SIGNAL TRANSDUCTION MACHINERY OF THE PARIETAL CELL SECRETORY SYSTEM

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ABSTRACT

It is well recognized that in cells of many higher eukaryotic organisms, Ca^{2+} ions and cyclic 3',5'-monophosphate (cAMP) are used as ubiquitous intracellular messengers in the regulation of cellular function*. The function of the gastric parietal cells is also regulated by these two different second messengers, leading to the secretion of HCl. In addition, multiple regulatory pathways might be involved in the parietal cell function to inhibit acid secretion. These pathways potentiate and interface with each other to modulate parietal cell secretion. This review will describe the possible mechanisms by which intracellular second messenger systems interact in the modulation of the parietal cell response.

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*For details, Molecular Biology of the Cell, 3rd ed.,

Alberts B., Bray D., Lewis J., Raff M., Roberts K. and Watson J. D. eds., Garland Publishing Inc., New York and London, is the most recommendable textbook. See references 147, 180, 185, 186 and 209 for details with respect to receptors and intracellular signal transduction.

I. INTRODUCTION

Gastric parietal cells synthesize and secrete HCl. There is a 10^6 -fold concentration gradient of protons across the plasma membrane of the parietal cell. The mechanism by which parietal cells secrete acid has been reviewed in detail by Forte and Wolosin (51), by Hanson and Hatt (68), by Hersey (73) and by Hersey and Sachs (74). In brief, acid is secreted across the apical plasma membrane of the parietal cell by a H^+ - K^+ pump which, in the resting state, is present in tubulovesicles. Consequent upon stimulation by chemical mediators (gastrin, acetylcholine, and histamine), these tubulovesicles fuse with the apical membrane to form secretory canaliculi. The fusion process requires a drastic and dramatic morphological change in the parietal cell and involves numerous cytoskeletal elements. In addition, secretagogue-stimulated parietal cells undergo a prominent elaboration of their apical membrane which is necessary for the translocation of H^+ - K^+ pump from the tubulovesicles to the apical membrane (Fig. 1). Stimulated vesicles possess a higher Cl^- conductance and a permeability to K^+ , which enable K^+ and Cl^- ions to extrude across the apical surface. The K^+ effluxed is recycled back into the cell by the H^+ - K^+ pump and the Cl^- is secreted with H^+ to reach a final HCl concentration of 150 mM in the gastric lumen. H^+ is thought to be produced from H_2O and CO_2 by the catalytic activity of the enzyme carbonic anhydrase ($H_2O + CO_2 = H^+ + HCO_3^-$). The HCO_3^- so produced is effluxed by a HCO_3^- - Cl^- exchanger in the basolateral membrane, while the entrant Cl^- from the extracellular space functions as a counter ion of HCO_3^- and balances intracellular Cl^- concentration since it is secreted with H^+ from the apical surface. A Na^+ - K^+ pump provides additional K^+ to replace that which fails to recycle back from the apical surface (15 mM leak).

All these events are initiated by the activation of receptors (Fig. 2). In general, secretion of the parietal cell is activated by at least three distinct extracellular signalling molecules which include acetylcholine (ACh), gastrin, and histamine. Ca^{2+} appears to be the second messenger for ACh and gastrin, whereas cAMP is a major second messenger for histamine (Table 1). These two components (Ca^{2+} and cAMP) act synergistically in inducing acid secretion. In addition, it appears that there exist additional regulatory agents that may inhibit parietal cell secretion. These include epidermal growth factor (EGF), transforming growth factor

alpha (TGF_{α}), somatostatin and prostaglandins. All of these agents interact initially with receptors on the plasma membrane of the parietal cell and therefore utilize intracellular messengers to regulate secretion in a process referred to as 'stimulus-secretion' coupling (46). This review describes and integrates some recent views of the intracellular messenger system obtained by studies of the parietal cell system. A further purpose of this review is to describe the crosstalk of the signal transduction systems involved in 'stimulus-secretion' coupling.

II. INTRACELLULAR MESSENGER, Ca^{2+} , IN PARIETAL CELLS

II-A. Ca^{2+} Release from Intracellular Stores

Over the last two decades it has become clear that the activation of many cell types is accompanied by an increase in intracellular Ca^{2+} . This process has been recognized as an important component in the sequence of events initiated during cell activation. Numerous hormones bind to cell surface receptors and lead to an increase in cytoplasmic free calcium concentration ($[Ca^{2+}]_i$). Ca^{2+} has been reported to function in a variety of cell types, that are involved in 'stimulus-secretion' coupling (s-s coupling). In s-s coupling, the turnover of plasma membrane phosphatidylinositol (PI) during the receptor activation is the major transducing element in the Ca^{2+} messenger system. Thus many agents act by stimulation of membrane-bound phospholipase C (PLC) through the activation of

Fig. 1 Stereo-image of high voltage micrograph of the parietal cell (A and B) and chief cell (C) (guinea pig). Parietal and chief cells were stimulated with gastrin (10 nM) and CCK-8 (10 nM), respectively, for 10 min at 37°C. Stimulated parietal cells undergo a prominent elaboration of their apical membranes (secretory canaliculus). Note that secretory processes of the parietal cell are different from those of the chief cell, which possesses abundant secretory granules. Tilting angle, $\pm 8^\circ$; $\times 6,200$ for parietal cells (panel A; diameter 20–25 μm) and $\times 6,700$ for chief cells (panel C; diameter 15 μm). Panel B is a magnification of the secretory canaliculus in which abundant microvilli are observed. See reference 80 for % of the cell volume of organelles in parietal cells (i.e., mitochondria are large and abundant with 30% of the cell volume). Abbreviations: SC, secretory canaliculus; N, nucleus; M, mitochondria; SG, secretory granules; AC, apical cytoplasm. Use a stereoscope for three dimensional views (courtesy of S. Sasaki and I. Nakagaki).

Fig. 1

Table 1 *Final Mediators of Parietal Cell Acid Secretion and Their Intracellular Messengers*

Original cells of stimulators	Stimulators	Parietal cell/second messengers
ECL cells (paracrine)	Histamine	cAMP (Ca^{2+})
G cells (endocrine/paracrine)	Gastrin	Ca^{2+}
Vagus (neurocrine)	Acetylcholine	Ca^{2+}

heterotrimeric guanosine nucleotide-binding protein (G protein). PLC activated by G protein catalyzes the hydrolysis of phosphatidylinositol 4,5-bisphosphate (PIP_2) to produce myo-inositol 1,4,5-trisphosphate (IP_3) and diacylglycerol (DG), as initially proposed by Berridge and Irvine (13) and Nishizuka (122). In s-s coupling, the major G protein and PLC in the mediation of Ca^{2+} signal transduction systems appear to be G_q and $\text{PLC}_{\beta-1}$, respectively (12, 14, 29, 159, 177). IP_3 and DG act as intracellular second messengers to mobilize Ca^{2+} from intracellular stores and to stimulate Ca^{2+} - and phospholipid-dependent protein kinase (protein kinase C/PKC), respectively. In addition to PKC, the IP_3 -activated $[\text{Ca}^{2+}]_i$ increase regulates many other cellular processes directly, e.g. Ca^{2+} -activated K^+ and Cl^- channels, or indirectly through interaction of Ca^{2+} -binding protein, cal-

Fig. 2 Diagrammatic scheme of stimulatory parietal cell pathways. Abbreviations: $\text{H}_2\text{-R}$, histamine H_2 receptor (358–359 amino acids/7 TM); $\text{M}_3\text{-R}$, muscarinic type 3 receptor (589–590 amino acids/7 TM); Gastrin/CCKB-R , gastrin/CCK_B receptor (447–453 amino acids/7 TM); Ach, acetylcholine; G_s , stimulatory G protein; AC, adenylyl cyclase; G_i , inhibitory G protein; PIP_2 , phosphatidylinositol 4,5-bisphosphate; $\text{PLC}_{\beta-1}$, phospholipase C $_{\beta-1}$; DG, diacylglycerol; PKC, protein kinase C; IP_3 , inositol 1,4,5-trisphosphate; $\text{IP}_3\text{-R}$, IP_3 receptor; PKA, protein kinase A; CaM, calmodulin; CaMK II, calmodulin-dependent protein kinase II; CA, carbonic anhydrase; PM, plasma membrane; SER, smooth endoplasmic reticulum or tubulovesicular membrane; N, nucleus; Mt, mitochondria; TV, tubulovesicles; MT, microtubulus; MF, microfilaments; SC, secretory canaliculus; wave lines, energy-dependent ion pumps. See text for phosphorylated proteins (II-E and III-B). See references 15, 50, 51, 68, 73, 74, 79, 80 and 204 for parietal cell morphology and acid secretion mechanisms.

modulin (CaM) which in turn acts upon Ca^{2+} /CaM-dependent protein kinases. This process leads a cascade of cellular reactions. Thus, the Ca^{2+} response evoked by IP_3 -mediated Ca^{2+} release from intracellular Ca^{2+} stores is coupled to the receptor activation through the PIP_2 turnover and functions as a ubiquitous intracellular messenger.

As in other non-excitable cells, intracellular Ca^{2+} release as a signal transducer has been implicated in the regulation of the parietal cell function. Acetylcholine (ACh) and gastrin have been recognized as Ca^{2+} -mobilizing agents in parietal cells. The parietal cell possesses muscarinic receptors: $K_d = 7.8 \times 10^{-10}$ M and receptor number = 4,900 per rat parietal cell (47). The muscarinic receptor in parietal cells from various species is M_3 which comprises 589/590 amino acids and seven transmembrane domains (TM) (93, 132, 209, 210). Activation of the parietal cell M_3 receptor by ACh is associated with the activation of G_q , PLC and subsequent PIP_2 turnover to produce IP_3 and DG. Thus, ACh stimulation has been reported to increase the inositol polyphosphates and the DG contents in parietal cells from various species, including rabbit, rat and dog (34, 39, 129, 131, 138). Hence, ACh elicits a large Ca^{2+} transient due to intracellular Ca^{2+} release from the stores (33, 117, 118).

The parietal cell possesses gastrin/CCK_B receptors (88): $K_d = 0.2 \times 10^{-10}$ – 3.2×10^{-10} M and receptor number = 64,000 sites per canine parietal cell (106, 165); $K_d = 4.1 \times 10^{-9}$ M and receptor number = 79,000 per guinea pig parietal cell (195). The parietal cell gastrin/CCK_B receptor (452 amino acids in rat and rabbit; 453 amino acids in canine; 452 amino acids, long isoform, and 447 amino acids, short isoform, in human), which is a seven-pass transmembrane molecule homologous to other members of the G protein-linked receptor superfamily (17, 88, 169, 209), is thought to be coupled to the G_q /PLC pathway (see reference 208 for details). Thus gastrin has been reported to increase formation of inositol trisphosphate in canine and rabbit parietal cells (39, 145). In rabbit parietal cells, gastrin-elicited IP_3 production may be biphasic with a peak increase of 2–5 fold occurring at 30 s and 5–10 min (145). The rapid increase in IP_3 production evoked by gastrin may be insensitive to pertussis toxin (PTX) which causes an ADP-ribosylation of the alpha subunit of G_i (inactivation of G_i) and to extracellular Ca^{2+} ($[\text{Ca}^{2+}]_o$), whereas the sustained IP_3 increase may be PTX-sensitive and requires $[\text{Ca}^{2+}]_o$ (146). The mecha-

Fig. 3 $^{45}\text{Ca}^{2+}$ release brought about by IP_3 and gastrin in saponin-permeabilized parietal cells (guinea pig). Isolated parietal cells were permeabilized with saponin in the cytosol buffer (high K^+ and low Na^+) in the presence of ATP regenerating system. $^{45}\text{Ca}^{2+}$ was incorporated in the cells in an ATP-dependent manner. IP_3 and gastrin transiently released $^{45}\text{Ca}^{2+}$ from the cells. The total sum of the $^{45}\text{Ca}^{2+}$ release by gastrin and IP_3 is the same as that released by IP_3 alone. Concentration of reagents: 1.5 mM ATP, 5 μM IP_3 and 10 nM gastrin (from reference 195)

nism of the sustained $[\text{IP}_3]$ production is, however, unknown. In saponin-permeabilized guinea pig parietal cells, gastrin and IP_3 can cause the release 25% and 53% of $^{45}\text{Ca}^{2+}$, respectively, which is previously incorporated into the non-mitochondrial stores when ATP and its regenerating system are applied (195). When IP_3 is added after gastrin, there is a further release of $^{45}\text{Ca}^{2+}$ and the sum of the $^{45}\text{Ca}^{2+}$ release by gastrin plus IP_3 is the same as that released by IP_3 alone, suggesting that gastrin and IP_3 share the same pathway to release Ca^{2+} from non-mitochondrial stores (195) (Fig. 3). This IP_3 response was visualized by a fluorescence digital image analyzer. Fig. 4 shows the IP_3 -mediated Ca^{2+} release in fura-2-loaded individual permeabilized parietal cells (guinea pig) (198). These results indicate that the PIP_2 turnover is increased by gastrin stimulation. Consequently, gastrin stimulation induces an initial large Ca^{2+} transient due to the release of Ca^{2+} from intracellular stores of the fura-2 (or quin 2)-loaded parietal cell in both dog (43) and guinea pig (188, 195). Similar observation has been noted in aequorin-loaded guinea-pig parietal cells (195, 196). The change in $[\text{Ca}^{2+}]_i$ elicited by gastrin can be directly related to activation of a

Fig. 4 Spatiotemporal changes in $[Ca^{2+}]_i$ induced by IP_3 in saponin-permeabilized and ATP supplied single parietal cells (guinea pig). Isolated parietal cells were loaded with fura-2 and acutely permeabilized with saponin in the cytosol buffer (high K^+ and low Na^+). Acutely permeabilized cells still retained fura-2 for measurement of $[Ca^{2+}]_i$. Exposure of ATP and its regenerating system to parietal cells resulted in an incorporation of Ca^{2+} into the stores (upper panel). When the cells were stimulated with $5 \mu M$ IP_3 , the $[Ca^{2+}]_i$ plateau (130 nM) enlarged to the entire cytosol and a new higher $[Ca^{2+}]_i$ plateau (250 nM) was created (middle panel). After 30 s of IP_3 addition, the new higher $[Ca^{2+}]_i$ plateau (250 nM) enlarged toward the cytosol (lower panel). The results indicated the IP_3 -mediated Ca^{2+} release from intracellular stores (from reference 198).

'gastrin-type' receptor since the gastrin/ CCK_B receptor antagonist L-365, 260 is capable of abolishing Ca^{2+} spiking induced by gastrin (43, 144). Cholecystokinin octapeptide ($CCK-8$), as well as gastrin, leads to an increase in $[Ca^{2+}]_i$ in rabbit parietal cells via mediation of the gastrin/ CCK_B receptor (34). Canine parietal cells secrete acid

equally well in response to both CCK and gastrin *in vitro* (CCK is, however, a weak stimulant to secrete acid *in vivo* because it also acts on D cells to secrete somatostatin, which results in inhibition of acid secretion) (154). Gastrin on the other hand fails to increase $[Ca^{2+}]_i$ and pepsinogen secretion from canine gastric chief cells (154, 197). This suggests that the CCK receptor on chief cells may differ from the closely related gastrin receptors on parietal cells, via mediation of the CCK_A receptor. Thus the CCK_B receptors have equal affinities for both gastrin and $CCK-8$, whereas the CCK_A receptors have a high affinity for only $CCK-8$ (sulfated form) (208). A major difference of eight amino acids of the carboxy termini between $CCK-8$ and gastrin is that the sulfate moiety of Tyr²⁷ and Met²⁸ in $CCK-8$ are lacking in gastrin, suggesting an importance of Tyr[SO₃H]²⁷ and Met²⁸ to differentially recognize and bind to the CCK_A and CCK_B receptors (200). The deduced amino acid sequences of the CCK_A and CCK_B receptors in rats are 429 and 452 amino acids, respectively (88, 208). As expected on the basis of the shared ligand affinity, they are highly homologous, having 48% amino acid identity (88, 208).

The initial Ca^{2+} transient is independent of the presence of extracellular Ca^{2+} and not influenced by the Ca^{2+} channel blocker La^{3+} (114, 116, 118, 196). It is therefore probable that the source of Ca^{2+} utilized for the initial Ca^{2+} response originates from intracellular stores of the parietal cell. In guinea-pig parietal cells, electron probe X-ray microanalysis and subcellular fractionation studies ($100,000 \times g$ interface of the sucrose gradient) suggest that the IP_3 -sensitive Ca^{2+} pool may reside in apical portions in which smooth ER and tubulovesicles are included (181, 195, 196). There exists a precedent for such a location, since in several cell types, including Purkinje cells (126), hepatocytes (158) and insulinoma cells (133), the IP_3 receptor resides in the smooth ER. In parietal cells, the attachment of the IP_3 -sensitive Ca^{2+} stores to the plasma membrane has been suggested based on the sensitivity of microtubular-microfilament disrupting agents: colchicine and cytochalasins inhibit IP_3 -induced Ca^{2+} mobilization in guinea-pig parietal cells (181, 182, 190). Thus the release of Ca^{2+} from the IP_3 -sensitive pool requires an appropriate organization of the cytoskeletal system. In other words, the integrity of the microtubular-microfilamentous system is essential for this Ca^{2+} response. These cytoskeletal-attached IP_3/Ca^{2+} channels, which have first been reported

in parietal cells, have increasingly been accepted in other cell types: the IP_3 receptor might be anchored to ankyrin, a component of the cytoskeleton in the brain and T-lymphocytes; the hepatic IP_3 receptor might attach to microfilaments by anchoring to unidentified cytoskeletal proteins (90).

The time interval of the initial Ca^{2+} transient is 2–3 min and the amplitude of $[Ca^{2+}]_i$ reaches 300–800 nM from a basal $[Ca^{2+}]_i$ of 50–150 nM. Thereafter, the Ca^{2+} released is extruded from the cell either by a CaM-dependent Ca^{2+} pump (116, 183) or a Na^+/Ca^+ antiporter (183, 196), or a combination of both. The Ca^{2+} pool, that is depleted during the receptor activation, is replenished by extracellular Ca^{2+} after the removal of agonist (118, 119). Since this type of Ca^{2+} release is transient, sustained acid secretion further requires the sustained influx of Ca^{2+} across the plasma membrane (11, 163, 183) (see II-C).

It is of interest to note that the cAMP-mobilizing agonist histamine can cause the release of Ca^{2+} from the stores of the rabbit parietal cell, although the amplitude of Ca^{2+} spikes observed is quantitatively less than that to carbachol (ACh analogue) (33, 121). Single cell measurement of $[Ca^{2+}]_i$ has revealed that histamine-responsive cells comprise only 35–50% of the total parietal cell fraction (99, 117, 121). There is no explanation for such heterogeneity. Since short-term cultured rabbit parietal cells respond to histamine at a rate of 75% (99), this difference may be attributed to cells being either in a different stage of the cell cycle or to differences in differentiation or metabolic state. The histamine-induced $[Ca^{2+}]_i$ response is mediated by the H_2 -receptor since it is inhibited by the specific H_2 -receptor antagonist cimetidine, but not by an H_1 -antagonist (99, 117). Of further note is the observation that aminopyrine accumulation [an index of acid secretion *in vitro* as developed by Berglinth *et al.* (10)] is partially inhibited by intracellular Ca^{2+} chelator BAPTA when cells are stimulated with histamine (121). This suggests that the histamine pathway may be partially dominated by a Ca^{2+} signalling pathway, at least in rabbit parietal cells. Histamine at the supramaximal dose (10^{-4} M) evokes a minimal Ca^{2+} spiking in less than 10% of individual canine parietal cells (43). In contrast, several reports have suggested that histamine alone is unable to generate IP_3 and DG in parietal cells from rabbit (34), rat (138) and dog (39, 129). Forskolin, an agent that acts directly on the catalytic component of the adenylyl cyclase

system (58), increases $[Ca^{2+}]_i$ in rabbit and pig gastric glands (34, 103), while a cell-permeant cAMP analogue dibutyryl cAMP (dbcAMP) fails to increase $[Ca^{2+}]_i$ (103, 117). It therefore appears to be likely that the activation of some component of the adenylyl cyclase system rather than cAMP and subsequent A-kinase activation may play a role in the regulation of the Ca^{2+} signalling pathway. This pathway is clearly different from the cAMP-gated Ca^{2+} channel superfamily II (olfactory cells) and G_s -coupled L type Ca^{2+} channels (cardiac muscle cells) (186) because of its voltage insensitivity. On the other hand, an alternative pathway may be involved in the histamine pathway: single histamine/ H_2 receptors may be differentially but directly coupled to adenylyl cyclase and Ca^{2+} signalling pathways: histamine stimulates cAMP, inositol phospholipid turnover and $[Ca^{2+}]_i$ increase in a rat hepatoma-derived cell line that has been transfected to express the H_2 receptor cDNA (44). All the effects are inhibited by the H_2 -receptor antagonist cimetidine. The increased membrane inositol phospholipid turnover and $[Ca^{2+}]_i$ increase are abolished by cholera toxin, which causes an ADP-ribosylation of the alpha subunit of G_s (activation of G_s), but not by PTX. However, this mechanism remains to be determined. In contrast to rabbit and pig gastric glands, forskolin fails to increase $[Ca^{2+}]_i$ in transfected cells. Thus histamine-stimulated Ca^{2+} signalling pathways show a species difference and cellular heterogeneity. Current information is insufficient to allow deduction of the mechanism of histamine-stimulated Ca^{2+} release.

II-B. Ca^{2+} Oscillation

The recent development of microspectrofluorometry and digital imaging analysis has facilitated the measurement of $[Ca^{2+}]_i$ in single cells. Such studies utilizing a variety of living cells and intracellular probes (quin 2, fura-2, indo-1, indo-3 and aequorin) have confirmed the oscillation of the Ca^{2+} signal in cells (185, 186). Individual rabbit parietal cells exhibit Ca^{2+} oscillation in response to histamine and low dose of carbachol (99). The primary source of Ca^{2+} utilized in the Ca^{2+} oscillation is intracellular. The frequency appears relatively slow (0.2–0.4 pulses/1 min) as compared with other cell types (1–12 cycles/min) (185, 186, 193). In single guinea-pig gastric glands, the oscillatory Ca^{2+} transient propagates to some adjacent cells in response to gastrin, via junctional regions connect-

ing the parietal cell to the central gland cells (199). This study also indicates that only 40% of parietal cells in the gastric gland respond to gastrin with a 2-fold increase in $[Ca^{2+}]_i$ over basal. In addition, only 30% of the rat parietal cell respond to gastrin to increase $[Ca^{2+}]_i$ (26). These findings suggest the existence of a subpopulation of gastrin-responsive cells. In individual canine parietal cells, lower concentrations of gastrin (10^{-10} M) induce Ca^{2+} oscillations (0.9 cycles/min) (43). Similarly, canine chief cells adjacent to parietal cells exhibit repetitive Ca^{2+} spikes in response to CCK and carbachol; gastrin on the other hand does not cause Ca^{2+} oscillations in chief cells (197).

The biological significance of the Ca^{2+} oscillation has not yet been ascertained in parietal cells or any other cell types. Cells may convey information by varying the frequency of Ca^{2+} spikes and the oscillatory mechanism may utilize frequency instead of amplitude at the low level of $[Ca^{2+}]_i$ (12). Indeed, lower and physiological concentrations of agonists cause repetitive Ca^{2+} spikes in a majority of cells (185, 186, 211). It would therefore seem probable that this signalling mode may be universal. Of further interest, would be the relationship of the Ca^{2+} oscillation to vesicular traffic, protein phosphorylation and dephosphorylation, and the activation of ion flux (153, 186).

The mechanism by which intracellular Ca^{2+} oscillates remains to be determined. In brief, there are two categories: firstly an IP_3 -induced Ca^{2+} release (IICR) and secondly a Ca^{2+} -induced Ca^{2+} release (CICR). IICR has been divided into two subclasses (12, 211). The first model (IICR) has proposed that the Ca^{2+} oscillation is dependent on a periodic release (or production) of IP_3 and that PKC activation might be involved through phosphorylation of G protein to inhibit IP_3 production (negative feedback regulation). The second model for IICR has suggested that the Ca^{2+} released by IP_3 activates PLC, resulting in PIP_2 turnover and the subsequent reproduction of IP_3 to release further Ca^{2+} from intracellular stores. CICR has similarly been divided into two subclasses (12). In the first (negative feedback regulation of Ca^{2+}), it is proposed that the release of stored Ca^{2+} inhibits the IP_3 -induced Ca^{2+} release by interfering with the binding of IP_3 to its receptor. The sequestration of Ca^{2+} in the stores then results in the re-binding of IP_3 to the receptor and the subsequent further release of Ca^{2+} . The second is the extension of positive feedback regulation of Ca^{2+} . Thus Ca^{2+} released by IP_3 would trigger the further release of

Ca^{2+} from the IP_3 -insensitive pool, as has originally been described in both cardiac sarcoplasmic reticulum and sympathetic neurons (excitation-contraction coupling). In permeabilized rabbit parietal cells, caffeine, known to release Ca^{2+} from the IP_3 -insensitive/ Ca^{2+} -sensitive stores, provokes a rapid increase in $[Ca^{2+}]_i$, suggesting that the CICR mechanism may be involved in the parietal cell Ca^{2+} oscillation (145). In contrast, caffeine and ryanodine (a blocker of the sarcoplasmic reticulum) do not have any effects on basal $[Ca^{2+}]_i$ or on changes in $[Ca^{2+}]_i$ induced by histamine, carbachol or gastrin in intact and cultured rabbit parietal cells, suggesting that there is no evidence of CICR (36). These working hypotheses have not conclusively been determined in parietal cells as well as other cell types, and other alternative pathways to describe the Ca^{2+} oscillation mechanism (phospholipase A_2 /arachidonic acid pathways, other second messengers, proton gradients, potassium conductance, ATP fluctuations and the Ca^{2+} pump mechanism) may mutually be involved (186).

II-C. Ca^{2+} Influx from the Extracellular Space

The 10,000-fold concentration gradient of Ca^{2+} across the plasma membrane requires the strict regulation of Ca^{2+} permeability. Cells utilize the large Ca^{2+} gradients across their plasma membrane for transducing external Ca^{2+} signals. The Ca^{2+} entering from the extracellular space acts as an intracellular messenger. The quantity of Ca^{2+} which enters from the extracellular space is smaller than that released from intracellular stores in the majority of cells. This influx of Ca^{2+} occurs following the release of intracellular Ca^{2+} (136, 186). The process by which Ca^{2+} enters into the cell is voltage-insensitive and Mn^{2+} permeable in rabbit parietal cells as seen in a majority of non-excitabile cells (145, 186). In parietal cells from various species, the influx of Ca^{2+} is mediated by the Ca^{2+} channels of the plasma membrane since the sustained plateau of $[Ca^{2+}]_i$ is totally eliminated by application of the Ca^{2+} channel blockers, La^{3+} and Ni^{2+} (114, 118, 196), but not by that of the voltage-sensitive Ca^{2+} channel blocker (145). It is therefore the receptor-operated and voltage-insensitive Ca^{2+} entry mechanism. The amplitude of the plateau of sustained increase in $[Ca^{2+}]_i$ is 50–100 nM above the prestimulation level, whereas the initial transient increase in $[Ca^{2+}]_i$ is 300–800 nM in parietal cells (117, 118). In general, this small sustained plateau of $[Ca^{2+}]_i$ is, however, a neces-

sary intermediate and is sufficient for maintenance of cellular response at the maximal rate in the majority of cells (186, 194). This does not mean that the small elevation in $[Ca^{2+}]_i$ alone is sufficient to drive the maximal cell response, but rather that it may 'lock in' the signal initiated by the larger increase in $[Ca^{2+}]_i$ which thereafter becomes less Ca^{2+} -dependent (and/or highly sensitive to Ca^{2+}). In other words, cells are capable of maintaining the sustained response even in the low level of $[Ca^{2+}]_i$. This might involve, for example, autophosphorylation of the Ca^{2+} /calmodulin type II kinase, which becomes less Ca^{2+} -dependent (110). Thus, the Ca^{2+} second messenger system may function by varying the signal produced by the initial transient increase, relative to that produced during the sustained plateau. Therefore, Ca^{2+} in the sustained phase may still function as a messenger system. In fact, in carbachol- and gastrin-induced acid secretion, extracellular Ca^{2+} is absolutely required for maintenance of the long-term parietal cell response (11, 163, 183). When extracellular Ca^{2+} is removed, this secretagogue-induced acid secretion exhibits a transient pattern, without sustained Ca^{2+} responses.

The process of Ca^{2+} influx across the plasma membrane is insensitive to voltage in the majority of cell types which are included in s-s coupling and is termed 'receptor-operated Ca^{2+} influx (ROCI)', as proposed by Tsien and Tsien (180). Parietal cells exhibit a Ca^{2+} influx mechanism which appears to be ROCI in nature. The precise mechanism by which Ca^{2+} enters via the receptor-operated process has not yet been ascertained. There are two possibilities: 1) direct activation of Ca^{2+} influx mechanism by the receptor or G protein; and 2) activation by an intracellular second messenger. These alternations would be respectively mediated by ligand-operated Ca^{2+} channels (LOCs) or second messenger-operated Ca^{2+} channels (SMOCs) (180).

The *N*-methyl-D-aspartate receptor (NMDAR) and ATP receptor (ATPR) channels might be determined LOCs, as has been reported in neurons and smooth muscle cells (180). In these channels, the influx of Ca^{2+} is directly associated with receptor. This is not the case in non-excitable cells including parietal cells (186). Alternatively, evidence consistent with the role of either PTX-sensitive G proteins (G_i/G_o) or $G_{s-\alpha}$ in the modulation of ROCs has been suggested in several non-muscle cells (14). This is, however, not universal in s-s coupling and predominant in voltage-operated Ca^{2+} channels (VOCs), as has been described in cardiac

and skeletal muscle T tubules (14, 22). Indeed, in rabbit parietal cells, neither the $[Ca^{2+}]_i$ response nor the Mn^{2+} entry stimulated by gastrin or carbachol is altered by PTX treatment (145).

The influx of Ca^{2+} via SMOCs has been proposed in several cell types. In T-lymphocytes IP_3 has been proposed as a putative messenger in mediating the opening of Ca^{2+} channels of the plasma membrane by mitogens (91). In neutrophils plasma membrane Ca^{2+} channels are activated by the rise in $[Ca^{2+}]_i$ when Ca^{2+} is released from intracellular stores (179). These two examples are, however, not universal among all cell types. Inositol 1,3,4,5-tetrakisphosphate (IP_4) with IP_3 are second messenger candidates for transducing external $[Ca^{2+}]_i$ signal in some cell types (78, 112). In parietal cells, there are no reports to support these three second messengers to describe the Ca^{2+} entry mechanism. Putney (135) has proposed that an IP_3 -mediated empty pool may serve as a signal for Ca^{2+} influx: discharge of the Ca^{2+} pool after IP_3 response becomes a trigger for Ca^{2+} influx initiated by electrochemical gradients (capacitative Ca^{2+} entry). The influx of Ca^{2+} may therefore be a direct consequence of the discharge of intracellular Ca^{2+} stores rather than a direct consequence of the presence of an agonist. For example, application of the microsomal Ca^{2+} ATPase inhibitor, thapsigargin, which activates internal Ca^{2+} release due to the leak of Ca^{2+} from intracellular stores, results in the subsequent Ca^{2+} influx without an increase of inositol phosphates (178). This depleted Ca^{2+} pool-induced Ca^{2+} influx is inhibited by La^{3+} , suggesting that the Ca^{2+} entry process from the extracellular space is mediated by Ca^{2+} channels (92). The thapsigargin-induced Ca^{2+} influx and Mn^{2+} entry have similarly been reported in rabbit parietal cells (38, 145). The $[Ca^{2+}]_i$ kinetic study has indicated that, in rabbit gastric glands, influx generated by the emptying pool is approximately equal to that stimulated by carbachol (119). It is therefore likely that in parietal cells the Ca^{2+} entry mechanism may be regulated by the capacitative Ca^{2+} entry mechanism (see II-D). This Ca^{2+} channel in rabbit parietal cells is sensitive to pH outside the cells; a decrease in extracellular pH results in inhibition of carbachol-stimulated Ca^{2+} entry (120). Current information is, however, insufficient to allow deduction of the mechanism of 'ROCI', which may be multiple. Alternative pathways may be involved in the regulation of the plasma membrane Ca^{2+} channel, such as Ca^{2+} influx factor (CIF), serine/threonine phosphatases, tyrosine

kinases, GTP hydrolysis occurring on small GTP binding proteins, cytoskeletons, arachidonic acid and eicosanoids (136, 186).

II-D. Ca^{2+} Refilling

The refilling of Ca^{2+} into the depleted store is a necessary step to evoke the second cellular response. This process requires both the presence of extracellular Ca^{2+} and the removal of the agonist from the receptor. Once the agonist is removed, the Ca^{2+} refilling process occurs in variety of cell types (136, 172, 186). This refilling process has also been observed in rabbit parietal cells (117, 118). The removal of the agonist or the application of an antagonist to stimulated cells leads to an immediate decrease in plateau of $[\text{Ca}^{2+}]_i$. The Ca^{2+} influx is, however, still maintained to replenish the agonist-sensitive pool. In rabbit parietal cells, the refilling time for intracellular stores after exposure to extracellular Ca^{2+} is 3–5 min (117, 118). Using a new mag-fura-2, which can detect $[\text{Ca}^{2+}]$ in intracellular Ca^{2+} pools, it has been reported that the ATP-dependent Ca^{2+} uptake into the stores (Ca^{2+} refilling) is reduced by Cl^- -free solutions in permeabilized rabbit gastric glands, although this mechanism is obscure (76). We have observed that the ATP-dependent $^{45}\text{Ca}^{2+}$ uptake by parietal cell microsomes is inhibited by increasing concentrations of extravesicular (cytosol) Na^+ , suggesting an involvement of Na^+ - Ca^{2+} counter-transport across the ER membrane as well as the plasma membrane (183).

The process by which Ca^{2+} enters the cells across the plasma membrane during refilling is blocked by polyvalent cations. La^{3+} and Ni^{2+} inhibit Ca^{2+} refilling in parietal cells as well as other cell types (86, 117, 118, 127, 174). Thus, the Ca^{2+} influx process during refilling period may be mediated by the opening of Ca^{2+} channels in the plasma membrane. Since Ca^{2+} influx is required for refilling and since there is no detectable increase in $[\text{Ca}^{2+}]_i$ during the refilling process, two possible mechanisms by which Ca^{2+} refills the agonist-sensitive pool have been suggested (136): 1) Ca^{2+} influx from the extracellular space and Ca^{2+} sequestration into the stores are highly synchronized; and 2) direct refilling of Ca^{2+} from the extracellular space to the stores may occur via direct contact between the plasma membrane and the stores.

Recent studies using Mn^{2+} influx as an index of the plasma membrane permeability have indicated

that Mn^{2+} may enter into the cytosol during refilling, thereby resulting in a fura-2 fluorescence quench (66, 67, 115). This results from the observation that fura-2 exhibits a high affinity for Mn^{2+} and its fluorescence is quenched on binding this cation (64). Since the Ca^{2+} pump transports Mn^{2+} poorly, Mn^{2+} derived from the extracellular space during the refilling process results in diffusion throughout the entire cytosol with subsequent fluorescence quench of cytosolic fura-2. These results indicate that the Ca^{2+} refilling occurs through cytosolic but not a direct route between the plasma membrane and the stores. An alternative explanation for the lack of increase in $[\text{Ca}^{2+}]_i$ during refilling is that Ca^{2+} may enter into the restricted region of the cytosol in which the plasma membrane and the ER are closely opposed. In favour of this hypothesis, guinea-pig parietal cells possess high $[\text{Ca}^{2+}]_i$ just beneath the plasma membrane, which enlarges after gastrin stimulation (199). Ca^{2+} which has entered in this region is then taken up by the Ca^{2+} pump of the ER whose gain control of influx and uptake of Ca^{2+} is highly synchronized. The term applied to this putative process is 'capacitative Ca^{2+} entry' (174). It has not yet been ascertained whether or not 'capacitative Ca^{2+} entry' with agonists (receptor-operated Ca^{2+} channels) and without agonists (refilling Ca^{2+} channels) is the same or different (186). Activation of the plasma membrane Ca^{2+} channel operated by either receptors or refilling processes seems to be functionally different: the receptor operated Ca^{2+} entry accompanies a small elevation in $[\text{Ca}^{2+}]_i$, requires the presence of agonists and functions as a second messenger; the refilling process in turn does not accompany the $[\text{Ca}^{2+}]_i$ plateau, requires the dissociation of agonists from their receptors and does not function as a second messenger (172, 186, 194).

II-E. Biological Effects of Ca^{2+} Signal in Parietal Cells

The $[\text{Ca}^{2+}]_i$ increase serves as a second messenger to trigger the cell response. Ca^{2+} -binding protein calmodulin (CaM) is a major regulator for Ca^{2+} signalling molecules in a wide variety of tissues. Involvement of CaM in parietal cells has been implicated since non-specific CaM inhibitors, such as trifluoperazine, pimozide, and naphthalenesulfonamide, inhibit carbachol-induced aminopyrine accumulation (205). In addition, CaM-activated protein has been detected in a cytosolic extract of

isolated rabbit parietal cells (124). CaM-dependent protein kinase II (CaMK II) appears to be a major target for CaM in rabbit parietal cells coupling to the Ca^{2+} signal transduction since a specific CaMK II inhibitor (KN-62) inhibits aminopyrine accumulation evoked by carbachol in isolated rabbit parietal cells (187). KN-62, on the other hand, has no or little effect on aminopyrine accumulation induced by histamine or forskolin, which mainly acts on the cAMP/PKA pathways (187). In contrast, in rat parietal cells the CaM antagonist W-7 inhibits histamine-stimulated acid secretion, again suggesting the complexity of the histamine pathway (156). CaMK II has been implicated in the modulation of secretion and regulation of cytoskeleton in a number of cells including parietal cells (40). In rabbit parietal cells, it is present in association with light membrane fraction with a holoenzyme of 250 KDa (52). The presence of a CaMK II has also been demonstrated in rat gastric mucosal cells, where both parietal and chief cells appear to express the gamma and delta (but not alpha and beta) subtypes (107). Tubulin (55 KDa) is a well-known substrate for CaMK II (16) and is prominent in parietal cells (frog) (171), suggesting the possibility of an involvement of cytoskeletal elements regulated by CaMK II. Furthermore, the Ca^{2+} pump of the plasma membrane in parietal cells of dog and guinea pig has been reported to be dependent on CaM (116, 183) and the lack of ability of ATP-dependent Ca^{2+} uptake into microsomes ($100,000 \times g$ pellet) by the treatment with EGTA/EDTA is restored by the addition of exogenous CaM (183). The ATP-dependent Ca^{2+} uptake into the parietal cell microsomes can also be inhibited by CaM inhibitors, such as trifluoperazine and compound 48/80 (183). This Ca^{2+} uptake is markedly enhanced by vesicles which have been pretreated with gastrin (stimulated vesicles), suggesting that the ATP and CaM-dependent Ca^{2+} uptake system is coupled to the gastrin cascade (183).

In addition to CaM, the $[\text{Ca}^{2+}]_i$ increase regulates many cellular processes directly, such as Ca^{2+} -activated Cl^- or K^+ channels. In guinea-pig parietal cells, an increase in $[\text{Ca}^{2+}]_i$ induced by gastrin may directly modulate Cl^- efflux across the apical membrane, since gastrin causes the membrane depolarization which has been inhibited by either BAPTA (an intracellular Ca^{2+} chelator) or by furosemide (Cl^- channel blocker) (188). There exists a precedent for such a mechanism since Ca^{2+} -activated Cl^- efflux occur in various tissues and because a Cl^- conductance distinct from a K^+

conductance is present in the apical membrane of the parietal cell (42, 100). Carbachol may be capable of activating outward K^+ currents in a Ca^{2+} -dependent and voltage-independent manner in guinea-pig parietal cells (89). Carbachol and gastrin, as well as histamine, are capable of inducing increases in mRNA levels encoding carbonic anhydrase II and H^+ - K^+ ATPase in isolated canine parietal cells, probably through the Ca^{2+} -dependent mechanism (28). In single guinea-pig parietal cells loaded with BCECF (intracellular pH indicator) and fura-2 ($[\text{Ca}^{2+}]_i$ indicator), a cytosolic acidification precedes an increase in $[\text{Ca}^{2+}]_i$ in response to gastrin (189). In addition, elimination of medium Na^+ by replacement with NMG-Cl, which causes the cytosolic acidification by inhibition of the Na^+/H^+ antiport, results in Ca^{2+} oscillations (189). Similarly, the K^+ - H^+ ionophore nigericin, which elicits cytosolic acidification due to H^+ uptake from the extracellular space as a counter ion of K^+ , causes intracellular Ca^{2+} release from the stores (189). These results suggest that the pH_i decrease elicited by gastrin may trigger intracellular Ca^{2+} release via mediation of $\text{Ca}^{2+}/\text{H}^+$ antiport across the ER membrane in parietal cells. The phenomenon that cytosolic acidification triggers intracellular Ca^{2+} release has increasingly been accepted in other cell types as well as parietal cells (134, 186).

The precise mechanism by which the Ca^{2+} signalling pathway regulates the cellular cascade reactions has not conclusively been ascertained (see reference 186 for details). Further investigations are needed to determine the relationship between the spatiotemporal change in $[\text{Ca}^{2+}]_i$ (Ca^{2+} release, oscillation, influx, and refilling) and subsequent cellular events in parietal cells as well as other cell types.

III. INTRACELLULAR MESSENGER, cAMP, IN PARIETAL CELLS

III-A. cAMP Pathway

Cyclic adenosine 3',5'-monophosphate (cAMP) is produced from ATP by adenylyl cyclase and is a ubiquitous second messenger for intracellular signal transduction (147). Numerous hormones may bind to cellular surface receptors with resultant activation of adenylyl cyclase and subsequent production of cAMP. The generation of cAMP for certain extracellular molecules, which bind to membrane receptors, involves a G protein that is an essential component of the transduction control of cellular function. Adenylyl cyclase activity is

regulated by a stimulatory G-protein (G_s) and an inhibitory G-protein (G_i), as reviewed by Birnbaumer *et al.* (14) and Gilman (58).

The histamine H_2 receptor (358–359 amino acids/7 TM), which is coupled to adenylyl cyclase via G_s proteins, is present on the plasma membrane of the parietal cell (54, 55, 209). Its receptor number is 40,000 per parietal cell (rat) (150). In parietal cells, several lines of evidence indicate that histamine utilizes cAMP as a second messenger via the mediation of H_2 receptor. First, histamine elevates the cAMP level in isolated parietal cells from various species (7, 155, 166). Second, increased cAMP precedes acid secretion (161, 192). Third, the cell permeant cAMP analogue dibutyryl cAMP (dbcAMP) and forskolin, an activator of adenylyl cyclase found in extracts of the root of the coleus plant, both induce an acid secretion (10, 162). In addition, the phosphodiesterase (PDE) inhibitor 3-isobutyl-1-methylxanthine (IBMX) potentiates both the increase in cAMP contents and the secretory rates that are induced by histamine (166). Fourth, cAMP levels increased by histamine are inhibited by the H_2 receptor antagonist cimetidine (44). As proposed by Sutherland and his associates (147), all of these phenomena satisfy four criteria of cAMP as a second messenger: i) the agonist should be capable of increasing production of cAMP; ii) increased cAMP should precede, or at least, not follow the cellular response; iii) it should be possible to mimic the physiological cell response to the agonist by addition of cAMP-elevating agents or cAMP analogs; and iv) it should be possible to attenuate the physiological cell response to the agonist by the presence of inhibitors of cAMP.

In rabbit parietal cells, the cAMP pathway appears to be a major signalling system. Thus the efficacy of secretagogues in inducing acid secretion is as follows: forskolin > histamine > dbcAMP > carbachol > gastrin (Fig. 5). The first three are cAMP-mobilizing agents and the remainder are Ca^{2+} -mobilizing secretagogues. Aminopyrine accumulation induced by gastrin (10^{-7} M) is only 15–20% of that induced by histamine (10^{-6} M) in rabbit parietal cells (35). Thus, cAMP is an important intermediate for acid secretion (83). This need not be interpreted as an indication that Ca^{2+} is not a major signalling for parietal cells but may indicate that there are species differences with respect to these two second messenger systems. Thus, different pathways predominate in different species (i.e. carbachol > histamine in canine pari-

Fig. 5 14 C-aminopyrine accumulation in isolated rabbit parietal cells. Cells were incubated with various concentrations of secretagogues for 60 min at 37°C. The potency of secretagogues to induce acid production from parietal cells was: forskolin > histamine > dbcAMP (dibutyryl cAMP) > CCh (carbachol). Data are mean $\pm$ SE from four separate experiments. US: unstimulated cells

etal cells). The cAMP level generated by histamine stimulation is steady from 5 min to 60 min after cell activation, suggesting its basis and the mechanism for long-term regulation of histamine receptors on parietal cells (71). Other secretagogues might involve the cAMP pathway and acid secretion, by directly acting upon parietal cells (68). In rat parietal cells, the glucagon-like peptide-1 (GLP-1-[7–36]) leads to an increase in cAMP level and subsequent H^+ accumulation only when IBMX is included in the medium, in a manner independent of histamine H_2 receptor (157). Unlike histamine, GLP-1 alone without IBMX cannot produce the H^+ accumulation. But the major stimulator which utilizes cAMP pathways to induce parietal cell acid secretion is histamine throughout all species (note that the mammals, birds and fishes possess parietal cells: in the amphibia acid and pepsinogen secreting cells are the same).

III-B. Biological Effect of the cAMP Pathway

Several studies have suggested that the presence of a histamine activated cAMP-dependent protein kinase (PKA) in gastric tissues (82, 102). Since gastric tissues include several different cell types and since different kinases predominate in different cell types, it is reasonable to examine the kinase activity in isolated cells. In isolated rabbit parietal cells, both types I and II PKA (Mr: 48 KDa for I and 58 KDa for II) have been identified (32).

Histamine stimulation may preferentially activate the type I PKA (32). Although the precise relationship between type I PKA and its substrate(s) has not yet been ascertained, several proteins present in cytosol and microsomes are phosphorylated by histamine (or forskolin) stimulation, e.g. a 30 kDa in the cytosol (rabbit) (123) and 130 kDa in the cytosol (dog) (101). Secretagogue-stimulated parietal cells undergo a prominent elaboration of their apical membrane which is necessary for the translocation of H^+-K^+ ATPase from the tubulovesicles to the apical membrane to initiate and maintain parietal cell acid secretion (41, 50). Therefore, inherent in these gross morphological rearrangements must exist critical alterations in cytoskeletal structure and cytoskeleton-membrane interaction (15). Indeed, cytoskeletal disrupting agents, such as colchicine, vinblastine and cytochalasins, inhibit histamine-stimulated parietal cell acid secretion (85, 149). There is a mobilization of actin and spectrin upon stimulation of secretion that results in their colocalization within the acid-secreting membranes (109, 170). It has been suggested that the association of the gastric H^+-K^+ ATPase with spectrin and actin is mediated by ankyrin with maintenance of the polarized distribution of the enzyme to the apical portion of parietal cells during acid secretion (rabbit) (160). Histamine-stimulated phosphoproteins may be involved in the assembly of the cytoskeletal system. For example, vinculin (cytoskeletal-associated protein) phosphorylation may represent the 130 kDa phosphoprotein in dog parietal cells (101). The identification of the 30 kDa phosphoprotein may need it function as a cytoskeletal modifier (rabbit parietal cells) (123). The activity of the H^+-K^+ ATPase is increased by an 80 kDa cytosolic protein, which has been purified from the apical membrane-rich fraction of rabbit parietal cells (70, 202). This protein is identical with the cytoskeletal-associated protein ezrin (212), which has originally been identified as an undercoat-constitutive protein of the microvilli plasma membrane (also called cytovillin or p81) (20). Ezrin has a sequence homology (75% identity in mouse) with radixin, an actin-binding protein and a novel member of the band 4.1 family (53). In rabbit parietal cells, the cytoskeletal protein ezrin is a novel substrate for calpain I, a Ca^{2+} -dependent protease (214). The calpain-ezrin interaction is similar to calpain with talin and radixin. Ezrin is also linked to the apical membrane by the spectrin-actin component in rabbit parietal cells (69). Stimulation of parietal cells with histamine

(+IBMX) results in a phosphorylation of ezrin, leading to a redistribution of H^+-K^+ ATPase in the same membrane in which ezrin is included (70, 202). Therefore, an 80 kDa protein (ezrin) in the apical surface may be an important substrate for the cAMP signalling pathway, as a cytoskeletal linker. Since ezrin is a tyrosine phosphorylated protein (substrate for tyrosine kinases) and because the histamine/cAMP/PKA pathway mainly involves serine/threonine kinase pathways, the precise link between them is obscure (21, 62). Ezrin may be coupled to small GTP binding proteins, such as Ras, Rab and Rho proteins, resulting in relay signals to various pathways (i.e., serine/threonine kinases $\rightarrow$ tyrosine kinases).

Upon stimulation with histamine and forskolin, the type II regulatory subunit of PKA (52 kDa) is dephosphorylated by phosphoprotein phosphatase activity in rabbit parietal cells (59). The serine/threonine phosphoprotein phosphatase type 2A (PrPase 2A) inhibitor, okadaic acid, reverses this effect. Thus protein phosphatase coupled to the histamine cascade may be a significant regulator of parietal cell function. Upon stimulation with histamine, parietal cells may undergo phosphorylation steps of the type I PKA and dephosphorylation steps of the type II PKA to mediate the cAMP-dependent acid secretion (32, 59). In rabbit gastric glands, calyculin A, an inhibitor of serine/threonine protein phosphatase type 1 (PrPase 1) (and also PrPase 2A), but not okadaic acid (PrPase 2A inhibitor), stimulates basal and dibutyryl cAMP-evoked acid secretion, suggesting an inhibitory pathway of PrPase 1 on parietal cell cAMP pathways (203).

A small GTP-binding protein (23 kDa), which belongs to the Ras superfamily, has been reported to be present in tubulovesicles of rabbit parietal cells along with the H^+-K^+ pump (6). Upon stimulation of cells with a combination of histamine and forskolin, this protein redistributes from tubulovesicles to secretory canaliculi in a pattern similar to that seen for the H^+-K^+ ATPase. This suggests a critical role of the monomeric GTP-binding protein in parietal cell vesicular traffic in a cAMP-dependent manner. Rab 2, a family of monomeric GTP-binding proteins (Rab proteins) which is located in transitional ER and cis Golgi, has recently been identified in rabbit parietal cells as a tubulovesicle-membrane associated protein (176).

SNAREs are thought to mediate the docking of vesicles at the apical membrane in preparation for exocytosis. Recent reports have suggested that

SNAREs are present in parietal cells and co-distribute into H^+K^+ ATPase-rich membranes (27, 213). SNAREs might be involved in vesicle trafficking, fusion and recycling on gastric tubovesicles during histamine stimulation (27, 213). This result recalls an involvement of Ca^{2+} signaling pathways in the histamine cascade since the fusion process generally requires high concentrations of cytosolic Ca^{2+} . In contrast, it is possible to prevent fusion while permitting docking by keeping the cytosolic concentration of Ca^{2+} very low (detailed roles of monomeric GTP-binding proteins and SNAREs have been reviewed in *Molecular Biology of the Cell*, 3rd ed., Chapter 13).

The cAMP-dependent pathway may in addition involve the activation of cation and anion channels. Studies using a patch clamp technique for the apical membrane of parietal cells (rabbit) have reported the presence of the histamine-activated single-class of voltage-dependent outward Cl^- channels (152). It has also been suggested that phosphorylation of the apical membrane protein is involved in the expression of a K^+ conductance (5, 77).

Both Na^+H^+ and $Cl^-HCO_3^-$ exchange systems have been described in parietal cells (rabbit) (128). The basolateral Na^+H^+ exchanger in rabbit parietal cells is activated by histamine and forskolin, suggesting that the cAMP pathway may be involved in the regulation of intracellular pH (pH_i) for maintenance of cell homeostasis (113). The biological role of pH_i in the cAMP signal transduction is still unknown, although the mutual change in pH_i and $[Ca^{2+}]_i$ appears to be closely related in several cell types, including parietal cells (134, 184, 189).

IV. CROSSTALK BETWEEN Ca^{2+} AND cAMP IN PARIETAL CELLS

In parietal cells (canine), Ca^{2+} -mobilizing hormones and cAMP-mobilizing hormones appear to act synergistically in inducing acid secretion. When either carbachol or gastrin is combined with histamine, the secretory response is greater than the additive response to either of the two individual secretagogues (canine) (164). This synergism is not observed between carbachol and gastrin themselves (164). This behavior appears to be based on the positive interaction between $[Ca^{2+}]_i$ and cAMP. Histamine and forskolin, but not dbcAMP, increase $[Ca^{2+}]_i$ without production of IP_3 (rabbit and pig) (34, 103), suggesting that the activation of adeny-

lyl cyclase rather than cAMP may regulate the components of Ca^{2+} signalling pathway without mediation of IP_3 . However, the effects of forskolin on Ca^{2+} signalling may need reevaluation because of species difference (44). On the other hand, thapsigargin or aspirin, which increases $[Ca^{2+}]_i$ in the parietal cell, potentiates histamine-induced acid secretion (rabbit) (38, 95), suggesting that increasing $[Ca^{2+}]_i$ may stimulate the cAMP pathway in inducing acid secretion without increase in cAMP content. Since aspirin which causes Ca^{2+} influx from the extracellular space can potentiate dbcAMP-induced acid secretion, increasing $[Ca^{2+}]_i$ may act at a site beyond adenylyl cyclase (rabbit) (95). In parietal cells, it remains to be determined which of the precise intermediate steps of the Ca^{2+} signalling pathway is potentiated by cAMP pathway, and vice versa, but it is likely that these potentiation processes occur in the downstream of the cellular cascade reaction (i.e., phosphorylated proteins), because there is no direct interaction between adenylyl cyclase and $[Ca^{2+}]_i$. For example, gastrin not only stimulates aminopyrine accumulation by itself, but also potentiates the action of histamine and dbcAMP (pig) (98). Gastrin, however, does not alter cAMP levels and its potentiating action is abolished by the PKA inhibitor. Therefore, secretagogues that elevate $[Ca^{2+}]_i$, such as gastrin and carbachol, may require a certain level of cAMP to be effective in stimulating acid secretion (56, 98).

V. UNIDENTIFIED PATHWAY OF INTRACELLULAR SIGNALLING IN PARIETAL CELLS

Although there is no doubt that cAMP and Ca^{2+} are ubiquitous intracellular messengers for signal transduction of parietal cell function, the levels of $[Ca^{2+}]_i$ and cAMP do not always parallel with acid secretory levels. As shown in Fig. 6, cAMP levels induced by a low concentration of forskolin are much higher than those induced by a high concentration of histamine, whereas acid secretion level induced by forskolin is only 60% of that induced by histamine. Similarly, although thapsigargin can induce an increase in $[Ca^{2+}]_i$ of an amplitude almost similar to that induced by carbachol, thapsigargin does not induce acid secretory responses of the same magnitude as those initiated by carbachol (38). The PKC activator 12-*O*-tetradecanoyl phorbol 13-acetate (TPA) potentiates the secretory response to thapsigargin, but even this combined response does not attain the same magnitude as the

Fig. 6 cAMP level and [¹⁴C]-aminopyrine accumulation in isolated rabbit parietal cells. Cells were incubated with histamine or forskolin for 30 min at 37°C. The forskolin-induced cAMP content was a 5-fold (% increase from unstimulated cells) than that induced by histamine, whereas acid production induced by forskolin was 60% of that induced by histamine. Thus, quantity of cAMP and secretion level did not parallel. Data are mean $\pm$ SE from four separate experiments. US: unstimulated cells

maximal carbachol response (30% of the carbachol response) (38). These results suggest that some unidentified components exist in addition to cAMP and Ca²⁺ (and PKC) might be involved in induction of secretion initiated by physiological ligands. An alternative possibility is that the fluctuation of second messenger levels (i.e., Ca²⁺ oscillations) elicited by physiological ligands (but not by forskolin and thapsigargin) may be required for the maintenance of cellular responses. In addition, a further increase over a certain level of cAMP and [Ca²⁺]_i may not be required for the full cellular response.

Since in rabbit parietal cells the tyrosine kinase inhibitor genistein is capable of potentiating histamine-stimulated acid secretion toward the maximal level without alteration of cAMP levels and because the tyrosine phosphatase inhibitor, molybdate, inhibits the action of histamine, a tyrosine kinase-protein phosphotyrosine phosphatase system may be involved in the potentiation of the histamine signal by a mechanism independent of adenylyl cyclase activation (192) (Fig. 7). In other words, activation of protein tyrosine kinase (PTK) pathways (by histamine) may result in an inhibition of acid secretion induced by histamine. This may be important to avoid the cellular over-response. Thus, the histamine pathway may involve the auto-regulation mechanism to enhance (by cAMP or Ca²⁺) and regulate (by PTK) acid

Fig. 7 Effects of the tyrosine kinase inhibitor, genistein, and the tyrosine phosphatase inhibitor, molybdate, on histamine-stimulated aminopyrine accumulation in rabbit parietal cells. A tyrosine kinase inhibitor genistein at 50 μ M potentiated [¹⁴C]aminopyrine accumulation in various doses of histamine, whereas the tyrosine phosphatase inhibitor molybdate at 25 μ M inhibited the action of histamine (panel A). Genistein (1–100 μ M) did not alter basal acid secretion (panel B), whereas genistein at 3–100 μ M potentiated histamine (3 μ M)-stimulated acid secretion in a concentration-dependent manner (panel C). In contrast, molybdate (3–100 μ M) dose-dependently inhibited the histamine (100 μ M) action (panel D). Both genistein and molybdate affect histamine-stimulated acid secretion without changing cAMP levels. Results suggest that the protein tyrosine kinase and phosphotyrosine phosphatase system may be involved in the regulation of the histamine signal by a mechanism independent of adenylyl cyclase activation. US: unstimulated cells (from reference 192, data of molybdate are mean $\pm$ SE from six separate experiments, not shown in reference 192)

secretion from parietal cells. Because the H_2 receptor is a G protein-coupled receptor (54, 55), the PTK pathway (nonreceptor PTK family) involved in the histamine cascade appears to be different from the 'receptor PTK family' which is coupled to the enzyme-linked receptor (i.e., EGF receptors) involving intrinsic PTK activities (201). A recent report has suggested that, in canine parietal cells, carbachol and EGF stimulate mitogen-activated protein (MAP) kinase activities [mitogen-activated protein kinase is activated by sequential phosphorylation of both tyrosine and threonine residues by either the dual tyrosine/threonine kinase MEK (MAP kinase kinase) alone or by MEK in conjunction with an as of yet unidentified kinases; see Molecular Biology of the Cell, Chapter 15]. Furthermore, the MAP kinase inhibitor PD98059 augments carbachol-stimulated acid secretion and reverses EGF inhibition. Therefore, carbachol may induce a cascade of events in parietal cells that results in MAP kinase activation to inhibit acid secretion for the autoregulation mechanism of acid secretion elicited by carbachol itself (175). It remains to be determined whether the MAP kinase (MAPK) pathway in response to carbachol is activated by either Ras ($Shc \rightarrow Grb2 \rightarrow SOS \rightarrow Ras \rightarrow Raf \rightarrow MEK \rightarrow MAPK$) or PKC ($PKC \rightarrow Raf \rightarrow MEK \rightarrow MAPK$).

It is well recognized that in the resting state the H^+K^+ -ATPase resides within tubulovesicles, which upon activation move to and fuse with the apical membrane (or its permanent extension, the secretory canaliculus), thereby bringing the H^+K^+ -ATPase in contact with the required K^+ channels to begin H^+ secretion (50, 51, 80). This membrane-recycling hypothesis may need re-investigation, re-evaluation or modification. Using cryopreparative techniques (fast freeze-fixation/freeze substitution), the new structure of the parietal cell secretory membrane has recently been reported (130). Discrete cytoplasmic tubulovesicles are not observed. Rather, in the resting state the subapical cytoplasm contains densely packed helical coils of tubule (each having an axial core of the cytoplasm, 120–150 nm in diameter), which contain the H^+K^+ ATPase and which, even in the resting state, appear to be in contact with the secretory canaliculus. Therefore, helical tubules may be permanent extensions of the secretory canaliculus. When parietal cells are stimulated to secrete H^+ , the helical coils distend, unwind, and are converted to the complex reticulum typical of the secretory canaliculus. They also suggest that tubulovesicles

are an artifact of chemical fixation utilizing conventional aldehyde primary fixation and osmium post-fixation. Thus an alternative model (130) would no longer invoke fusion events, although (as in the recycling model) it would retain cytoskeletal involvement in the expansion and contraction of the tubular membrane network. However, further careful studies are required in this area since tubulovesicles are the conventional organelle in parietal cells, in which several lines of evidence for their existence have been accumulated over the last two decades.

Although glycine-extended gastrin (G-Gly) has essentially no acute effect on Ca^{2+} mobilization and acid secretion, it shows enhancement of aminopyrine uptake by isolated canine parietal cells after a 2-day preincubation. Similar to gastrin, G-Gly increases H^+K^+ ATPase alpha-subunit gene expression and luciferase activity (transcriptional activity of the H^+K^+ ATPase alpha-subunit gene promotion). These effects induced by G-Gly are not inhibited by the gastrin/CCK_B receptor antagonist L365,260, but abolished by the tyrosine kinase inhibitor genistein. Specific binding of G-Gly to parietal cells is not displaced by gastrin. Therefore, G-Gly seems to show the functional role in chronic acid secretion via enhancement of the H^+K^+ ATPase gene expression. This pathway may be mediated by the tyrosine kinase cascade through the unidentified gastrin-like receptor, but not the gastrin/CCK_B receptor (84). This has further been confirmed by *in vitro* studies that show G-Gly potentiates gastrin-stimulated acid secretion in rats (75). However, intravenous administration of G-Gly alone for 2 h does not stimulate acid secretion. Interestingly, incompletely processed forms of another gastrointestinal hormone, secretin, have also been shown to be biologically active. Secretin-Gly and secretin-Gly-Lys-Arg can exert a potent stimulatory effect on the exocrine pancreas in the rat (167, 168). These results along with the G-Gly data indicate that amidation of the C terminal of the peptides may not always be essential for biological activity, but in some cases, the presence or lack of amidation determines the specific target. However, further careful studies will be required in these areas since the G-Gly receptor has not yet been identified and cloned throughout all cell types and because biologically active peptides generally possess the amidation form on their carboxy terminus (i.e., gastrin: Phe-NH₂ vs G-Gly: Phe-Gly-OH) if there are no basic amino acids (Lys, Arg and His) on the carboxy termini.

VI. REGULATORY PATHWAY OF THE PARIETAL CELL FUNCTION

VI-A. Growth Factor

It is well recognized that epidermal growth factor (EGF) inhibits histamine-stimulated acid secretion *in vivo* (48, 63). Indeed EGF has been reported to inhibit acid secretion from gastric glands and isolated parietal cells of many species; rabbit, rat, and dog (45, 71, 96). EGF acts directly on parietal cells to regulate acid secretion and EGF receptors have been identified on guinea-pig gastric glands (49), isolated canine parietal cells (30) and the basolateral membrane of human gastric parietal cells (111). Furthermore, EGF may inhibit cytoskeletal-related changes in the surface of the rat parietal cells (61). The exact mechanism by which EGF inhibits histamine-stimulated acid secretion remains to be determined. A major effect of EGF to inhibit acid secretion appears to be the inhibition of the production of cAMP (71, 96), although there exist a number of other possibilities. Since the inhibition of EGF on histamine-stimulated cAMP production is reversed by the PDE inhibitor (IBMX), EGF may decrease the cAMP content by increasing cAMP-dependent PDE activity (71). Similarly, since EGF inhibition of histamine-stimulated cAMP content and H^+ accumulation are reversed by PTX, the EGF pathway might be mediated by the activation of G_i which results in an inhibition of the adenylyl cyclase activity (96). Furthermore since EGF inhibition is reversed by the protein tyrosine kinase inhibitor (genistein), the EGF pathway may be mediated by a protein tyrosine kinase (PTK) (191). Indeed, in several cell types activation of the EGF receptor (enzyme-linked receptor) involves PTK and results in a tyrosine phosphorylation of the substrates of PTK, as occurs in a phosphorylation of PLC gamma, GTPase-activating protein (GAP) or phosphatidylinositol 3-kinase (PI 3-kinase), all of which possess the Src homology domains (201). These results obtained in parietal cells suggest that crosstalk between the PTK and adenylyl cyclase system is bi-directional. This is consistent with data that show inhibition of the tyrosine kinase activity by genistein results in a potentiation of histamine-stimulated acid secretion (192) (see Fig. 7). A similar bi-directional mechanism between PTK and adenylyl cyclase systems has been reported in several cells; murine Leydig tumor cell line, hepatocytes, kidney cells, platelets, and A431 cells (18, 19, 65, 81). By contrast, in several cell

types the nature of the mutual relationship between the PTK and adenylyl cyclase is uni-directional; A431 cells, luteal cells, ovarian granulosa cells. C3H10T_{2/1} cells, Swiss 3T3 cells and A31 fibroblasts (4, 24, 25, 86, 125, 139, 151). Although it is not clear why such different crosstalk predominates in different cells, the concept is emerging that certain effects of growth factor/PTK may require an interaction with the adenylyl cyclase system. Interactions between PTX-sensitive G proteins and PTKs in the EGF pathway are unknown since the EGF receptor is an enzyme-linked receptor (single-pass transmembrane proteins), which possesses intrinsic PTK activities, but it is not linked to G proteins. Of interest, a peptide corresponding to the cytosolic juxtamembrane domain of the EGF receptor (EGF-R) has recently been shown to anti-thetically regulate the G_s and G_i proteins in another cell type (173). Taken together, results suggest the existence of following possible pathways in parietal cells; EGF-R/PTK $\rightarrow G_i$ to inhibit cAMP signal transductions.

Transforming growth factor alpha (TGF_{alpha}) shares a 30–35% structural similarity with EGF and binds to the EGF receptor (104, 105). In human parietal cell enriched fraction, TGF_{alpha} mRNA and TGF_{alpha} protein have been qualified, suggesting that TGF_{alpha} may be produced by parietal cells and may serve as an autocrine factor regulating parietal cell function (autocrine signalling) (8). In various species, it too is capable of inhibiting histamine-induced acid secretion *in vivo* (143) and *in vitro* (60, 97, 192, 206) by a mechanism which is reversed by PTX and genistein (97, 192). This suggests a mechanism similar to that by which EGF inhibits acid secretion. The mechanism by which EGF and TGF_{alpha} inhibit carbachol-stimulated acid secretion (Ca^{2+} signalling pathway) is unknown (97, 192). Since TGF_{alpha} inhibition of the carbachol response is, however, reversed by genistein, the TGF_{alpha} inhibitory pathway on Ca^{2+} signal transduction may also be mediated by PTK activation (192) (Fig. 8). A recent report suggests that in cultured canine parietal cells, EGF inhibits carbachol-stimulated aminopyrine uptake in a PTX-insensitive but genistein-sensitive manner (207). Since EGF does not inhibit IP₃ formation and Ca^{2+} spiking induced by carbachol and because EGF increase translocation of alpha and beta isoforms of PKC, the inhibitory action of EGF on carbachol-stimulated parietal cell activity seems to involve PKC. Furthermore, the PKC, but not PKA, inhibitors reverse the inhibitory action of EGF

Fig. 8 Effects of TGF_α and genistein on carbachol (CCh)-stimulated aminopyrine accumulation in rabbit parietal cells. Cells were pretreated with 50 μM genistein and 100 nM TGF_α followed by a 30 min incubation with varying concentrations of CCh. TGF_α inhibited CCh-stimulated aminopyrine accumulation and this inhibition was reversed by genistein. **P* < 0.05 compared with CCh alone at each concentration. US: unstimulated cells (from reference 192)

(207). In addition, it has been reported that EGF stimulates MAP kinase activities (175). Results suggest a positive crosstalk between PKC and tyrosine kinase pathways to inhibit parietal cell acid secretion (i.e., EGF-R/PTK → PLC_γ → DG → PKC → MAP kinases pathways to inhibit Ca²⁺ signal transductions). A controversial thing of this pathway is that EGF is incapable of increasing [IP₃] (207). At the present time, there is no evidence that the EGF-R is alternatively coupled to phospholipase D or phospholipase A₂ pathways, other enhancer molecules of PKC activation.

All of these inhibitory effects by EGF and TGF_α *in vitro* have been observed in acutely isolated parietal cells, except reference 207. It is of interest that in cultured rabbit parietal cells EGF and TGF_α, in turn, enhance basal and histamine-stimulated acid secretion, in a PTX-insensitive manner (37). Since several tyrosine kinase inhibitors suppress this chronic effect and reduce phosphorylation of a 44–45 KDa protein (maybe the MAP kinase family), a soluble tyrosine kinase may be involved in the mediation of the chronic effect of growth factors to elicit acid secretion (37). Thus, protein tyrosine kinase pathways coupled to the growth factor receptor may change the signal transduction system in cultured parietal cells.

VI-B. Somatostatin and Eicosanoids

In the stomach, somatostatin is present in D cells (108). *In vivo*, somatostatin is capable of inhibiting acid secretion in a number of species, via paracrine signalling (108). Somatostatin receptors have been identified on rat parietal cells (142) and this may be the somatostatin I-V receptor subtypes (363–428 amino acids/7 TM) (209). Somatostatin has been noted to inhibit cAMP levels, adenylyl cyclase activity, and histamine-stimulated aminopyrine accumulation in isolated parietal cells and glands from a number of species (57, 129, 155). Since somatostatin inhibition of histamine-stimulated aminopyrine accumulation is reversed by pertussis toxin, it is likely that the somatostatin receptor (SS-R) is coupled with G_i in the parietal cell, at least in rat and dog, resulting in inactivation of histamine-stimulated adenylyl cyclase systems (3, 129). It has also been suggested that the anti-secretory effect of somatostatin is due to the activation of protein phosphatases (141). Therefore, there are two possibilities on somatostatin inhibitory pathways in parietal cells: SS-R → activation of G_i → inhibition of adenylyl cyclase; SS-R → activation of G_i → activation of protein phosphatases. In contrast, somatostatin has no effect on parietal cell membrane phospholipid turnover (Ca²⁺ signalling pathway) or PKC levels (129), thus being different from growth factors which are coupled with PTKs.

Eicosanoids (arachidonic acid metabolites), such as prostaglandin E series, prostacyclin, and leukotriene C₄, inhibit the histamine-induced aminopyrine accumulation and cAMP content in isolated parietal cells and glands from a number of species, via paracrine signalling (3, 87, 94, 148, 155, 162). There is no effect of prostaglandin E₂ on carbachol- and gastrin-induced aminopyrine accumulation in dog parietal cells, suggesting an inhibitory effect of these eicosanoids on only the cAMP pathway. Since pretreatment of parietal cells with PTX results in an attenuation of the inhibitory effect of prostaglandin E₂ (PGE₂), it is possible that similar to somatostatin the PGE₂ receptors are coupled with G_i (3, 31, 155). This area needs further studies since in general the EP₃ receptor is the only prostanoid receptor which decreases cAMP, whereas PGE₂ and leukotriene C₄ increase cAMP and IP₃/DG, respectively: PGE₂ is a cAMP-elevating autacoid through activation of G_s in several cells (209). These regulatory pathways are summarized in Table 2.

Table 2 *Inhibitory Factors of Parietal Cell Acid Secretion*

Original cells	Regulators	G protein	Mediators
Parietal and other gastric cells ^a	EGF, TGF _{alpha}	G _i ^b none	AC(-) PKC/PTK(+)
D cells	Somatostatin	G _i	AC(-)
Mucous neck cells	Prostaglandin E ₂	G _i	AC(-)

Abbreviations: G_i, inhibitory G protein; AC, adenylyl cyclase; PKC, protein kinase C; PTK, protein tyrosine kinase. (+) activation and (-) inhibition. ^aUnidentified and ^bno direct coupling

V-C. Protein Kinase C

PKC can be activated by DG and phosphatidylserine in the presence of Ca²⁺ (122). Membrane-bound and activated PKC might thus be less dependent on Ca²⁺ and would contribute to the maintenance of a sustained cell response. In several cell type, the Ca²⁺/CaM pathway and DG/PKC pathway act synergistically in inducing cellular responses (140). Furthermore, the tumor promoting phorbol ester, TPA can substitute for DG, leading to an activation of PKC (122). TPA is thus a useful tool to investigate the role of PKC.

PKC has been identified in parietal cells of a number of species (2, 9). In canine parietal cells, TPA, gastrin and carbachol increase the PKC activity (129). TPA stimulation results in a small aminopyrine accumulation in rabbit parietal cells (23). In addition, in combination with the Ca²⁺-mobilizing agent (thapsigargin), TPA act synergistically in inducing acid secretion (38). Therefore, in addition to the Ca²⁺/CaM pathway, the DG/PKC pathway may be involved in gastrinergic and muscarinic-cholinergic pathways. Activated PKC may, however, function as a negative feedback regulator during receptor activation. Thus, TPA has been reported to inhibit aminopyrine accumulation induced by the Ca²⁺-mobilizing agent carbachol in rat parietal cells (1) and in rabbit gastric glands (23, 114).

In several cell types, it remains to be determined which of the intermediate steps of the Ca²⁺ signalling pathway are regulated by PKC (186). The possible phosphorylation steps by PKC that could be so regulated include agonist-receptor binding, G-protein phosphorylation, IP₃ formation or degradation, IP₃-operated Ca²⁺ channels, the Ca²⁺ pump mechanism or a combination of these. Since TPA reduces the content of inositol trisphosphate produced by carbachol stimulation in rat parietal cells

(137), PKC may act at the level of IP₃ formation or its upstream. It is equally probable that PKC negative feedback regulation is due to receptor phosphorylation, resulting in a decrease of the receptor affinity for its cognate ligand. Although this possibility has not yet been ascertained in parietal cell gastrin/CCK_B receptor, it is known that the CCK_A receptor has three consequent sequence sites for PKC phosphorylation in the third intracellular loop (88, 208). In addition, it is equally probable that PKC negative feedback regulation might involve protein tyrosine kinase pathways (i.e., PKC → MAP kinase pathways) (see VI-A).

TPA also inhibits the cAMP pathway (histamine-stimulated aminopyrine accumulation) (1, 2, 23). Since TPA inhibits dbcAMP-induced aminopyrine accumulation with a similar protency to that for inhibition of histamine response in rat and guinea-pig parietal cells (9, 72), an inhibitory site for the action of PKC would appear to be post-cAMP formation or A-kinase. It is equally probable that PKC phosphorylates the H₂ receptor (54, 55). Thus PKC acts as a weak stimulant to induce acid secretion and as a negative feedback regulator on both Ca²⁺- and cAMP-mobilizing secretagogues. It shows diverse effects on the process of parietal cell secretory system and could be crucial to avoid the cellular over-response.

VII. CONCLUDING REMARKS

The signal transduction machinery of the parietal cell secretory system is a complex one and illustrates the numerous points of interaction between the known transduction events of secretory systems. However, it also points out significant gaps in knowledge and the necessity to define areas of the secretory mechanism which is at present ill understood. A complicating feature is the presence of a secretory mechanism for the macro-molecular

protein intrinsic factor which as yet has not been separately defined from proton secretion. Nevertheless, the major membrane movement which the parietal cell undergoes during secretion and the significant proton secretory events render this cell an exciting model for the exploration of secretory signal transduction machinery.

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It has recently been reported that, in alpha-toxin permeabilized rabbit gastric glands, a non-hydrolyzable guanosine nucleotide analogue, GTP[S] (guanosine 5'-3-thiotrisphosphate), inhibits acid secretion induced by secretagogues (histamine and carbachol). This inhibition occurs at a postreceptor site since acid secretion evoked by forskolin or 8Br-cAMP, which bypasses the receptor, is also inhibited by GTP[S]. GTP[S] does not inhibit the gastric ATPase directly nor does induce a H⁺ leak in the secretory membrane. Accordingly, GTP[S] may act within the signal transduction cascade at a terminal step. Given the known action of GTP[S] to bind to G proteins, GTP hydrolysis occurring on monomeric and small GTP binding proteins may be required for the terminal step of acid secretion [MILLER M. D. and HERSEY S. J. (1996) Differential effects of GTPs on acid and pepsinogen secretion by permeable gastric glands. *Amer. J. Physiol.* 270, G962–G968]. Note that GTP[S] generally stimulates the cell response since GTP[S] bound to the heterotrimeric G proteins cannot be hydrolyzed to GDP. An adenylyl cyclase or phospholipase C activated by GTP[S] thus remains in the active state indefinitely. Indeed, the same report indicates that GTP[S], in turn, stimulates pepsinogen secretion from chief cells.

REFERENCES

- ANDERSON N. G. and HANSON P. J. (1984) Inhibition of gastric acid secretion by a phorbol ester: effect of 12-*o*-tetradecanoylphorbol 13-acetate on aminopyrine accumulation by rat parietal cells. *Biochem. Biophys. Res. Commun.* **121**, 566–572
- ANDERSON N. G. and HANSON P. J. (1985) Involvement of calcium-sensitive phospholipid-dependent protein kinase in control of acid secretion by isolated rat parietal cells. *Biochem. J.* **232**, 609–611
- ATWELL M. M. and HANSON P. J. (1988) Effect of pertussis toxin on the inhibition of secretory activity by prostaglandin E₂, somatostatin, epidermal growth factor and 12-*o*-tetradecanoylphorbol 13-acetate in parietal cells from rat stomach. *Biochim. Biophys. Acta* **971**, 282–288
- BALL R. L., TANNER K. D. and CARPENTER G. (1990) Epidermal growth factor potentiates cyclic AMP accumulation in A-431 cells. *J. Biol. Chem.* **265**, 12836–12845
- BANDOPADHYAY S., DAS P. K., WRIGHT V. W., NANDI J., BHATTACHARYAY D. and RAY T. K. (1987) Characteristics of a pure endogenous activator of the gastric H⁺, K⁺-ATPase system: evaluation of the role as a possible intracellular regulator. *J. Biol. Chem.* **262**, 5664–5670
- BASSON M. D., GOLDENRING J. R., TANG L. H., LEWIS J. J., PADFIELD P., JAMIESON J. D. and MODLIN I. M. (1991) Redistribution of 23 KDa tubulovesicle-associated GTP-binding proteins during parietal cell stimulation. *Biochem. J.* **279**, 43–48
- BATZRI S. and DYER J. (1981) Aminopyrine uptake by guinea-pig gastric mucosal cells: mediation by cyclic AMP and interaction among secretagogues. *Biochim. Biophys. Acta* **675**, 416–426
- BEANCHAMP R. D., BARNARD J. A., MCCUTCHEN C. M., CHERNER J. A. and COFFEY R. JR. (1988) Localization of transforming growth factor alpha and its receptor in gastric mucosal cells. Implications for a regulatory role in acid secretion and mucosal renewal. *J. Clin. Invest.* **84**, 1017–1023
- BEIL W., MANNSCHEDEL W. and SEWING K.-FR. (1987) Protein kinase C and parietal cell function. *Biochem. Biophys. Res. Commun.* **149**, 720–728
- BERGLINDH T., HELANDER H. F. and OBRINK K. J. (1976) Effects of secretagogues on oxygen consumption, aminopyrine accumulation and morphology in isolated gastric glands. *Acta Physiol. Scand.* **97**, 401–414
- BERGLINDH T., SACHS G. and TAKEGUCHI N. (1980) Ca²⁺-dependent secretion in isolated rabbit gastric glands. *Amer. J. Physiol.* **239**, G90–G94
- BERRIDGE M. J. and GALLIONE A. (1988) Cytosolic calcium oscillators. *FASEB J.* **2**, 3074–3082
- BERRIDGE M. J. and IRVINE R. F. (1984) Inositol trisphosphate, a novel second messenger in cellular signal transduction. *Nature* **312**, 315–321
- BIRNBAUMER L., ABRAMOWITZ J. and BROWN A. M. (1990) Receptor-effector coupling by G proteins. *Biochim. Biophys. Acta* **1031**, 163–224
- BLACK J. A., FORTE T. M. and FORTE J. G. (1982) The effects of microfilament disrupting agents on HCl secretion and ultrastructure of piglet oxyntic cells. *Gastroenterology* **83**, 595–604
- BLACKSHARE P. J., NARIAN A. C. and KUO J. F. (1988) Protein kinases 1988: a current perspective. *FASEB J.* **2**, 2957–2969
- BLANDIZZI C., SONG I. and YAMADA T. (1994) Molecular cloning and structural analysis of the rabbit gastrin/CCK_B receptor gene. *Biochem. Biophys. Res. Commun.* **202**, 947–953
- BOSCH F., BOUSCAREL B., SLATON J., BLACKMORE P. F. and EXTON J. H. (1986) Epidermal growth factor mimics insulin effects in rat hepatocytes. *Biochem. J.* **239**, 523–530
- BOYAJIAN C. L., GARRITSEN A. and COOPER D. M. F. (1991) Bradykinin stimulates Ca²⁺ mobilization in NCB-20 cells leading to direct inhibition of adenylyl cyclase. A novel mechanism for inhibition of cAMP production. *J. Biol. Chem.* **266**, 4995–5003
- BRESCHER A. (1983) Purification of an 80,000-dalton protein that is a component of the isolated microvillus cytoskeleton, and its localization in non-muscle cells. *J. Cell*

- Biol.* **97**, 425–432
21. BRESCHER A. (1989) Rapid phosphorylation and reorganization of ezrin and spectrin accompany morphological changes induced in A-431 cells by epidermal growth factor. *J. Cell Biol.* **108**, 921–930
 22. BROWN A. M. and BIRNBAUMER L. (1988) Direct G protein gating of ion channels. *Amer. J. Physiol.* **254**, H401–H410
 23. BROWN M. R. and CHEW C. S. (1987) Multiple effects of phorbol ester on stimulated gastric glands and parietal cells. *Can. J. Physiol. Pharmacol.* **65**, 1840–1847
 24. BUDNIK L. T. and MUKHOPADHYAY A. K. (1991) Epidermal growth factor, a modulator of luteal adenylate cyclase. Characterization of epidermal growth factor receptors and its interaction with adenylate cyclase system in bovine luteal cell membrane. *J. Biol. Chem.* **266**, 13908–13913
 25. BUSHMAN W. A., WILSON L. K., LUTTRELL D. K., MOYERS J. S. and PARSONS S. J. (1990) Overexpression of *c-src* enhances β -adrenergic-induced cAMP accumulation. *Proc. Natl. Acad. Sci. USA* **87**, 7462–7466
 26. CABERO J. L., GRAPENGIESSER E., GLYTE E., LI Z.-Q. and MARDH S. (1992) Effects of gastrin on cytosolic free Ca^{2+} in individual, acid-secreting rat parietal cells. *Biochem. Biophys. Res. Commun.* **183**, 1097–1102
 27. CALHOUN B. C. and GOLENRING J. R. (1996) Immunolocalization of tubulovesicles: identification of SCAMPs and SNAREs. *Gastroenterology* **110**, A73
 28. CAMPBELL V. W. and YAMADA T. (1989) Acid secretagogue-induced stimulation of gastric parietal cell gene expression. *J. Biol. Chem.* **264**, 11381–11386
 29. CHICKCROFT S. and THOMAS G. M. H. (1992) Inositol-lipid-specific phospholipase C isoenzymes and their differential regulation by receptors. *Biochem. J.* **288**, 1–14
 30. CHEN M. C., DEBORAH A., AMIRIAN A. and SOLL A. H. (1984) Epidermal growth factor (EGF) binding and inhibitory effect on acid secretion in isolated canine parietal cells. *Federation Proceedings* **43**, 1073
 31. CHEN M. C. Y., AMIRIAN D. A., TOOMEY M., SANDERS M. J. and SOLL A. H. (1988) Prostanoid inhibition of canine parietal cells: mechanism by the inhibitory guanosine triphosphate-binding protein of adenylate cyclase. *Gastroenterology* **94**, 1121–1129
 32. CHEW C. S. (1985) Parietal cell protein kinases. Selective activation of type I cAMP-dependent protein kinase by histamine. *J. Biol. Chem.* **260**, 7540–7550
 33. CHEW C. S. (1986) Cholecystokinin, carbachol, histamine and forskolin increase $[\text{Ca}^{2+}]_i$ in gastric glands. *Amer. J. Physiol.* **250**, G814–G823
 34. CHEW C. S. and BROWN M. R. (1986) Release of intracellular Ca^{2+} and elevation of inositol trisphosphate by secretagogues in parietal and chief cells isolated from rabbit gastric mucosa. *Biochim. Biophys. Acta* **888**, 116–125
 35. CHEW C. S. and HERSEY S. J. (1982) Gastrin stimulation of isolated gastric glands. *Amer. J. Physiol.* **242**, G504–G512
 36. CHEW C. S., NAKAMURA K. and LJUNGSTROM M. (1992) Calcium signaling mechanisms in the gastric parietal cell. *Yale J. Biol. Med.* **65**, 561–526
 37. CHEW C. S., NAKAMURA K. and PETROPOULOS A. C. (1994) Multiple actions of epidermal growth factor and TGF_α on rabbit gastric parietal cell function. *Amer. J. Physiol.* **267**, G818–G826
 38. CHEW C. S. and PETROPOULOS A. C. (1991) Thapsigargin potentiates histamine-stimulated HCl secretion in gastric parietal cells but does not mimic cholinergic responses. *Cell Regulation* **2**, 27–39
 39. CHIBA T., FISHER S. K., PARK J., SEGUIN E. B., AGRANOFF B. W. and YAMADA T. (1988) Carbamylcholine and gastrin induce inositol lipid turnover in canine gastric parietal cells. *Amer. J. Physiol.* **255**, G99–G105
 40. COIBRAN R. J., SCHWORER C. M., HASHIMOTO Y., FONG Y.-L., RICH D. P., SMITH J. K. and SODERLING T. R. (1989) Ca^{2+} /calmodulin-dependent protein kinase II. *Biochem. J.* **258**, 313–325
 41. CROTHERS J. M., CHOW D. C., SCALLEY M. L. and FORTE J. G. (1995) *In vivo* trafficking of nascent H^+/K^+ ATPase in rabbit parietal cells. *Amer. J. Physiol.* **269**, G883–G891
 42. CUPPOLETTI J. and SACHS G. (1984) Regulation of gastric acid secretion via modulation of a chloride conductance. *J. Biol. Chem.* **259**, 14952–14959
 43. DELVALLE J., TSUNODA Y., WILLIAMS J. A. and YAMADA T. (1992) Regulation of $[\text{Ca}^{2+}]_i$ by secretagogue stimulation of canine gastric parietal cells. *Amer. J. Physiol.* **262**, G420–G426
 44. DELVALLE J., WANG L., GANTS I. and YAMADA T. (1992) Characterization of H_2 histamine receptor: linkage to both adenylate cyclase and $[\text{Ca}^{2+}]_i$ signaling system. *Amer. J. Physiol.* **263**, G967–G972
 45. DEMBINSKI A., DROZDOWICZ D., GREGORY H., KONTUREK S. J. and WARZECHA Z. (1984) Inhibition of acid formation by growth factor in the isolated rabbit gastric glands. *J. Physiol.* **378**, 347–357
 46. DOUGLAS W. W. (1968) Stimulus-secretion coupling: the concept and clues from chromaffin and other cells. *Brit. J. Pharmacol.* **34**, 451–474
 47. ECKNAVER R., THOMPSON W. J., JOHNSON L. R. and ROSENFELD G. C. (1980) Isolated parietal cell: $[\text{H}^3]\text{QNB}$ binding to putative cholinergic receptors. *Amer. J. Physiol.* **239**, G204–G209
 48. ELDER J. B., GANGULI P. C., GILLESPIE I. E., GERRING E. L. and GREGORY H. (1982) Effects of urogastone on gastric secretion and plasma levels in normal subjects. *Gut* **16**, 887–893
 49. FORGUE-LAFITTE M.-E., KOBARI L., GESPACH G., CHAMBLIER M.-C. and ROSSELIN G. (1984) Characterization and repartition of epidermal growth factor-urogastrone receptors in gastric glands from young and adult guinea pigs. *Biochim. Biophys. Acta* **798**, 192–198
 50. FORTE J. G., BLACK J. A., FORTE T. M., MACHEN T. E. and WOLOSIN J. M. (1981) Ultrastructural changes related to functional activity in gastric oxyntic cells. *Amer. J. Physiol.* **241**, G349–G358
 51. FORTE J. G. and WOLOSIN J. M. (1987) HCl secretion by the gastric oxyntic cell. In *Physiology of the Gastrointestinal Tract* (2nd ed.) (ed. JOHNSON L. R.) Raven Press, New York, pp. 853–863
 52. FUNASAKA M., TSUNODA Y., SOROKA C. J., FOX L. M., MODLIN I. M. and GOLDENRING J. R. (1991) Identification of gastric Ca^{2+} /calmodulin dependent protein kinase II (CaMK II). *Gastroenterology* **100**, A541
 53. FUNAYAMA N., NAGAFUCHI A., SATO N., TSUKITA S. and TSUKITA S. (1991) Radixin is a novel member of the band 4.1 family. *J. Cell Biol.* **115**, 1039–1048
 54. GANTZ I., DELVALLE J., WANG L., TASHIRO T., MUNZERT G., GUO Y.-J., KONDA Y. and YAMADA T. (1992) Molecular basis for the interaction of histamine with the histamine H_2 receptor. *Proc. Natl. Acad. Sci. USA* **267**, 20840–20843
 55. GANTZ I., MUNZERT G., TASHIRO T., SCHAFFER M., WANG

- L., DELVALLE J. and YAMADA T. (1991) Molecular cloning of the human histamine H₂ receptor. *Biochem. Biophys. Res. Commun.* **178**, 1386–1392
56. GEIBEL J., ABRAHAM R., MODLIN I. and SACHS G. (1995) Gastrin-stimulated changes in Ca²⁺ concentration in parietal cells depends on adenosine 3',4'-cyclic monophosphate levels. *Gastroenterology* **109**, 1060–1067
57. GESPACH C., BATAILLE D., DUTRILLAUX M.-C. and ROSSELIN G. (1982) The interaction of glucagon, gastric inhibitory peptide and somatostatin with cyclic AMP production systems present in rat gastric glands. *Biochim. Biophys. Acta* **720**, 7–16
58. GILMAN G. (1987) G proteins: transducers of receptor-generated signals. *Annu. Rev. Biochem.* **56**, 615–649
59. GOLDENRING J. A., ASHER V. A., BARREUTHER M. F., LEWIS J. J., LOHMANN S. M., WALTER U. and MODLIN I. M. (1992) Dephosphorylation of cAMP-dependent protein kinase regulatory subunit in stimulated parietal cells. *Amer. J. Physiol.* **262**, G763–G773
60. GOLDENRING J. A., TSUNODA Y., STOCH S. A., COFFEY R. J. and MODLIN I. M. (1993) Transforming growth factor- α inhibition of parietal cell secretion: structural requirements for activity. *Regul. Pept.* **43**, 37–47
61. GONZALEZ A., GARRIDO J. and VIAL J. D. (1981) Epidermal growth factor inhibits cytoskeleton-related changes in the surface of parietal cells. *J. Cell Biol.* **88**, 108–114
62. GOULD K. L., COOPER J. A., BRESCHER A. and HUNTER T. (1986) The protein-tyrosine kinase substrate, p81, is homologous to a chicken microvillar core protein. *J. Cell Biol.* **102**, 660–669
63. GREGORY H. G., THOMAS C. E., YOUNG J. A., WILLSHIRE I. R. and GARNER A. (1988) The combination of the C-terminal undecapeptide sequence of urogastrone-epidermal growth factor to its biological action. *Regul. Pept.* **22**, 217–226
64. GRYNKIEWICZ G., POENIE M. and TSIEN R. Y. (1985) A new generation of Ca²⁺ indicators with greatly improved fluorescence properties. *J. Biol. Chem.* **260**, 3440–3450
65. HAFEZ M. M. and ASCOLI M. (1990) Epidermal growth factor desensitizes the gonadotropin-responsive adenylate cyclase in membranes isolated from MA-10 Leydig tumor cells and luteinized rat ovaries. *Endocrinology* **127**, 394–402
66. HALLAM T. J., JACOB R. and MERRITT J. E. (1988) Evidence that agonists stimulate bivalent-cation influx into human endothelial cells. *Biochem. J.* **255**, 179–184
67. HALLAM T. J., JACOB R. and MERRITT J. E. (1989) Influx of bivalent cations can be independent of receptor stimulation in human endothelial cells. *Biochem. J.* **259**, 125–129
68. HANSON P. J. and HATT J. F. (1989) Intracellular signaling and regulation of gastric acid secretion. *Quart. J. Exp. Physiol.* **74**, 607–634
69. HANZEL D., REGGIO H., BRETSCHER A., FORTE J. G. and MANGEAT P. (1991) The secretion-stimulated 80K phosphoprotein of parietal cells is ezrin, and has the properties of a membrane cytoskeletal linker in the induced apical microvilli. *EMBO J.* **10**, 2363–2373
70. HANZEL D. H., URISHIDANI T., USINGER W. R., SMOLKA A. and FORTE J. G. (1989) Immunological localization of an 80 KDa phosphoprotein to the apical membrane of gastric parietal cells. *Amer. J. Physiol.* **256**, G1082–G1089
71. HATT J. F. and HANSON P. J. (1988) Inhibition of gastric acid secretion by epidermal growth factor. Effects of cyclic AMP and prostaglandin production in rat isolated parietal cells. *Biochem. J.* **255**, 789–794
72. HATT J. F. and HANSON P. J. (1989) Sites of action of protein kinase C on secretory activity in rat parietal cells. *Amer. J. Physiol.* **256**, G129–G138
73. HERSEY S. J. (1974) Interactions between oxidative metabolism and acid secretion in gastric mucosa. *Biochim. Biophys. Acta* **344**, 157–203
74. HERSEY S. J. and SACHS G. (1995) Gastric acid secretion. *Physiol. Rev.* **75**, 155–189
75. HIGASHIDE S., GOMEZ G., GREELEY G. M., TOWNSEND C. and THOMPSON J. C. (1996) *Amer. J. Physiol.* **270**, G220–G224
76. HOFER J. A. and MACHEN T. E. (1993) Technique for *in situ* measurement of calcium in intracellular inositol 1,4,5-trisphosphate-sensitive stores using the fluorescent indicator mag-fura-2. *Proc. Natl. Acad. Sci. USA* **90**, 2598–2602
77. IM W. B., BLAKEMAN D. P., BLEASDALE J. E. and DAVIS J. P. (1987) A protein phosphatase associated with rat heavy gastric membranes enriched with (H⁺-K⁺)-ATPase influences membrane transport activity. *J. Biol. Chem.* **262**, 9865–9871
78. IRVINE R. F. and MOOR R. M. (1986) Microinjection of inositol 1,3,4,5-tetrakisphosphate activates sea urchin eggs by a mechanism dependent on extracellular Ca²⁺. *Biochem. J.* **24**, 917–920
79. ITO S., MUNRO D. R., SCHOFIELD G. C. and PHIL D. (1977) Morphology of the isolated mouse oxyntic cell and some physiological parameters. *Gastroenterology* **73**, 887–898
80. ITO S. and SCHOFIELD G. C. (1974) Studies on the depletion and accumulation of microvilli and changes in the tubulovesicular compartment of mouse parietal cell in relation to gastric acid secretion. *J. Cell Biol.* **63**, 364–382
81. IWASHITA S., MATSUI K., SHOJI-KASAI Y. and SENSU-MIYAKE M. (1990) cAMP-mediated modulation of signal transduction of epidermal growth factor (EGF) receptor systems in human epidermoid carcinoma A431 cells. Depression of EGF-dependent diacylglycerol production and EGF receptor phosphorylation. *J. Biol. Chem.* **265**, 10702–10708
82. JACKSON R. J. and SACHS G. (1982) Identification of gastric cyclic AMP binding proteins. *Biochim. Biophys. Acta* **717**, 453–458
83. JACOBSON E. D. and THOMPSON W. J. (1976) Cyclic AMP and gastric secretion: the illusive second messenger. *Adv. Cyclic Nucleotide Res.* **7**, 199–224
84. KAISE M., MURAOKA A., SEVA C., TAKEDA T., DICKINSON C. and YAMADA T. (1995) Glycine-extended progastrin processing intermediates induce H⁺,K⁺-ATPase gene expression through a novel receptor. *J. Biol. Chem.* **270**, 11155–11160
85. KASBERKER D. K. and GORDON G. S. (1979) Effects of colchicine and vinblastine on *in vitro* gastric secretion. *Amer. J. Physiol.* **236**, E550–E555
86. KNECHT M. and CATT K. J. (1983) Modulation of cAMP-mediated differentiation in ovarian granulosa cells by epidermal growth factor and platelet-derived growth factor. *J. Biol. Chem.* **258**, 2789–2794
87. KONTUREK S. J., BILSKI J., DEMBINSKI A., WARZECHA A., BECK J. and JENDRALLA H. (1987) Effects of leukotrienes on gastric acid and alkaline secretions. *Gastroenterology* **92**, 1209–1214
88. KOPIN A. S., LEE Y.-M., MCBRIDE E. W., MILLER L. J., LU M., LIN H. Y., KOLAKOWSKI L. F. and BEINBORN M. (1992) Expression, cloning and characterization of the canine pari-

- etal cell receptor. *Proc. Natl. Acad. Sci. USA* **89**, 3605–3609
89. KOTERA T., HASHIMOTO A., UEDA S. and OKADA Y. (1991) Whole-cell K^+ current activation in response to voltages and carbachol in gastric parietal cells isolated from guinea pig. *J. Membrane Biol.* **124**, 43–52
 90. KRAUS-FRIEDMANN N. (1994) Signal transduction and calcium: a suggested role for the cytoskeleton in inositol 1,4,5-trisphosphate action. *Cell Motil. Cytoskeleton* **28**, 279–284
 91. KUNO M. and GARDNER P. (1987) Ion channels activated by inositol 1,4,5-trisphosphate in plasma membrane of human T-lymphocytes. *Nature* **326**, 301–304
 92. KWAN C. Y., TAKEMURA H., OBIE J. F., THASTRUP O. and PUTNEY J. W. (1990) Effects of methacholine, thapsigargin and La^{3+} on plasmalemmal and intracellular Ca^{2+} transport in lacrimal acinar cells. *Amer. J. Physiol.* **258**, C1006–C1015
 93. LEONARD A., CUQ P., MAGOUS R. and BALI J-P. (1991) M_3 -subtype muscarinic receptor that controls intracellular calcium and inositol phosphate accumulation in gastric parietal cells. *Biochem. Pharmacol.* **42**, 839–845
 94. LEVINE R. A., KOHEN K. R., SCHWARTZEL E. H. and RAMSAY C. E. (1982) Prostaglandin E_2 -histamine in interactions on cAMP, cGMP, and acid production in isolated fundic glands. *Amer. J. Physiol.* **242**, G21–G26
 95. LEVINE R. A., NANDI J. and KING R. L. (1990) Aspirin potentiates prestimulated acid secretion and mobilizes intracellular calcium in rabbit parietal cells. *J. Clin. Invest.* **86**, 400–408
 96. LEWIS J. J., GOLDENRING J. R., ASHER V. A. and MODLIN I. M. (1990) Effects of epidermal growth factor on signal transduction in rabbit parietal cells. *Amer. J. Physiol.* **258**, G476–G483
 97. LEWIS J. J., GOLDENRING J. R., MODLIN I. M. and COFFEY R. J. (1990) Inhibition of parietal cell H^+ secretion by transforming growth factor alpha: a possible autocrine regulatory mechanism. *Surgery* **119**, 220–227
 98. LI Z-Q., CABERO J. L. and MARDH S. (1995) Gastrin and carbachol require cAMP to elicit aminopyrine accumulation in isolated pig and rat parietal cells. *Amer. J. Physiol.* **268**, G82–G89
 99. LJUNGSTROM M. and CHEW C. S. (1991) Calcium oscillations and morphological transformations in single cultured gastric parietal cells. *Amer. J. Physiol.* **260**, C67–C78
 100. MALINOWSKA D. H., CUPPOLETTI J. and SACHS G. (1983) Cl^- requirement of acid secretion in isolated glands. *Amer. J. Physiol.* **245**, G573–G581
 101. MALINOWSKA D. H., SACHS G. and CUPPOLETTI G. (1988) Gastric H^+ secretion: histamine (cAMP-mediated) activation of protein phosphorylation. *Biochim. Biophys. Acta* **972**, 95–109
 102. MANGEAT P., MARCHIS-MOUREN G., CHERET A. M. and LEWIN M. J. M. (1980) Specific activity of cyclic AMP-dependent protein kinases by H_2 -histamine antagonists in isolated gastric mucosal cells from guinea pig. *Biochim. Biophys. Acta* **629**, 604–608
 103. MARDH S., SONG Y. H., CARLSSON C. and BJORKMAN J. (1987) Mechanisms of stimulation of acid secretion in parietal cells isolated from the guinea pig gastric mucosa. *Acta Physiol. Scand.* **131**, 589–598
 104. MARQUARDT H., HUNKAPILLER M. W., HOOD L. E. and TODARO G. J. (1984) Transforming growth factor type I: structure and relation to epidermal growth factor. *Science* **223**, 1079–1082
 105. MASSAGUE J. (1983) Epidermal growth factor-like transforming growth factor. II. Interaction with epidermal growth factor receptors in placenta membranes and A431 cells. *J. Biol. Chem.* **258**, 13614–13620
 106. MATSUMOTO M., PARK J. and YAMADA T. (1987) Gastrin receptor characterization: affinity cross-linking of the gastrin receptor in canine gastric parietal cells. *Amer. J. Physiol.* **252**, G143–G147
 107. MAYER P., MOHLIG M., SEIDLER U., RUCHLITZ H., FAHRMANN M., SCHATZ H., HIDAKA H. and PFEIFFER A. (1994) Characterization of gamma- and delta-subunits of Ca^{2+} /calmodulin-dependent protein kinase II in rat gastric mucosal cell populations. *Biochem. J.* **297**, 157–162
 108. MCINTOSH C. H. S. (1985) Gastrointestinal somatostatin: distribution, secretion and physiological significance. *Life Sci.* **37**, 2043–2058
 109. MERCIER F., REGGIO H., DEVILLIERS G., BATAILLE D. and MANGEAT P. (1989) Membrane-cytoskeleton dynamics in rat parietal cells: mobilization of actin and spectrin upon stimulation of gastric acid secretion. *J. Cell Biol.* **108**, 441–453
 110. MILLER S. G. and KENNEDY M. B. (1986) Regulation of brain type II Ca^{2+} /calmodulin dependent protein kinase by autophosphorylation: a Ca^{2+} -triggered molecular switch. *Cell* **44**, 861–870
 111. MORI S., MORISHITA Y., SASAKI K., KURIMOTO S., OKAMOTO M., KAWAMOTO T. and KUROKI T. (1987) Electron microscopic evidence for epidermal growth factor receptor (EGF-R)-like immunoreactivity associated with the basolateral surface of gastric parietal cells. *Acta Pathol. Japon.* **37**, 1909–1917
 112. MORRIS A. P., GALLACHER D. J., IRVINE R. T. and PETERSON O. H. (1987) Synergism of inositol trisphosphate and tetrakisphosphate in activating Ca^{2+} -dependent K^+ channels. *Nature* **330**, 653–655
 113. MUALLEM S., BLISSARD D., CRAGOE E. J. and SACHS G. (1989) Activation of the Na^+/H^+ and Cl^-/HCO_3^- exchange by stimulation of acid secretion in the parietal cells. *J. Biol. Chem.* **263**, 14703–14711
 114. MUALLEM S., FIMMEL C. J., PANDOL S. J. and SACHS G. (1986) Regulation of free cytosolic Ca^{2+} in the peptic and parietal cells of the rabbit gastric gland. *J. Biol. Chem.* **261**, 2660–2667
 115. MUALLEM S., KHADEMAZAD M. and SACHS G. (1990) The route of Ca^{2+} entry during reloading of the intracellular Ca^{2+} pool in pancreatic acini. *J. Biol. Chem.* **265**, 2011–2016
 116. MUALLEM S. and SACHS G. (1985) Ca^{2+} metabolism during cholinergic stimulation of acid secretion. *Amer. J. Physiol.* **248**, G216–G228
 117. NEGULESCU P. A. and MACHEN T. E. (1988) Intracellular Ca^{2+} regulation during stimulus-secretion coupling in the parietal cell. *Amer. J. Physiol.* **254**, C130–C140
 118. NEGULESCU P. A. and MACHEN T. E. (1988) Release and reloading of intracellular Ca^{2+} stores after cholinergic stimulation of the parietal cell. *Amer. J. Physiol.* **254**, C498–C504
 119. NEGULESCU P. A. and MACHEN T. E. (1993) Ca^{2+} transport by plasma membrane and intracellular stores of gastric cells. *Amer. J. Physiol.* **264**, C843–C851
 120. NEGULESCU P. A. and MACHEN T. E. (1995) La^{3+} and pH sensitivity of Ca^{2+} entry and intracellular store filling in

- gastric parietal cells. *Amer. J. Physiol.* **269**, G770-G778
121. NEGULESCU P. A., REENSTRA W. W. and MACHEN T. E. (1989) Intracellular Ca^{2+} requirements for stimulus-secretion coupling in parietal cell. *Amer. J. Physiol.* **256**, C241-C251
 122. NISHIZUKA Y. (1995) The role of protein kinase C in cell surface signal transduction and tumor production. *Nature* **308**, 693-698
 123. ODDSDOTTIR M., GOLDENRING J. R., ADRIAN T. E., ZDON M. J., ZUCKER K. A. and MODLIN I. M. (1988) Identification and characterization of a cytosolic 30 KDa histamine stimulated phosphoprotein in parietal cell cytosol. *Biochem. Biophys. Res. Commun.* **154**, 489-496
 124. ODDSDOTTIR M., MODLIN I. M., ZUCKER K. A., ZDON M. J. and GOLDENRING J. R. (1987) A calmodulin-dependent protein kinases in parietal cells. *Biochem. Biophys. Res. Commun.* **148**, 1390-1397
 125. OLASHAW N. E. and PLEDGER W. J. (1988) Epidermal growth factor stimulates formation of inositol phosphates in BALBc/3T3 cells pretreated with cholera toxin and isobutyl-methyl-xanthine. *J. Biol. Chem.* **263**, 1111-1114
 126. OTSU H., YAMAMOTO A., MAEDA N., MIKOSHIBA K. and TASHIRO Y. (1990) Immunogold localization of inositol 1,4,5-trisphosphate (InsP_3) receptor in mouse cerebellar Purkinje cells using three monoclonal antibodies. *Cell Struct. Funct.* **15**, 163-173
 127. PANDOL S. J., SCHOEFFIELD M. S., FIMMEL C. J. and MUALLEM S. (1987) The agonist-sensitive calcium pool in the pancreatic acinar cell: activation of plasma membrane Ca^{2+} influx mechanism. *J. Biol. Chem.* **262**, 16963-16968
 128. PARADISO A. M., TSIEN R. Y. and MACHEN T. E. (1987) Digital image processing of intracellular pH in gastric oxyntic and chief cells. *Nature* **325**, 447-450
 129. PARK J., CHIBA T. and YAMADA T. (1987) Mechanisms for direct inhibition of canine gastric parietal cells by somatostatin. *J. Biol. Chem.* **262**, 14190-14196
 130. PETTITT J. M., HUMPHRIS D. C., BARRETT S. P., TOH B. H., VAN DRIEL I. R. and GLEESON P. A. (1995) Fast freeze-fixation/freez-substitution reveals the secretory membranes of the gastric parietal cell as a network of helically coiled tubules. A new model for parietal cell transformation. *J. Cell Sci.* **108**, 1127-1141
 131. PFEIFFER A., ROCHLITZ H., HERZ A. and PAUMGARTNER G. (1988) Stimulation of acid secretion and phosphoinositol production by rat parietal cell muscarinic M_2 receptors. *Amer. J. Physiol.* **254**, G622-G629
 132. PFEIFFER A., ROCHLITZ H., NOELKE B., TACKE R., MOSER U., MUTSCHLER E. and LAMBRECHT G. (1990) Muscarinic receptors mediating acid secretion in isolated rat gastric parietal cells are of M_3 type. *Gastroenterology* **98**, 218-222
 133. PRENTKI M., BIDEN T. J., JANJIC D., IRVINE R. F., BERRIDGE M. J. and WOLLHEIM C. B. (1984) Rapid mobilization of Ca^{2+} from rat microsomes by inositol-1,4,5-trisphosphate. *Nature* **309**, 562-564
 134. PUCEAT M., CLEMENT O., SCAMPS F. and VASSORT G. (1991) Extracellular ATP-induced acidification leads to cytosolic calcium transient rise in single rat cardiac myocytes. *Biochem. J.* **274**, 55-62
 135. PUTNEY J. W. JR. (1990) Capacitative calcium entry revisited. *Cell Calcium* **11**, 611-624
 136. PUTNEY J. W. JR. and BIRD G. ST. J. (1993) The inositol phosphate-calcium signaling system in nonexcitable cells. *Endocrine Rev.* **14**, 610-631
 137. PUURUNEN J., LOHSE M. J. and SCHWABE U. (1987) Interactions between intracellular cyclic AMP and agonist-induced inositol phospholipid breakdown in isolated gastric mucosal cells of the rat. *Naunyn-Schmied. Arch. Pharmacol.* **336**, 471-477
 138. PUURUNEN J. and SCHWABE U. (1987) Effect of gastric secretagogues on the formation of inositol phosphates in isolated gastric cells of the rat. *Brit. J. Pharmacol.* **90**, 479-490
 139. RAN W., DEAN M., LEVINE R. A., HENKLE C. and CAMPISI J. (1986) Induction of c-fos and c-myc mRNA by epidermal growth factor or calcium ionophore is cAMP dependent. *Proc. Natl. Acad. Sci. USA* **83**, 8216-8220
 140. RASMUSSEN H. and BARRETT P. Q. (1984) Calcium messenger system: an integrated view. *Physiol. Rev.* **64**, 938-984
 141. REYL F. J. and LEWIN M. J. M. (1982) Intracellular receptor for somatostatin in gastric mucosal cells; decomposition and reconstitution of somatostatin-stimulated phosphoprotein phosphatases. *Proc. Natl. Acad. Sci. USA* **79**, 978-982
 142. REYL F. J., SILVE C. and LEWIN M. J. M. (1979) Somatostatin receptors on isolated gastric glands. In *Hormone Receptors in Digestion and Nutrition* (ed. ROSSELIN G., FROMAGEOT O. and BONFILS S.) Elsevier/North-Holland Biomedical Press, Amsterdam, pp. 341-400
 143. RHODES J. A., TAM J. P., FINKE U., SAUNDERS M., BERNANKE J., SILEN W. and MURPHY A. (1986) Transforming growth factor alpha inhibits secretion of gastric acid. *Proc. Natl. Acad. Sci. USA* **83**, 3844-3846
 144. ROCHE S., BALI J.-P., GALLEYRAND J.-C. and MAGOUS R. (1991) Characterization of a gastric type receptor on rabbit gastric parietal cells using L 365, 260 and L 364, 718. *Amer. J. Physiol.* **260**, G182-G188
 145. ROCHE S., BALI J.-P. and MAGOUS P. (1993) Receptor-operated Ca^{2+} channels in gastric parietal cells: gastrin and carbachol induce Ca^{2+} influx in depleting intracellular Ca^{2+} stores. *Biochem. J.* **289**, 117-124
 146. ROCHE S., GUSDINAR A., BALI J.-P. and MAGOUS R. (1991) Biphasic kinetics of inositol 1,4,5-trisphosphate accumulation in gastrin-stimulated parietal cells. Effects of pertussis toxin and extracellular calcium. *FEBS Lett.* **282**, 147-151
 147. RODINSON G. A., BUTCHER R. W. and SUTHERLAND E. W. (1971) *Cyclic AMP*, Academic Press, New York
 148. ROSENFELD G. C. (1986) Prostaglandin E_2 inhibition of secretagogue-stimulated [^{14}C]aminopyrine accumulation in rat parietal cells: a model for its mechanism of action. *J. Pharmacol. Exp. Ther.* **237**, 513-518
 149. ROSENFELD G. C., MCALLISTER E. and THOMPSON W. J. (1981) Cytochalasin inhibition of isolated rat gastric parietal cell function. *J. Cell. Physiol.* **109**, 53-57
 150. ROSENFELD G. C., STRADA S. J., DIAL E. J., CYNTHIA D., BEARER F. and THOMPSON W. J. (1980) Histamine, cyclic nucleotides, and gastric parietal cell function. *Adv. Cyclic Nucleotides Res.* **12**, 255-266
 151. ROZENGURT A., LEGG A., STRANG G. and COURTENAY-LUCK N. (1981) Cyclic AMP: a mitogenic signal for Swiss 3T3 cells. *Proc. Natl. Acad. Sci. USA* **78**, 4392-4396
 152. SACCOMANI G., PSARRAS C. G., SMITH P. R., KIRK K. L. and SHOEMAKER R. L. (1991) Histamine-induced chloride channels in apical membrane of isolated rabbit parietal

- cells. *Amer. J. Physiol.* **260**, C1000–C1011
153. SAMBROOK J. F. (1990) The involvement of calcium in transport of secretory patterns from the endoplasmic reticulum. *Cell* **61**, 197–199
 154. SANDERS M. J., AMIRIAN D. A., AYALON A. and SOLL A. H. (1983) Regulation of pepsinogen release from canine chief cells in primary monolayer culture. *Amer. J. Physiol.* **245**, G641–G646
 155. SCHEPP W., HEIM H.-K. and RUOFF H.-J. (1983) Comparison of the effect of PGE₂ and somatostatin on histamine-stimulated [¹⁴C]aminopyrine uptake and cAMP formation in isolated rat gastric mucosal cells. *Agents Actions* **13**, 200–206
 156. SCHEPP W., SCHNEIDER J., HEIN H.-K., ROUFF H.-J., SCHUSDZIARRA V. and CLASSEN M. (1987) A calmodulin antagonist inhibits histamine-stimulated acid production by isolated rat parietal cells. *Regul. Pept.* **17**, 209–220
 157. SCHMIDTLER J., SCHEPP W., JANCZEWSKA I., WEIGERT N., FURLINGER C., SCHUSDZIARRA V. and CLASSEN M. (1991) GLP-1-(7–36)amide, -(1–37), and -(1–36)amide: potent cAMP-dependent stimuli of rat parietal cell function. *Amer. J. Physiol.* **260**, G940–G950
 158. SHOSAN-BURMATZ V., ZHANG G. H., GARRETSON L. and KRAUS-FRIDEMANN N. (1990) Distinct ryanodine- and inositol 1,4,5-trisphosphate-binding sites in hepatic microsomes. *Biochem. J.* **268**, 699–705
 159. SMERCA A. V., HEPLER J. R., BROWN K. O. and STERNWEIS P. C. (1991) Regulation of poly-phosphoinositides-specific phospholipase C activity by purified G_q. *Science* **251**, 804–807
 160. SMITH P. R., BRADFORD A. L., JOE E.-H., ANGELIDES K. J., BENOS D. J. and SACCOMANI G. (1993) Gastric parietal cell H⁺-K⁺-ATPase microsomes are associated with isoforms of ankyrin and spectrin. *Amer. J. Physiol.* **264**, C63–C70
 161. SOLL A. H. (1980) Secretagogue stimulation of [¹⁴C]aminopyrine accumulation by isolated canine parietal cells. *Amer. J. Physiol.* **238**, G366–G375
 162. SOLL A. H. (1980) Specific inhibition by prostaglandin E₂ and I₂ of histamine-stimulated [¹⁴C]aminopyrine accumulation and cyclic adenosine monophosphate generation by isolated canine parietal cells. *J. Clin. Invest.* **65**, 1222–1229
 163. SOLL A. H. (1981) Extracellular calcium and cholinergic stimulation of isolated canine parietal cells. *J. Clin. Invest.* **68**, 270–278
 164. SOLL A. H. (1982) Potentiating interactions of gastric stimulants on [¹⁴C]aminopyrine accumulation by isolated canine parietal cells. *Gastroenterology* **83**, 216–223
 165. SOLL A. H., AMIRIAN D. A., THOMAS L. P., REEDY T. J. and ELASHOFF J. D. (1984) Gastrin receptors on isolated canine parietal cells. *J. Clin. Invest.* **73**, 1434–1447
 166. SOLL A. H. and WOLLIN A. (1979) Histamine and cAMP in isolated canine parietal cells. *Amer. J. Physiol.* **237**, E444–E450
 167. SOLOMON T. E., SHIN Y. W., MCCORMACK P., BUSSJAEGER L. J., VARGA G., CAMPBELL D., HAMILTON J. W. and REEVE J. R. (1995) Bioactivity and immunoreactivity of naturally occurring C-terminally extended forms of secretion. *Gastroenterology* **108**, A391
 168. SOLOMON T. E., SHIN Y. W., MCCORMACK P., BUSSJAEGER L. J., VARGA G., CAMPBELL D. and REEVE J. R. (1995) Processing of amidation consensus sequence alters potency and specificity of secretin for pancreatic and gastric secretion. *Gastroenterology* **108**, A1007
 169. SONG I., BROWN D. R., WILTSHIRE R. N., GANTZ I., TRENT J. M. and YAMADA T. (1993) The human gastrin/cholecystokinin type B receptor gene: alternative splice donor site in exon 4 generates two variant mRNAs. *Proc. Natl. Acad. Sci. USA* **90**, 9085–9089
 170. SOROKA C. J., CHEW C. S., HANZEL D. K., SMOLKA D. K., MODLIN I. M. and GOLDENRING J. R. (1993) Characterization of membrane and cytoskeletal compartments in cultured parietal cells: immunofluorescence and confocal microscopic examination. *Eur. J. Cell Biol.* **60**, 76–87
 171. STEWART H. E. and KASBEKAR D. K. (1981) Gastric oxyntic cell tubulin: characterization and possible significance. *Amer. J. Physiol.* **240**, G317–G323
 172. STUNKEL E. D., TSUNODA Y. and WILLIAMS J. A. (1989) Secretagogue induced calcium mobilization in single pancreatic acinar cells. *Biochem. Biophys. Res. Commun.* **158**, 863–869
 173. SUN H., SEYER J. M. and PATEL T. B. (1995) A region in the cytosolic domain of the epidermal growth factor receptor antithetically regulates the stimulatory and inhibitory guanine nucleotide-binding regulatory proteins of adenylyl cyclase. *Proc. Natl. Acad. Sci. USA* **92**, 2229–2233
 174. TAKEMURA H. and PUTNEY J. W. (1989) Capacitative calcium entry in parotid acinar cells. *Biochem. J.* **258**, 409–412
 175. TAKEUCHI Y., TODISCO A., YAMADA J., POST L. and YAMADA T. (1996) Functional roles of mitogen activated protein kinases in gastric acid secretion. *Gastroenterology* **110**, A272
 176. TANG L. H., STOCH A., MODLIN I. M. and GOLDENRING J. R. (1992) Identification of rab 2 as a tubulovesicle-membrane associated protein in rabbit gastric parietal cells. *Biochem. J.* **285**, 715–719
 177. TAYLOR S. J., CHAE H. Z., RHEE S. G. and EXTON J. H. (1991) Activation of the β_1 isozyme of phospholipase C by alpha subunits of the G_q class of G proteins. *Nature* **350**, 516–518
 178. THASTRUP O. B., FORDER B. and SCHARFF O. (1987) The calcium mobilizing tumor promoting agent, thapsigargin elevates platelet cytoplasmic free calcium concentration to a higher steady state level. A possible mechanism of action for the tumor promotion. *Biochem. Biophys. Res. Commun.* **142**, 654–660
 179. TSCHANER V. V., PROD'HOM B., BAGGIOLINI M. and REUTER H. (1986) Ion channels in human neutrophils activated by a rise in free cytosolic calcium concentration. *Nature* **324**, 369–372
 180. TSIEN R. W. and TSIEN R. Y. (1990) Calcium channels, stores and oscillations. *Annu. Rev. Cell Biol.* **6**, 715–760
 181. TSUNODA Y. (1986) Gastrin induces intracellular Ca²⁺ and acid secretion regulation by the microtubular-microfilamentous system. *Biochim. Biophys. Acta* **855**, 186–188
 182. TSUNODA Y. (1986) An ATP-dependent and inositol trisphosphate-sensitive Ca²⁺ pool linked with microfilaments of the parietal cell. *FEBS Lett.* **207**, 47–52
 183. TSUNODA Y. (1987) Ca²⁺ currents and acid secretion in the isolated parietal cell involved in response to gastrin, compound 48/80 and ethylenediamine tetracetic acid. *Biochem. Cell Biol.* **65**, 144–162
 184. TSUNODA Y. (1990) Ca²⁺ spiking affected by intracellular pH change. *Exp. Cell Res.* **188**, 294–301
 185. TSUNODA Y. (1991) Oscillatory Ca²⁺ signaling and its cel-

- lular function. *New Biol.* **3**, 3–17
186. TSUNODA Y. (1993) Receptor-operated Ca^{2+} signaling and crosstalk in stimulus secretion coupling. *Biochim. Biophys. Acta* **1154**, 105–156
 187. TSUNODA Y., FUNASAKA M., MODLIN I. M., HIDAKA H., FOX L. M. and GOLDENRING J. R. (1992) An inhibitor of Ca^{2+} /calmodulin-dependent kinase II inhibitor, KN-62, inhibits cholinergic stimulated parietal cell secretion. *Amer. J. Physiol.* **262**, G118–G122
 188. TSUNODA Y. and MATSUMIYA H. (1987) Calcium-activated membrane depolarization via a modulation of chloride efflux from parietal cells during gastrin stimulation. *FEBS Lett.* **222**, 149–153
 189. TSUNODA Y., MATSUNO K. and TASHIRO Y. (1991) Cytosolic acidification leads to Ca^{2+} mobilization from intracellular stores in single and populational parietal cells and platelets. *Exp. Cell Res.* **193**, 356–363
 190. TSUNODA Y. and MIZUNO T. (1985) Participation of the microtubular-microfilamentous system on intracellular Ca^{2+} transport and acid secretion in dispersed parietal cells. *Biochim. Biophys. Acta* **820**, 189–198
 191. TSUNODA Y., MODLIN I. M. and GOLDENRING J. R. (1991) Tyrosine kinase activity is required for EGF or TGF- α inhibition of parietal cell secretion. *Gastroenterology* **100**, A541
 192. TSUNODA Y., MODLIN I. M. and GOLDENRING J. R. (1993) Tyrosine kinase activities in the modulation of stimulated parietal cell secretion. *Amer. J. Physiol.* **264**, G351–G356
 193. TSUNODA Y., STUENKEL E. L. and WILLIAMS J. A. (1990) Oscillatory mode of calcium signaling in rat pancreatic acinar cells. *Amer. J. Physiol.* **258**, C147–C155
 194. TSUNODA Y., STUENKEL E. L. and WILLIAMS J. A. (1990) Characterization of sustained $[\text{Ca}^{2+}]_i$ increase in pancreatic acinar cells and its relation to amylase secretion. *Amer. J. Physiol.* **259**, G792–G801
 195. TSUNODA Y., TAKEDA H., ASAKA M., NAKAGAKI I. and SASAKI S. (1988) Initial and sustained calcium mobilizations in the parietal cell during stimulations with gastrin, inositol trisphosphate, phorbol ester and exogenous diacylglycerol. *FEBS Lett.* **232**, 83–90
 196. TSUNODA Y., TAKEDA H., OTAKI T., ASAKA M., NAKAGAKI I. and SASAKI S. (1988) Intracellular Ca^{2+} shift and signal transduction from the tubulovesicular portion of gastric parietal cells during gastrin stimulation or Ca^{2+} ionophore treatment: comparison between luminescent and fluorescent probes, and electron probe X-ray microanalyzer. *Biochem. Cell Biol.* **66**, 279–287
 197. TSUNODA Y., WILLIAMS J. A. and DELVALLE J. (1991) Secretagogue induced Ca^{2+} oscillations in isolated canine gastric chief cells. *Biochim. Biophys. Acta* **1091**, 251–254
 198. TSUNODA Y., YODOZAWA S. and TASHIRO Y. (1988) Fluorescence digital image analysis of the inositol trisphosphate-mediated calcium transient in single permeabilized parietal cells. *FEBS Lett.* **231**, 29–35
 199. TSUNODA Y., YODOZAWA S. and TASHIRO Y. (1989) Heterogeneous distribution of free calcium and propagation of calcium transient in gastric parietal cells revealed by digital imaging microscopy. *J. Histochem. Cytochem.* **37**, 999–1005
 200. TSUNODA Y., YOSHIDA H. and OWYANG C. (1996) Structural requirements of CCK analogues to differentiate second messengers and pancreatic secretion. *Amer. J. Physiol.* **271**, G8–G19
 201. ULLRICH A. and SCHLESSINGER J. (1990) Signal transduction by receptors with tyrosine kinase activity. *Cell* **61**, 203–212
 202. URUSHIDANI T., HANZEL D. K. and FORTE J. G. (1989) Characterization of an 80-KDa phosphoprotein involved in parietal cell stimulation. *Amer. J. Physiol.* **256**, G1070–G1081
 203. URUSHIDANI T. and NAGAO T. (1996) Calculin A, a phosphoprotein phosphatase inhibitor, stimulates acid secretion in isolated gastric glands. *Amer. J. Physiol.* **270**, G103–G112
 204. VIAL J. D. and GARRIDO J. (1976) Actin-like filaments and membrane arrangement in oxyntic cells. *Proc. Natl. Acad. Sci. USA* **73**, 4032–4036
 205. WALKER W., VINIK A., HELDSINGER A. and KAVEH R. (1983) Role of calcium and calmodulin in activation of the oxyntic cell by histamine and carbamylcholine in the guinea pig. *J. Clin. Invest.* **72**, 955–964
 206. WANG L., LUCEY M. R., FRAS A. M., WILSON E. J. and DELVALLE J. (1993) Epidermal growth factor and transforming growth factor- α directly inhibit parietal cell function through a similar mechanism. *J. Pharmacol. Exp. Ther.* **265**, 308–313
 207. WANG L., WILSON E. J., OSBURN J. and DELVALLE J. (1996) Epidermal growth factor inhibits carbachol-stimulated canine parietal cell function via protein kinase C. *Gastroenterology* **110**, 469–477
 208. WANK S. A. (1995) Cholecystokinin receptors. *Amer. J. Physiol.* **269**, G628–G646
 209. WATSON S. and GIRDLESTONE D. (1996) 1996 Receptor and ion channel nomenclature supplement. *Trends Pharmacol. Sci.* 7th ed., 1–81
 210. WILKES J. M., SCOTT D. R., HERSEY S. J. and SACHS G. (1991) Muscarinic receptors of gastric parietal cells. *J. Membrane Biol.* **122**, 97–110
 211. WOODS N. M., CUTHBERTSON K. S. R. and COBBOLD P. H. (1986) Repetitive transient rises in cytoplasmic free calcium in hormone-stimulated hepatocytes. *Nature* **319**, 600–602
 212. YANG Q. and TONKS N. K. (1991) Isolation of a cDNA clone encoding a human protein-tyrosine phosphatase with homology to the cytoskeletal-associated proteins band 4.1, ezrin and talin. *Proc. Natl. Acad. Sci. USA* **88**, 5949–5953
 213. YAO X., PENG X., CHENG L. C., BANNET M. K. and FORTE J. G. (1996) SNAREs are involved in the secretory activation of parietal cells. *Gastroenterology* **110**, A302
 214. YAO X., THIBODEAV A. and FORTE J. E. (1993) Ezrin-calpain I interactions in gastric parietal cells. *Amer. J. Physiol.* **265**, C36–C46