Guanine Nucleotides Activate Multiple Signaling Pathways in Permeabilized Gastric Chief Cells

EVIDENCE FOR GTP\(\gamma\)S-INDUCED CALCIUM-INDEPENDENT PEPSSINOGEN SECRETION

(Received for publication, October 27, 1992)

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Nonhydrolyzable guanine nucleotide analogues were used to evaluate the role of guanine nucleotide binding (G) proteins in regulating pepsinogen secretion from streptolysin O-permeabilized chief cells from guinea pig stomach. In the presence of 100 nM calcium, 100 \(\mu\)M guanosine 5'-\((\beta,\gamma\text{-imido})\)triphosphate or guanosine 5'-3-O-\((\text{thio})\)triphosphate (GTP\(\gamma\)S) caused a 2- to 4-fold increase in pepsinogen secretion. GTP\(\gamma\)S-stimulated secretion in the absence of calcium (up to 10 mM EGTA). With or without added calcium, GTP analogues caused a 2- to 3-fold increase in cAMP, whereas guanosine 5'-O-2-(\(\text{thio})\)diphosphate and calcium alone had no effect on cAMP levels. GTP analogue-induced activation of phospholipase C was evidenced by a calcium-independent increase in cytidine diphospho-1,2-diacylglycerol levels (50% above basal). Phorbol ester and GTP\(\gamma\)S-stimulated phosphorylation of a 72-kDa acidic protein was abolished by an inhibitor of protein kinase C (CGP 41251). However, GTP\(\gamma\)S-induced pepsinogen secretion was only partially inhibited by adding CGP 41251 or a protein kinase C inhibitor peptide. These results indicate that guanine nucleotides activate major signaling pathways in gastric chief cells. Nevertheless, GTP\(\gamma\)S can induce pepsinogen secretion independently of changes in calcium, cAMP, or activation of protein kinase C.

At least two major cellular mechanisms are involved in agonist-induced pepsinogen secretion from dispersed chief cells from guinea pig stomach (for review see Ref. 1). Interaction of agents like secretin, vasoactive intestinal peptide, prostaglandins, or cholera toxin with cell membrane receptors results in activation of adenylylcyclase and the production of cAMP (1–3). Interaction of agents like cholecystokinin, carbachol, and gastrin with cell membrane receptors results in activation of phospholipase C and the production of inositol trisphosphate and diacylglycerol (1, 4–6). Inositol trisphosphate increases intracellular calcium concentration, whereas diacylglycerol activates protein kinase C (PKC)\(^1\) (5, 6). Increases in cellular levels of cAMP and calcium result in activation of protein kinases and/or phosphatases that, by currently unknown mechanisms, mediate the final steps that result in pepsinogen secretion (1). The possibility that, as in other cells, guanine nucleotide binding (G) proteins are part of these signaling cascades has been suggested by studies indicating that cholera toxin (3) and sodium fluoride (6) activate adenylylcyclase and phospholipase C, respectively, and stimulate pepsinogen secretion.

The introduction of nonhydrolyzable guanine nucleotide analogues into permeabilized secretory cells, thereby modulating the activity of G proteins, has been used by several investigators to determine the role of G proteins in exocytosis (for review see Ref. 7). It has been demonstrated that guanine nucleotides are able to activate known effector proteins such as adenylylcyclase and phospholipase C (8, 9) and, in some cases, stimulate calcium-independent secretion (8, 10). Moreover, the use of guanine nucleotides in this fashion resulted in the observation that secretion from some cells (e.g. mast cells) is triggered by calcium plus guanine nucleotides, and cannot be attributed to known cellular mechanisms. It has been proposed, therefore, that another G protein, designated \(G_x\), is involved in mediating some forms of exocytosis (11). At present, the existence of this G protein remains speculative.

In the present study, we used streptolysin O (SLO)-permeabilized chief cells from guinea pig stomach to characterize the actions of guanine nucleotides on cellular signaling mechanisms and pepsinogen secretion. The permeability of the cell membrane in this model is evidenced by the egress of more than 60% of cellular lactate dehydrogenase activity, whereas the cellular content of pepsinogen, maintained within zymogen granules, is not altered (12). Nevertheless, SLO-permeabilized chief cells retain several important functional characteristics, including the ability to secrete pepsinogen when stimulated with cellular mediators, like calcium or cAMP, and secretagogues, like carbachol and phorbol esters (12).

**EXPERIMENTAL PROCEDURES**

**Materials**—Male Hartley guinea pigs (150–200 g) were obtained from CAMM Research Lab Animals (Wayne, NJ); collagenase (type I), bovine serum albumin (fraction V), EGTA, phorbol 12-myristate 13-acetate (PMA), cAMP, ATP, and 3-isobutyl 1-methylxanthine (IBMX) from Sigma; guanine nucleotide analogues from Calbiochem, SLO, basal medium (Eagle's) amino acids, and essential vitamin solution from Gibco; Percoll from Pharmacia LKB Biotechnology.

\(^1\) The abbreviations used are: PKC, protein kinase C; Gpp(NH)p, 5'-(\(\beta,\gamma\text{-imido})\)triphosphate; GTP\(\gamma\)S, guanosine 5'-3-O-\((\text{thio})\)triphosphate; GDP\(\beta\)S, guanosine 5'-2-O-\((\text{thio})\)diphosphate; SLO, streptolysin O; PMA, phorbol 12-myristate 13-acetate; IBMX, 3-isobutyl 1-methylxanthine; CDP-DAG, cytidine 5'-diphospho-1,2-diacylglycerol.
Inc.; protein kinase C inhibitor peptide (PKC(19-31)) from Upstate Biotechnology, Inc. (Lake Placid, NY); Iso-Lytes and 129-albumin from ICN; and [32P]ATP from Du Pont-New England Nuclear. CGP 41251 was kindly provided by Ciba-Geigy (Basel, Switzerland).

**Incubation Solution**—For the preparation of chief cells, standard incubation solution contained 24.5 mM HEPES (pH 7.4), 98 mM NaCl, 6 mM KCl, 2.5 mM KH2PO4, 1 mM MgCl2, 11.5 mM glucose, 5 mM sodium fumarate, 5 mM sodium pyruvate, 5 mM sodium glutamate, 1.5 mM CaCl2, 2 mM glutamine, 0.1% (w/v) albumin, 1% (v/v) amino acid mixture, and 1% (v/v) essential vitamin mixture. The standard incubation solution was equilibrated with 100% O2, and all incubations were performed with 100% O2 as the gas phase.

**Tissue Preparation and Permeabilization**—Dispersed chief cells from guinea pig stomach (>90% chief cells) were described previously (13) and were kept in standard incubation solution containing Ca2+ and Mg2+ for 10 min before proceeding. The cells were then washed three times and resuspended in permeabilization solution which consisted of the following: 120 mM KCl, 30 mM NaCl, 1 mM MgCl2, 1 mM KH2PO4, 10 mM FIPES, 1 mM ATP, and 1 mg/ml bovine serum albumin (pH 7.0). Free calcium concentrations were adjusted using an EGTA:CaCl2 buffering system (14). SLO (30 IU/ml) was adjusted using an EGTACaC12 buffering system (14). SLO (30 IU/ml) and agonists were added simultaneously to the cell suspensions.

**Pepsinogen Secretion**—Peptic activity was determined as described previously (4) using 129-albumin as substrate. Pepsinogen secretion was expressed as the percentage of total cellular pepsinogen at the start of the incubation that was released into the medium during the incubation.

**Cellular cAMP**—Chief cell cAMP was determined by radioimmunoassay using the procedure described previously (2). In these experiments, 100 μM IBMX was included in the incubation medium.

**Cellular Cytidine-5-diphospho-1,2-diacylglycerol (CDP-DAG)**—CDP-DAG in chief cells was determined according to the method of Godfrey (15). Briefly, dispersed chief cells were labeled with [3H]cytidine for 1 h. The cells were washed with permeabilization buffer without SLO and incubated in the presence of 10 mM LiCl for an additional 10 min. The cells were then permeabilized in the presence of various additions for 10 min after which the lipids were extracted and 3H content was determined by liquid scintillation counting.

**Phosphorylation of Endogenous Proteins in Permeabilized Chief Cells**—To examine the effects of guanine nucleotides on chief cell phosphorylation, cells were permeabilized in the presence of 10 μM [32P]ATP (500 mCi/ml) and 100 nM calcium in the presence or absence of 100 μM GTPyS or 100 μM PMA. Following a 3-min incubation at 37 °C, the cell suspension was placed on ice and adjusted to 2% Nonidet P-40, 9.5 M urea, 5% 2-mercaptoethanol, and 5% Iso-Lytes (consisting of 0.8% PEG, 3.5% urea, 5% 2-mercaptoethanol, and 5% Iso-Lytes) by a pH 5.8-6.0 containing buffer (16). Gels were fixed, dried and exposed for 3-10 days to Kodak X-Omat film. The pH gradient of the isoelectric focusing gels was determined by placing segments of the tube gels in 2 ml of water for 3-4 h with constant agitation. In some cases, proteins were visualized by silver staining and molecular weights of chief cell phosphoproteins were determined by comparison to positions of standards. Protein phosphorylation was quantified using an LKB densitometer. Phosphoproteins that were not altered by agonists were used as reference standards for proteins that clearly underwent agonist-dependent changes in phosphorylation.

**Statistical Analysis**—Significance between two means was determined by Student's t test. Differences between several means were determined by analysis of variance, followed by Dunnett's test. Values of p < 0.05 were considered significant.

**RESULTS**

**Effect of Guanine Nucleotides on Pepsinogen Secretion from Permeabilized Chief Cells**—In addition to allowing the passage of guanine nucleotides into cells, permeabilization also offered the advantage of maintaining intracellular calcium concentration ([Ca2+]i) at a set value using an EGTA:Ca2+ buffering system (14). Basal chief cell [Ca2+]i is approximately 100 nM (4, 6). Therefore, the ability of guanine nucleotides to induce pepsinogen secretion from permeabilized chief cells was first examined by permeabilizing the cells in the presence of 100 nM calcium, 1 mM ATP, and increasing concentrations of the nucleotide to be tested. Pepsinogen secretion with concentrations of GTPyS ≥ 3 μM and Gpp(NH)p ≥ 100 μM was greater than control (p < 0.05) (Fig. 1A). Maximal concentrations of GTPyS and Gpp(NH)p caused a 4- and 2-fold increase in pepsinogen secretion, respectively (Fig. 1A). In contrast, GDPβS did not alter basal secretion at any concentration tested.

Previously, we demonstrated that adjusting [Ca2+]i ≥ 300 nM stimulates pepsinogen secretion from permeabilized chief cells (12). Fig. 1B, shows the effect of different [Ca2+]i on guanine nucleotide-induced pepsinogen secretion. GTPyS (100 μM) caused a 4-fold increase in pepsinogen secretion in the absence of calcium (no added Ca2+ plus 0.4 mM EGTA) or in the presence of 100 mM Ca2+. Pepsinogen secretion was increased by GTPyS and Gpp(NH)p over the entire range of [Ca2+]i tested. With [Ca2+]i ≥ 300 nM, Gpp(NH)p was as efficacious as GTPyS in enhancing calcium-induced secretion. The addition of GDPβS (100 μM) alone did not alter pepsinogen secretion at any [Ca2+]i tested.

![Fig. 1. Effect of guanine nucleotide analogues on pepsinogen secretion from permeabilized chief cells. A, dose response. Dispersed chief cells were permeabilized in the presence of 100 μM calcium and increasing concentrations of GTPyS ( ), Gpp(NH)p ( ), GDPβS ( ) at 37 °C for 10 min, B, [Ca2+]i dependence. Dispersed chief cells were permeabilized in the presence of guanine nucleotides (100 μM) and increasing calcium concentrations at 37 °C for 10 min. Values for pepsinogen secretion are expressed as the percent of the total cellular pepsinogen present at the start of the incubation that was released into the medium after 10 min. Each experiment, values were determined in duplicate and represent means ± S.E. from at least three separate experiments.](image-url)
To characterize further the specificity of guanine nucleotide-induced pepsinogen secretion, we examined the effect of increasing concentrations of GDPβS on GTPγS-induced secretion. As shown in Fig. 2, whereas up to 1 mM GDPβS did not alter basal pepsinogen secretion, concentrations ≥300 μM caused progressive inhibition of GTPγS-induced secretion. Maximal inhibition of GTPγS-induced pepsinogen secretion (72%) was observed with 1 mM GDPβS.

To determine whether GTPγS-induced pepsinogen secretion was truly calcium-independent, cells were permeabilized in the presence of increasing concentrations of EGTA, with or without adding 100 μM GTPγS. As shown in Fig. 3, neither basal nor GTPγS-induced pepsinogen secretion was altered by adding up to 10 mM EGTA. Hence, we conclude that calcium is not required for GTPγS to stimulate pepsinogen secretion.

To examine the ATP requirements of GTPγS-induced secretion, chief cells were permeabilized in the presence or absence of 1 mM ATP. As shown in Table I, addition of 1 mM ATP caused a consistent (30-50%) increase in pepsinogen secretion. However, this increase was statistically significant only in the presence of 1 μM calcium and 100 μM GTPγS.

**Effect of Guanine Nucleotides on Cellular cAMP**—In an initial attempt to understand the cellular mechanisms that mediate guanine nucleotide-induced secretion, we examined the effects of guanine nucleotides on cellular cAMP. Chief cells were permeabilized in the presence of a phosphodiesterase inhibitor, IBMX (100 μM), and increasing concentrations of guanine nucleotides. As shown in Fig. 4, up to 3 μM calcium alone did not alter chief cell cAMP. GTPγS caused a 2-3-fold increase in cAMP in the absence (0.4 mM EGTA) or presence (100 nM) of calcium. This increase gradually declined with increasing calcium concentrations. In the presence of 100 nM calcium, Gpp(NH)p also increased chief cell cAMP (2-fold), whereas GDPβS did not (data not shown).

**Effect of Guanine Nucleotides on Cellular CDP-DAG**—To examine the effects of guanine nucleotides on phosphoinositide metabolism, we measured changes in cellular CDP-DAG, a metabolite of inositol phospholipid hydrolysis. In the presence of 0.4 mM EGTA or 100 nM calcium, GTPγS induced a 1.4- and 1.5-fold increase in CDP-DAG, respectively (Table II). The small increase in CDP-DAG with 1 μM calcium alone was not statistically significant. However, in the presence of 1 μM calcium, GTPγS induced nearly a 1.6-fold increase in CDP-DAG. These data indicate that, as in the case of GTPγS-induced activation of adenylate cyclase, GTPγS-induced activation of phospholipase C is calcium-independent.

**Effect of PKC Inhibitors on PMA- and GTPγS-induced Pepsinogen Secretion**—In intact chief cells, the addition of 1 μM CGP 41251, a specific inhibitor of PKC (17), inhibits carbachol- and cholecystokinin-induced pepsinogen secretion by 20–33% (p < 0.05).2 The effects of this inhibitor on PMA- and GTPγS-induced secretion are shown in Table III. In the absence or presence of calcium, PMA caused a 2-fold increase in pepsinogen secretion. R. D. Raffaniello and J.-P. Raufman, unpublished observation.
FIG. 4. Effect of GTPγS and calcium on cellular cAMP levels in permeabilized chief cells. Chief cells were permeabilized in the presence or absence of GTPγS (100 μM), IBMX (100 μM), and increasing calcium concentrations for 10 min. Incubations were terminated by the addition of cold ethanol and cellular cAMP was determined as described under "Methods." In each experiment, values were determined in duplicate and represent the means ± S.E. from at least four separate experiments.

**TABLE II**

**Effect of GTPγS on CDP-DAG production in permeabilized chief cells**

Dispersed gastric chief cells were incubated with [3H]cytidine for 1 h at 37 °C and washed with permeabilization buffer. The cells were preincubated for 10 min with 10 mM LiCl and then permeabilized with or without GTPγS (100 μM) for an additional 10 min in the presence of the indicated calcium concentration. Lipids were extracted and [3H] content was determined. Values represent means ± S.E. from four separate experiments.

<table>
<thead>
<tr>
<th>pCa</th>
<th>CDP-DAG</th>
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<tr>
<td></td>
<td>Alone</td>
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<tr>
<td>0</td>
<td>100 ± 12</td>
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<tr>
<td>7</td>
<td>108 ± 2</td>
</tr>
<tr>
<td>6</td>
<td>130 ± 4</td>
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*Significantly greater than basal value (p < 0.05).

In pepsinogen secretion from permeabilized chief cells (data not shown (12)). Addition of CGP 41251 reduced PMA-induced secretion, in the presence and absence of calcium, by 83 (p < 0.01) and 75% (p < 0.01), respectively. In contrast, in the presence of 0.4 mM EGTA (no added calcium) and 100 nM calcium, GTPγS-induced pepsinogen secretion, was inhibited by only 13 (not significant) and 31% (p < 0.02), respectively. Similar results were obtained when a PKC inhibitor peptide, PKC(19-31), was added under conditions identical to those shown in Table III (data not shown). In the absence of calcium (0.4 mM EGTA), GTPS-induced pepsinogen secretion was not altered by adding a Ca2+-dependent protein kinase inhibitor peptide, PKI(6-22) amide (7.5 ± 2.3 versus 7.6 ± 4.5%, without and with the inhibitor, respectively). Similar results were observed in the presence of calcium (100 nM) (8.6 ± 0.6 vs. 8.8 ± 0.4%). These data suggest that in the absence of calcium, GTPγS-induced pepsinogen secretion is partially mediated by PKC and not by Ca2+-dependent protein kinases.

**TABLE III**

**Effect of a PKC inhibitor, CGP 41251, on GTPγS- and PMA-induced pepsinogen secretion**

Dispersed chief cells were preincubated with CGP 41251 (1 μM) or dimethyl sulfoxide:H2O (1:1) for 15 min. The cells were then permeabilized in the presence of GTPγS (100 μM) or PMA (100 nM) at the indicated calcium concentration. Results are expressed as pepsinogen secretion observed in the presence of the inhibitor (experimental) divided by that observed in the absence of the inhibitor (control). Values represent means ± S.E. from four separate experiments.

<table>
<thead>
<tr>
<th>pCa</th>
<th>Additions</th>
<th>Pepsinogen secretion</th>
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<tbody>
<tr>
<td></td>
<td></td>
<td>CGP 41251 (1 μM)</td>
</tr>
<tr>
<td>0</td>
<td>PMA</td>
<td>25.4 ± 9.8*</td>
</tr>
<tr>
<td>7</td>
<td>PMA</td>
<td>16.7 ± 6.7*</td>
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<tr>
<td>0</td>
<td>GTPγS</td>
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</tr>
<tr>
<td>7</td>
<td>GTPγS</td>
<td>69.2 ± 3.7*</td>
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*Indicate values that are significantly less (p < 0.01 and 0.02, respectively) than those in the absence of the inhibitor.
signaling pathways that are currently thought to mediate chief cell responses. Guanine nucleotides can activate these pathways (7), and these results in permeabilized chief cells are not particularly surprising.

In the present study, we have demonstrated that calcium-independent GTPγS-induced activation of phospholipase C in permeabilized chief cells is evidenced by diacylglycerol production. Moreover, GTPγS-induced phosphorylation of the apparent PKC substrate, pp72, indicates that GTPγS-induced increases in diacylglycerol result in activation of PKC. Nevertheless, other experiments suggest that the role of PKC in GTPγS-induced secretion is relatively minor. First, whereas PMA and GTPγS cause an approximately 2-fold increase in phosphorylation of pp72, maximal concentrations of PMA cause a relatively small secretory response in permeabilized chief cells (2-fold) when compared to the response observed with GTPγS (> 4-fold) under identical conditions. Second, although the PKC inhibitor CGP 41251 abolishes GTPγS-induced phosphorylation of pp72, it causes only a partial inhibition of GTPγS-induced secretion. These data indicate that the majority of GTPγS-induced pepsinogen secretion is not mediated by calcium, cAMP, or activation of PKC. This suggests that, in gastric chief cells, GTPγS plays a role in another signal transduction mechanism that remains to be determined. Nonetheless, in permeabilized chief cells, G proteins are components of these signal transduction pathways (7).

Another important observation in the current study is that, although calcium enhances the GTPγS-induced secretory response, the nucleotide analogue can stimulate pepsinogen secretion in the absence of this cation. In these experiments, the absence of calcium was assured by adding as much as 10 mM EGTA. The ability of GTPγS to cause a calcium-independent process (12). Furthermore, the addition of a CAMP-dependent protein kinase inhibitor peptide does not alter GTPγS-induced pepsinogen secretion. These observations indicate that the calcium-independent component of GTPγS-induced secretion is not mediated by activation of adenylcyclase and a consequent increase in cAMP.

In terms of the phospholipase C signaling cascade, it has been reported that activation of certain PKC isozymes may be calcium-independent (24). Hence, we examined the possibility that such a PKC isozyme mediates calcium-independent GTPγS-induced pepsinogen secretion. Calcium-independent GTPγS-induced activation of phospholipase C in permeabilized chief cells is evidenced by diacylglycerol production. Moreover, GTPγS-induced phosphorylation of the apparent PKC substrate, pp72, indicates that GTPγS-induced increases in diacylglycerol result in activation of PKC. Nevertheless, other experiments suggest that the role of PKC in GTPγS-induced secretion is relatively minor. First, whereas PMA and GTPγS cause an approximately 2-fold increase in phosphorylation of pp72, maximal concentrations of PMA cause a relatively small secretory response in permeabilized chief cells (2-fold) when compared to the response observed with GTPγS (> 4-fold) under identical conditions. Second, although the PKC inhibitor CGP 41251 abolishes GTPγS-induced phosphorylation of pp72, it causes only a partial inhibition of GTPγS-induced secretion. These data indicate that the majority of GTPγS-induced pepsinogen secretion is not mediated by calcium, cAMP, or activation of PKC. This suggests that, in gastric chief cells, GTPγS plays a role in another signal transduction mechanism that remains to be determined. Nonetheless, in permeabilized chief cells, G proteins are components of these signal transduction pathways (7).

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**DISCUSSION**

The results of the present study indicate that in gastric chief cells, guanine nucleotides can activate the two major signaling pathways that are currently thought to mediate agonist-induced pepsinogen secretion (1). That is, in permeabilized chief cells, GTPγS activates adenylyl cyclase, as evidenced by an increase in cellular CAMP, and phospholipase C, as evidenced by an increase in diacylglycerol. Moreover, maximal concentrations of GTPγS cause a 4-fold increase in pepsinogen secretion that is of the same magnitude as the secretory response observed when carbachol is added to permeabilized chief cells (15). These effects are specific in that they are observed only with GTP analogues and are inhibited by GDPβS, a nucleotide that maintains G proteins in the inactive state. In view of evidence in other tissues that G proteins modulate many different signal transduction pathways (7), these results in permeabilized chief cells are not particularly surprising.

Nonetheless, several important conclusions regarding signal transduction in gastric chief cells can be drawn from the effects of changes in cellular calcium concentration on guanine nucleotide-induced pepsinogen secretion. For example, GDPβS does not alter calcium-induced pepsinogen secretion, suggesting that the major sites of action of calcium are distal to guanine nucleotide-mediated events. In contrast to its actions in chief cells, it has been reported that GDPβS inhibits calcium-induced secretion in other cells (8, 10, 19). Hence, the present data indicate that activated G proteins are required for calcium-induced secretion in some cell types but not in others.

Another important observation in the current study is that, although calcium enhances the GTPγS-induced secretory response, the nucleotide analogue can stimulate pepsinogen secretion in the absence of this cation. In these experiments, the absence of calcium was assured by adding as much as 10 mM EGTA. The ability of GTPγS to cause a calcium-independent secretory response is not a phenomenon that is unique to gastric chief cells. Similar results have been reported with other permeabilized cell models, including basophilic leukemia cells (19), neutrophils (20), RINm5F cells (10), and adrenal chromaffin cells (8). Although studies using SLO-permeabilized mouse pancreatic acini indicate that GTPγS-induced amylase secretion is calcium-dependent (21, 22), calcium-independent GTPγS-induced secretion was observed using SLO-permeabilized acini from rat pancreas (23).

The ability of GTPγS to stimulate pepsinogen release from permeabilized chief cells in the absence of calcium implicates the involvement of a calcium-independent signal transduction mechanism(s). We considered but, for reasons that follow, excluded the possibility that a component of the adenylcyclase class or phospholipase C signaling cascades could account for this calcium-independent mechanism. In terms of adenylcyclase, although guanine nucleotides cause a calcium-independent increase in cellular cAMP, we have demonstrated previously that cAMP-mediated pepsinogen secretion is a calcium-dependent process (12). Furthermore, the addition of a CAMP-dependent protein kinase inhibitor peptide does not alter GTPγS-induced pepsinogen secretion. These observations indicate that the calcium-independent component of GTPγS-induced secretion is not mediated by activation of adenylcyclase and a consequent increase in cAMP.

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One must be circumspect in attempting to draw conclusions regarding the role of G proteins in mediating agonist-induced pepsinogen secretion from these studies using permeabilized chief cells. Although permeabilized chief cells maintain several important structural and functional characteristics of intact chief cells (12), it is clear that leakage of cytosolic constituents after permeabilization must alter cellular physiology to some extent. Nonetheless, without demonstrating directly that G proteins mediate the actions of agents that activate either adenylcyclase or phospholipase C, we can conclude from the present studies that, in gastric chief cells, G proteins are components of these signal transduction pathways.

In the present study, we have identified a phosphoprotein, pp72, that appears to be an endogenous substrate of PKC in gastric chief cells. Based on its size and isoelectric point, it is likely that this protein is a form of the MARCKS protein, a prominent PKC substrate in brain and other mammalian tissues (25, 26). Although the MARCKS protein is a major PKC substrate in many tissues, its role in signal transduction remains uncertain. It has been suggested that the MARCKS protein may be involved in modulating cellular calmodulin levels because its affinity for calmodulin is altered by activation of PKC (27). It has also been suggested that the ability
of the MARCKS protein to bind actin may be of functional significance (28, 29). Further work is required to determine whether pp72 is in fact a MARCKS protein and to determine its role in mediating pepsinogen secretion from gastric chief cells.

REFERENCES