of $LaCl_3$ (10^{-6} M) on H- and C-stimulated HIP. The asterisk indicates significant differences between C alone and C plus $LaCl_3$.

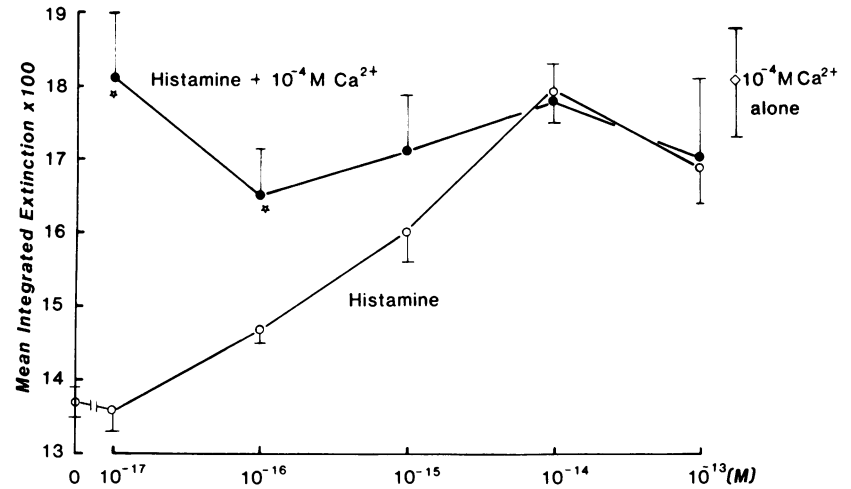

FIGURE 5 The effects of a maximally effective dose of Ca^{2+} (10^{-4} M) on H-stimulated oxyntic cell HIP. The asterisk indicates significant differences between H alone and H plus 10^{-4} M Ca^{2+}.

of the agents to the cell membrane, as occurs in the cytochemistry bioassay, is one unlikely possibility. Release of endogenous acetylcholine or H may result in higher effective levels at the cell than those applied in the buffer. This effect, however, is repeatedly and consistently observed and similar sensitivities have been reported in similar bioassays for adrenocorticotropic hormone, parathyroid hormone, and thyrotropin in sections of adrenal, kidney, and thyroid tissue, respectively (21–23). For example, the cytochemical bioassay for bovine parathyroid hormone is sensitive to ~0.1 fg/ml (23) while that for adrenocorticotropic hormone is ~5 fg/ml (or 10^{-12} M) (24).

The role of Ca^{2+} in the action of neurotransmitters and in hormone release is well established (25–28). Ca^{2+} caused significant stimulation of HIP when applied in the range of 2.1×10^{-5}–2.1×10^{-3} M. The most likely mechanism for this stimulation is an ele-

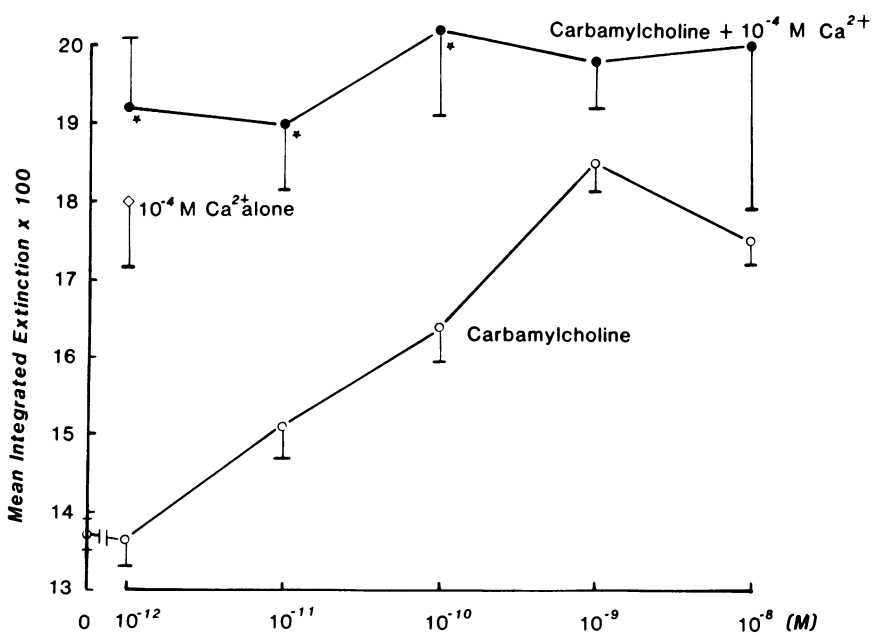

FIGURE 6 The effects of Ca^{2+} (10^{-4} M) on C-stimulated oxyntic cell HIP. The asterisk indicates significant differences between C alone and C plus 10^{-4} M Ca^{2+}.

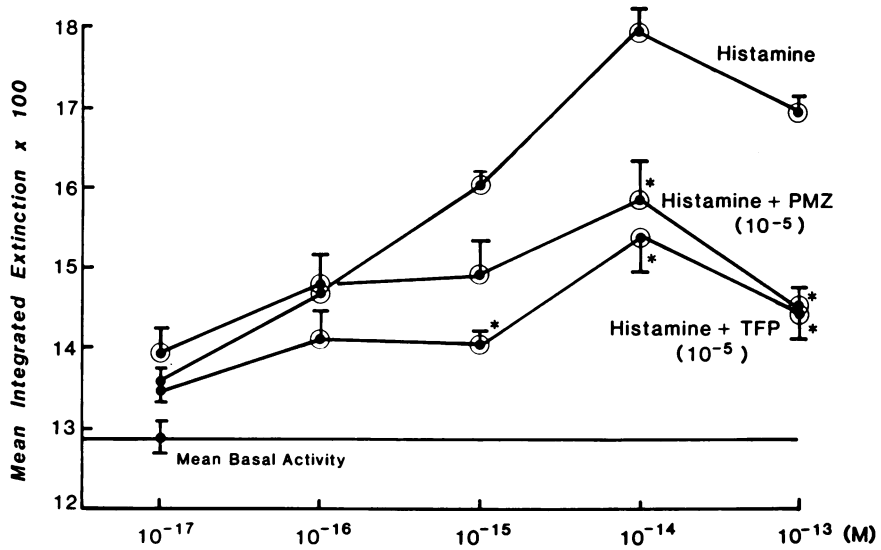

FIGURE 7 The effect of TFP (10^{-5} M) and PMZ (10^{-5} M) on H-stimulated guinea pig oxyntic cell HIP. O = significant increase above base line. *Significant difference from H alone.

vation in the intracellular Ca^{2+} concentration with activation of the Ca^{2+}-dependent pathways within the cell, which result in increased HIP. These pathways may involve Ca^{2+} as a second messenger, perhaps in the form of a Ca^{2+}-calmodulin complex, which activates processes leading to increased HIP. The reason for the reduction in HIP at the highest dose tested is unknown but may represent end-product inhibition or activation of antagonistic pathways. Removal of the medium allows further maximal stimulation by the agonist.

H and C caused stimulation of HIP over a range of concentrations and to a magnitude very similar to those reported in our earlier studies (11). The role of Ca^{2+} in the mediation of the action of these two secretagogues was derived from studies using EGTA, $LaCl_3$, and exogenous Ca^{2+}. EGTA chelates extracellular Ca^{2+} and renders it biologically inactive. $LaCl_3$

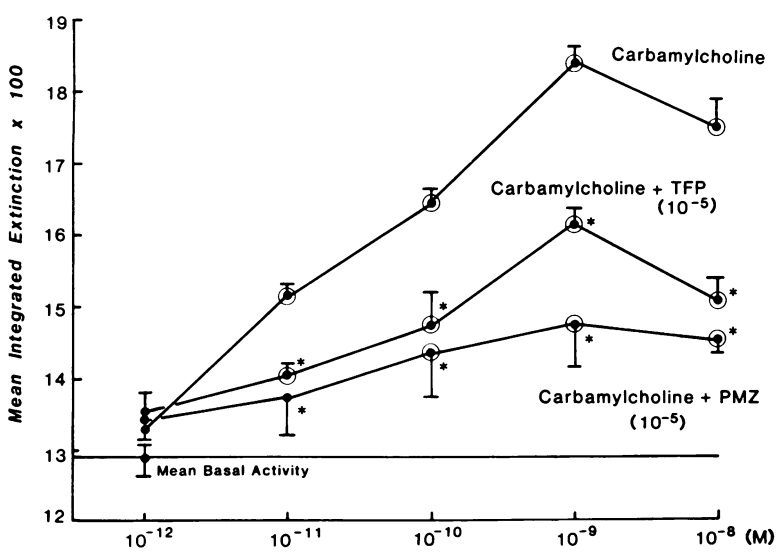

FIGURE 8 The effects of TFP (10^{-5} M) and PMZ (10^{-5} M) on C-stimulated guinea pig oxyntic cell HIP. O = significant increase above base line. *Significant difference from C alone.

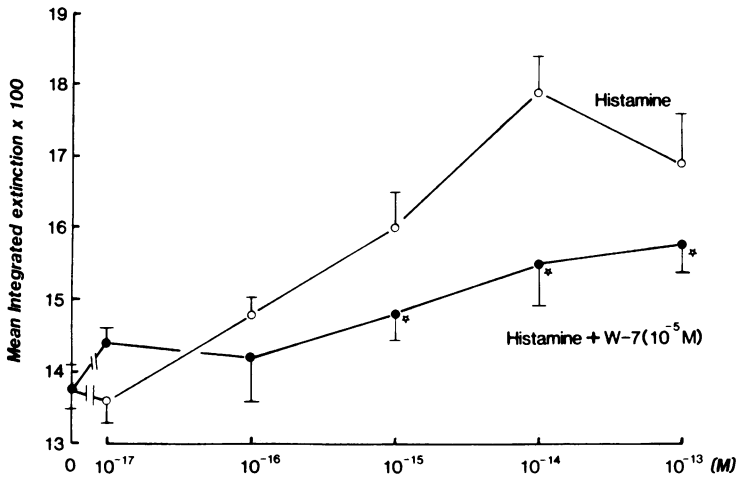

FIGURE 9 The effects of W-7 (10^{-5} M) on H-stimulated oxyntic cell HIP. The asterisk indicates significant differences between H alone and H plus W-7.

displaces membrane-bound calcium and prevents flux of Ca^{2+} across the membrane without penetrating intracellularly (29–32). C-induced HIP was completely inhibited by $LaCl_3$ and significantly by EGTA. This is consistent with other studies that report Ca^{2+} dependence of the action of C in [^{14}C]aminopyrine uptake in isolated canine parietal cells (25) and enzyme secretion from pancreatic acinar cells (26). The greater degrees of inhibition by $LaCl_3$ may be a function of its ability to block efflux as well as influx of Ca^{2+} (32) suggesting that Ca^{2+} efflux may be important in the action of C.

The addition of exogenous Ca^{2+} did not result in an increase of HIP above that expected for either C or Ca^{2+} alone. This suggests that both Ca^{2+} and C act via the same pathway, since neither potentiation nor inhibition is seen. This supports the concept that the action of C is primarily a Ca^{2+}-mediated event.

TABLE II
Effect of W-7 on C- and H-stimulated HIP

W-7 (M)	C (10^{-9} M)	H (10^{-14} M)
0	19.8±0.44	17.2±0.48*
10^{-10}	17.14±0.81*†	17.13±0.67*
10^{-9}	15.68±0.69†	16.3±0.44*
10^{-8}	15.63±1.35†	16.85±0.76
10^{-7}	14.13±0.53†	15.55±0.96†
10^{-6}	14.25±1.66†	15.33±0.69
10^{-5}	13.25±0.41†	15.46±0.39*†

Values are mean integrated extinction × 100.
* Significantly different from base line.
† Significantly different from D_{max}.

H-stimulated HIP was unaffected by either EGTA or $LaCl_3$, which indicates the Ca^{2+}-independent nature of H-stimulated HIP, where extracellular and membrane Ca^{2+} events are involved. This is consistent with other studies showing that H stimulates [^{14}C]aminopyrine uptake via a cyclic (c)AMP mechanism and is independent of extracellular Ca^{2+} (27). The addition of exogenous Ca^{2+} did not result in stimulation above that expected for either Ca^{2+} or H alone.

Since $LaCl_3$ and EGTA do not apparently affect intracellular Ca^{2+} events, the above studies do not exclude the possibility that H and C may act via changes of Ca^{2+} within the cell.

Calmodulin is an intracellular Ca^{2+}-binding protein that regulates a multitude of intracellular enzyme systems including those influencing cAMP levels and various Ca^{2+}-dependent protein kinases, which influence secretory processes (3, 33). If H and C require changes in intracellular Ca^{2+} to activate HIP, calmodulin may initiate this action in its role as a Ca^{2+} transducer. TFP and PMZ are two lipid-soluble drugs thought to be antagonists of the Ca^{2+}-calmodulin complex (3, 34). Both H- and C-stimulated HIP was substantially inhibited by TFP and PMZ. The magnitude of this inhibition was similar. The degree of inhibition was not significant at lower concentrations of H or C, suggesting that TFP and PMZ inhibit only stimulated HIP induced by H or C.

Although TFP and PMZ were among the first drugs shown to inhibit calmodulin in vitro, more recent studies indicate both may cause changes in cell membranes and disrupt the cytoskeleton, perhaps accounting for some of the observed events attributed to inhibition of calmodulin (35–37). Because of this possibility, additional studies were performed using W-7, a naphtha-

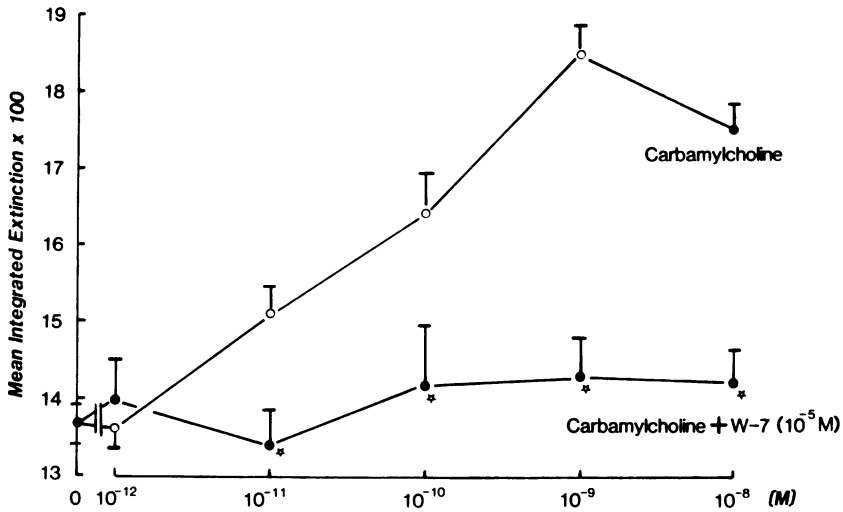

FIGURE 10 The effects of W-7 (10^{-5} M) on C-stimulated oxyntic cell HIP. The asterisk indicates significant differences between C alone and C plus W-7.

lenesulfonamide, which has also been reported to inhibit calmodulin, but as yet has not been shown to cause cell membrane or cytoskeletal changes (38, 39). W-7 caused a 99.9% inhibition of the maximally effective dose of C at doses as low as 10^{-10} M. The action of H was less affected, but inhibition by W-7 was 52.9%. If W-7 is specific for calmodulin this suggests that calmodulin plays a very important role in the action of C and a less important one in the action of H. This is consistent with our study and others, which show that H acts via a cAMP pathway, while C acts via a Ca^{2+} pathway, without observed effects on cAMP (40, 41). The differing effects found with TFP, PMZ, and W-7 suggest that TFP and PMZ are weak antagonists of calmodulin and are consistent with a nonspecific action of TFP and PMZ either on the cell membrane or elsewhere. This seems to indicate that TFP and PMZ may not be useful tools to study calmodulin.

Though the magnitude of inhibition of C seems to indicate a direct link between the action of C and calmodulin, the role of calmodulin in H stimulation may be that of a potentiator in a manner such as that described by Gardner et al. (42) for cAMP- and Ca^{2+}-dependent secretagogues in pancreatic acinar cells. Inhibition of calmodulin would therefore be expected to reduce somewhat the HIP caused by H, such as was found with W-7. Thus, although calmodulin may not mediate the direct pathway of H action, it may mediate parallel and potentiated pathways.

These observations fit well with Rasmussen's concept of synarchic regulation of cell function where different stimuli activate different intracellular messengers, which may interact with one another but lead to a common end result (41). In this case, C would activate the proton pump via a Ca^{2+}-calmodulin pathway culminating in acid secretion. H, which acts primarily through an adenylate cyclase-cAMP pathway, causing acid secretion, also seems to interact with pathways involving calmodulin. Understanding the mechanism of these interactions would aid greatly in understanding disorders of acid secretion occurring in clinical states such as peptic ulcer disease.

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