

Intracellular signal transduction during gastrin-induced histamine secretion in rat gastric ECL cells

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Zanner, Robert, Gerhard Hapfelmeier, Manfred Gratzl, and Christian Prinz. Intracellular signal transduction during gastrin-induced histamine secretion in rat gastric ECL cells. *Am J Physiol Cell Physiol* 282: C374–C382, 2002. First published October 3, 2001; 10.1152/ajpcell.00366.2001.—Activation of G_q protein-coupled receptors usually causes a biphasic increase in intracellular calcium concentration ([Ca²⁺]_i) that is crucial for secretion in nonexcitable cells. In gastric enterochromaffin-like (ECL) cells, stimulation with gastrin leads to a prompt biphasic calcium response followed by histamine secretion. This study investigates the underlying signaling events in this neuroendocrine cell type. In ECL cells, RT-PCR suggested the presence of inositol 1,4,5-trisphosphate receptor (IP₃R) subtypes 1–3. The IP₃R antagonist 2-aminoethoxydiphenyl borate abolished both gastrin-induced elevation of [Ca²⁺]_i and histamine release. Thapsigargin increased [Ca²⁺]_i, however, without inducing histamine secretion. In thapsigargin-pretreated cells, gastrin increased [Ca²⁺]_i through calcium influx across the plasma membrane. Both nimodipine and SKF-96365 inhibited gastrin-induced histamine release. The protein kinase C (PKC) activator phorbol 12-myristate 13-acetate induced histamine secretion, an effect that was prevented by nimodipine. In summary, gastrin-stimulated histamine release depends on IP₃R activation and plasmalemmal calcium entry. Gastrin-induced calcium influx was mediated by dihydropyridine-sensitive calcium channels that appear to be L-type channels activated through a pathway involving activation of PKC.

enterochromaffin-like cells; exocytosis; histamine; inositol 1,4,5-trisphosphate

ELEVATION OF INTRACELLULAR calcium concentration ([Ca²⁺]_i) has an important function in the regulation of many cellular processes. This increase in [Ca²⁺]_i can be achieved by release from internal stores, calcium entry across the plasma membrane, or both (5–7). The calcium response to a calcium-mobilizing agonist, e.g., a hormone, usually consists of two phases, an initial peak caused by calcium release from stores and a plateau phase caused by calcium influx.

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In excitable cells, calcium entry is mediated by voltage-operated calcium channels (VOCCs) after membrane depolarization and triggers, e.g., neurotransmitter release (25). In contrast, nonexcitable cells do not require a transient membrane depolarization for calcium influx. Hormones activate a phosphatidylinositol-specific phospholipase C to produce inositol 1,4,5-trisphosphate (IP₃) and diacylglycerol (DAG) (5, 6, 26). IP₃ releases calcium from internal stores, inducing calcium influx across the plasma membrane via store-operated calcium channels (40), also termed capacitative calcium entry (32). DAG activates protein kinase C (PKC), which is known to regulate several transduction pathways, including gene transcription, proliferation, and hormone secretion (6).

Gastric enterochromaffin-like (ECL) cells are histamine-producing neuroendocrine cells in the gastric epithelium that play an important role in the peripheral regulation of acid secretion (39, 45). The antral hormone gastrin directly stimulates histamine release from ECL cells (36, 38, 44). Incubation of permeabilized ECL cells with micromolar free calcium induces histamine secretion (12). Moreover, gastrin has been shown to induce production of inositol phosphates in ECL cells of *Mastomys natalensis* (15). These findings suggest that IP₃ and elevation of [Ca²⁺]_i play a key role in histamine secretion in this neuroendocrine cell type. Currently, the exact mechanisms triggering calcium influx in gastric endocrine cells are unclear. Because isolated ECL cells are highly enriched, viable, and nontransformed endocrine cells, they are especially well suited for investigating signal transduction pathways.

VOCCs present a potential candidate for mediating the calcium influx in ECL cells because L- and N-type channels have been detected in gastric ECL cells by patch-clamp experiments (8) and RT-PCR (57). VOCCs have also been detected in adrenal chromaffin and intestinal enterochromaffin cells (10, 20, 43), where they mediate exocytosis. Obviously, these channels are a common feature of chromaffin

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cells, but the importance and activation of these channels might be different in each cell type because of differences in excitability.

In the present study, we investigated the signaling events involved in histamine secretion from gastric ECL cells after gastrin receptor activation. We found that gastrin-induced secretion was dependent on IP₃ receptor (IP₃R) activation and plasmalemmal calcium entry. Mobilization of intracellular calcium stores alone was not sufficient to stimulate histamine release. Gastrin- and phorbol 12-myristate 13-acetate (PMA)-induced histamine secretion were inhibited by dihydropyridines. Thus we conclude that calcium influx after gastrin stimulation is mediated by L-type VOCCs that appear to be activated through PKC.

MATERIALS AND METHODS

Reagents and antibodies. All reagents were of analytical grade and were purchased from the indicated suppliers: Pronase (Roche Molecular Biochemicals, Mannheim, Germany); bovine serum albumin and HEPES (Serva, Heidelberg, Germany); Nycodenz (Accurate Chemicals, New York, NY); Cell-Tak and Matrigel (Becton-Dickinson, Heidelberg, Germany); fetal bovine serum (GIBCO, Eggenstein, Germany); fura 2-AM (Molecular Probes, Eugene, OR); 2-aminooxydiphenyl borate (2-APB; Tocris, Bristol, UK); SKF-96365 (Calbiochem, La Jolla, CA); thapsigargin (Calbiochem; RBI, Natick, MA); nimodipine and nifedipine (RBI); DMEM, PMA, and rat gastrin-17 (Sigma, Deisenhofen, Germany). For immunocytochemistry, a monoclonal antibody for IP₃R3 (Transduction Laboratories, Lexington, KY) was used. Secondary antibodies conjugated to Alexa Fluor 488 were purchased from Molecular Probes.

Cell isolation and primary culture. For each experiment, five female Sprague-Dawley rats (body wt 180–200 g; Charles River, Sulzfeld, Germany) were anesthetized by carbon dioxide and killed by cervical dislocation in accordance with the ethics guidelines of the Technische Universität München. The experiments comply with all relevant local and institutional regulations. Mucosal cells from the stomachs were isolated by pronase digestion (1.3 mg/ml) with an everted sac technique (36). The resulting crude cell preparation was fractionated by counterflow elutriation and subsequent density gradient centrifugation as described previously (12, 22, 36, 38). Cell viability (trypan blue exclusion) exceeded 95%. This enriched ECL cell fraction was then placed on six-well tissue culture plates precoated with Matrigel. Cells were cultured in Dulbecco's modified Eagle's medium (DMEM)-F12 medium supplemented with 2 mg/ml bovine serum albumin, 5% fetal bovine serum, 5 mg/l gentamicin, 5 mg/l insulin, 5 mg/l transferrin, 5 µg/l sodium selenite, 1 nM hydrocortisone, and 1 pM gastrin. After 48 h of culture, 90–95% of total adherent cells were identified as ECL cells by acridine orange uptake and antibody staining for histidine decarboxylase (12, 37).

Histamine release experiments. After 48-h short-term culture, ECL cells ($2.5\text{--}5 \times 10^4$ /well) were incubated in DMEM-F12 medium without supplements for 180 min to remove the influence of growth factors potentially present in the supplemented growth medium. Subsequently, growth medium was removed and replaced by 1 ml of elutriation buffer (Krebs-Ringer solution containing 0.1 mg/ml bovine serum albumin) for the indicated time periods under basal conditions or in the presence of inhibitor after a 60-min stimulation. In some experiments, CaCl₂ in the elutriation buffer was replaced with 0.5 mM EGTA to obtain nominally calcium-free medium. When solvents other than water were used, negative controls with solvent alone were conducted to exclude non-specific solvent effects on histamine secretion. Histamine concentration in the incubation medium was measured by a commercially available radioimmunoassay kit (Beckman Coulter, Krefeld, Germany). Data are expressed as picomoles of histamine produced from single six-well plates (corresponding to $2.5\text{--}5 \times 10^4$ cells/well) under basal conditions or in the presence of stimulants and/or inhibitors during 60 min of incubation.

Measurement of $[Ca^{2+}]_i$ in single ECL cells. Isolated ECL cells were placed on sterile glass coverslips coated with Cell-Tak diluted 1:1 with 0.5 M NaHCO₃ and cultured as described in *Cell isolation and primary culture*. After 48 h, the cells were incubated with fura 2-AM at a final concentration of 1 µg/ml for 15 min at 37°C and subsequently transferred into a heated microchamber perfused with elutriation buffer (containing 1 mM CaCl₂ or 0.5 mM EGTA). Cells were observed with a Zeiss Fluor ×40 objective mounted on a Zeiss Axiovert 135 TV inverted microscope in the epifluorescence mode using a dichroic filter of 395 nm. Alternating wavelength excitation of 340 and 380 nm was provided by a motorized filter wheel. Light emissions were monitored by a video camera connected to the Attofluor digital recording system (Zeiss, Jena, Germany). The curves shown present the average of 9–15 cells investigated simultaneously.

RNA isolation, reverse transcription, and RT-PCR. Total RNA was isolated from enriched ECL cells with peqGOLD-TriFast reagent (Peqlab Biotechnologie, Erlangen, Germany) according to the manufacturer's instructions. Complementary DNA was generated from total RNA with *Taq* Man reverse transcription reagents from Perkin-Elmer (Weiterstadt, Germany). The coding sequences for IP₃R published in GenBank were used to generate specific oligonucleotide primers (Table 1). PCR was performed using the *Taq* PCR Master Mix Kit from Qiagen (Hilden, Germany) and the following temperature cycle profile: 2 min at 94°C followed by 30 cycles of 45 s at 94°C, 55 s at annealing temperature, and 1 min at 72°C. The profile was ended with a final extension of 10 min at 72°C. Annealing temperatures were 58°C for IP₃R1 and IP₃R2 and 56°C for IP₃R3. PCR products were subjected to horizontal agarose gel electrophoresis, and bands were visualized in a digital documentation system (MWG Biotech, Ebersberg, Germany). Subsequently, the amplification products were eluted from the gel, cloned into TOPO pCR 2.1

Table 1. Oligonucleotide primer sequences

Gene	Accession No.	Forward Primer	Reverse Primer	Product, bp
IP ₃ R1	J05510	5'-TGA CAG CAG TGG AGC ATC G-3'	5'-GCA GCA GCC ATA GGA GTC A-3'	393
IP ₃ R2	X61677	5'-CAA GTG CCA AGG TGC TGA G-3'	5'-TCA GCA CAT AGG CAC CAG G-3'	587
IP ₃ R3	L06096	5'-CGC TCC ATC CTG CTA ACT G-3'	5'-TTC CTC AGT CCG TGG TTC A-3'	317

IP₃R, inositol 1,4,5-trisphosphate receptor; bp, base pairs.

vector (Invitrogen, Groningen, The Netherlands), and sequenced for verification.

Immunocytochemistry. Isolated ECL cells were fixed in 4% paraformaldehyde at room temperature for 10 min and spun on glass coverslips using a Cytospin 3 cell preparation system (Shandon, Frankfurt, Germany). After blocking and permeabilization, the cells were incubated with a monoclonal antibody for IP₃R3 (1:100) overnight at 4°C. After washing, cells were incubated with anti-mouse secondary antibody conjugated to Alexa Fluor 488 for 1 h at room temperature and processed for immunofluorescence microscopy. In negative controls, cells were incubated with blocking buffer (10 mM PBS containing 10% normal goat serum) instead of the primary antibody.

Statistical analysis. Results are expressed as means ± SE. Data were analyzed by paired Student's *t*-test. Values of *P* < 0.05 were considered to be significant.

RESULTS

Importance of IP₃ and IP₃Rs. To investigate the role of IP₃ in gastrin-induced histamine release, ECL cells

were stimulated after 10-min preincubation with the IP₃ receptor antagonist 2-APB. This membrane-permeant drug has been shown to have no effect on IP₃ production through phospholipase C and does not modify the function of VOCCs and ryanodine receptors (23). 2-APB (100 μM) completely inhibited gastrin-induced histamine secretion (Fig. 1A). Moreover, 2-APB abolished the biphasic calcium signal in response to gastrin without affecting basal [Ca²⁺]_i (Fig. 1B).

The IP₃R subtypes present in ECL cells were determined. RT-PCR with subtype-specific primers produced bands of 393, 587, and 317 base pairs, corresponding to IP₃R1, IP₃R2, and IP₃R3, respectively (Fig. 1C). We used cDNA from cerebellum and kidney as positive controls (Fig. 1C). Because no quantitative PCR was used, the relative importance or concentration could not be determined for any of these subtypes. Nevertheless, the PCR products were identical to the original sequences as determined by subsequent clon-

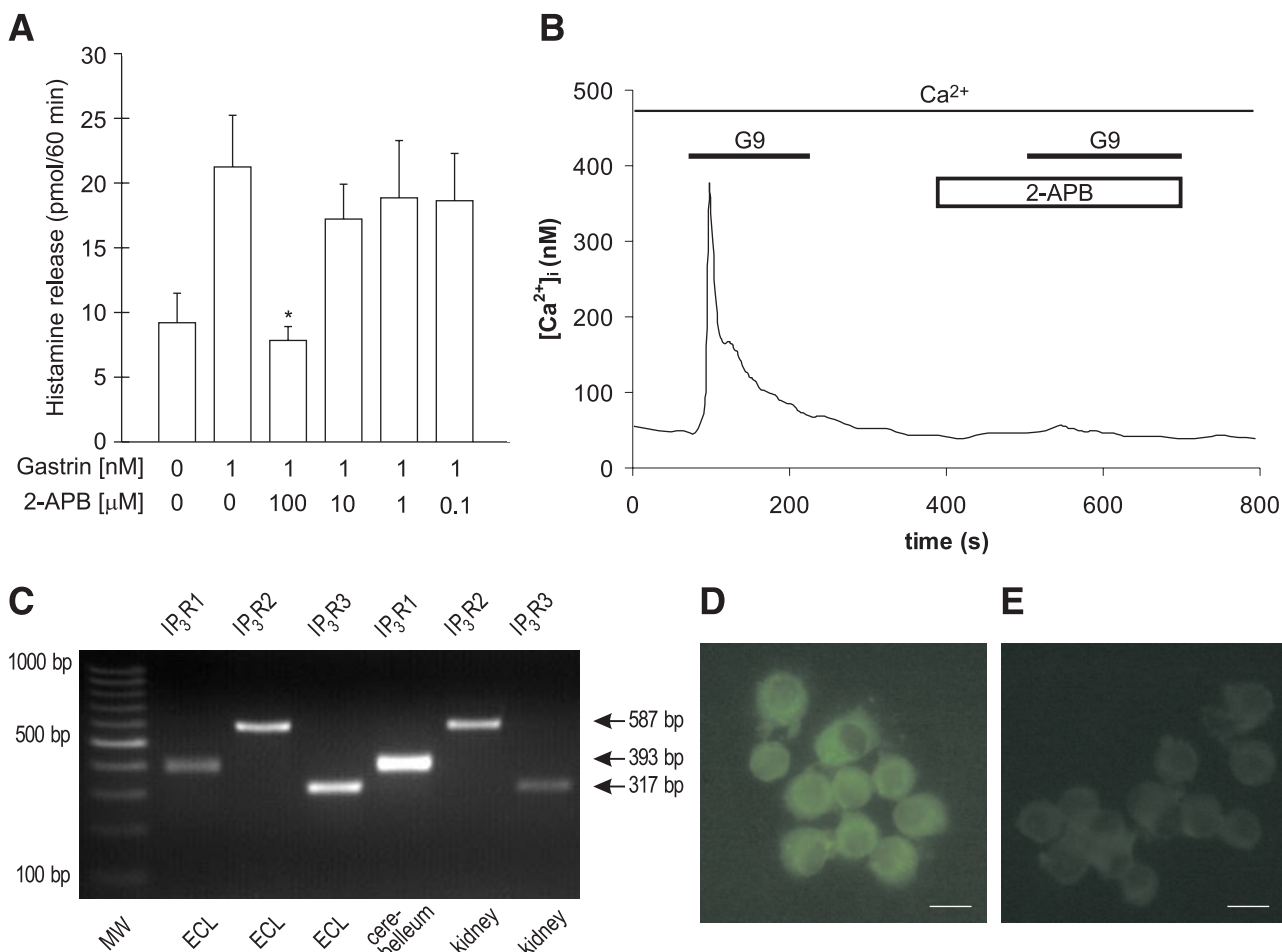


Fig. 1. Importance of inositol 1,4,5-trisphosphate (IP₃) and IP₃ receptors (IP₃Rs). **A**: effect of the IP₃R antagonist 2-aminoethoxydiphenyl borate (2-APB) on gastrin-stimulated histamine secretion. 2-APB treatment abolished gastrin-induced histamine secretion at a concentration of 100 μM (*n* = 7; **P* < 0.007 vs. gastrin stimulation). **B**: effect of 2-APB on intracellular calcium concentration ([Ca²⁺]_i) in enterochromaffin-like (ECL) cells. Gastrin produced a biphasic calcium signal. Addition of 2-APB rendered the cells insensitive to gastrin. G9, gastrin (1 nM). **C**: RT-PCR with subtype-specific primers detected all 3 IP₃R subtypes in ECL cells. Positive controls (cerebellum and kidney) are also shown. MW, molecular weight markers. **D**: antibody staining for the type 3 IP₃ receptor. **E**: negative control. Bar, 10 μm.

ing and sequencing, confirming the presence of IP₃R subtypes in ECL cells. Negative controls did not produce any products (data not shown).

In addition, we performed antibody staining in isolated ECL cells with a mouse monoclonal antibody for IP₃R3 (Transduction Laboratories). Fluorescence microscopy identified IP₃R3 in isolated ECL cells, confirming the results revealed by PCR (Fig. 1D). A negative control is shown in Fig. 1E.

Importance of calcium release from internal stores. Thapsigargin, a commonly used inhibitor of sarco(endo)plasmic reticulum calcium ATPase, was used to investigate the influence of calcium release from intracellular stores on histamine release. Incubation of ECL cells with thapsigargin (10^{-6} – 10^{-10} M) in the presence of extracellular calcium for 1 h did not cause histamine secretion (Fig. 2A). To further characterize the effect of thapsigargin, $[Ca^{2+}]_i$ was determined in single ECL cells loaded with fura 2. To exclude nonspecific effects, thapsigargin was used at a concentration of $\leq 0.5 \mu\text{M}$ (11). In nominally calcium-free medium, thapsigargin induced a transient increase in $[Ca^{2+}]_i$ followed by a quick recovery to basal level (Fig. 2B). Addition of 1 nM gastrin did not induce any further response in calcium-free medium. As shown in Fig. 2C, readdition of calcium (1 mM) after store mobilization with thapsigargin ($0.5 \mu\text{M}$) resulted in an increase in $[Ca^{2+}]_i$ caused by increased calcium influx through store-operated calcium channels and inhibition of calcium uptake through sarco(endo)plasmic reticulum calcium ATPase (3). We also investigated the effect of thapsigargin in the presence of extracellular calcium (Fig. 2D). Under these conditions, thapsigargin increased $[Ca^{2+}]_i$ more effectively than in calcium-free medium. Furthermore, a sustained increase of $[Ca^{2+}]_i$ above the basal level could be observed. Subsequent addition of gastrin resulted in a biphasic calcium response. Compared with the response seen with gastrin alone, the initial peak was decreased, whereas the plateau phase remained at a comparable level (see Figs. 1B, 2D, and 3B). Thapsigargin added to enriched ECL cells 60 min before stimulation with gastrin (1 nM) in calcium-containing medium did not affect gastrin-induced histamine secretion ($n = 3$; data not shown).

Importance of plasmalemmal calcium entry. The effect of gastrin on histamine secretion in nominally calcium-free medium was investigated to define the role of calcium influx through calcium channels in the

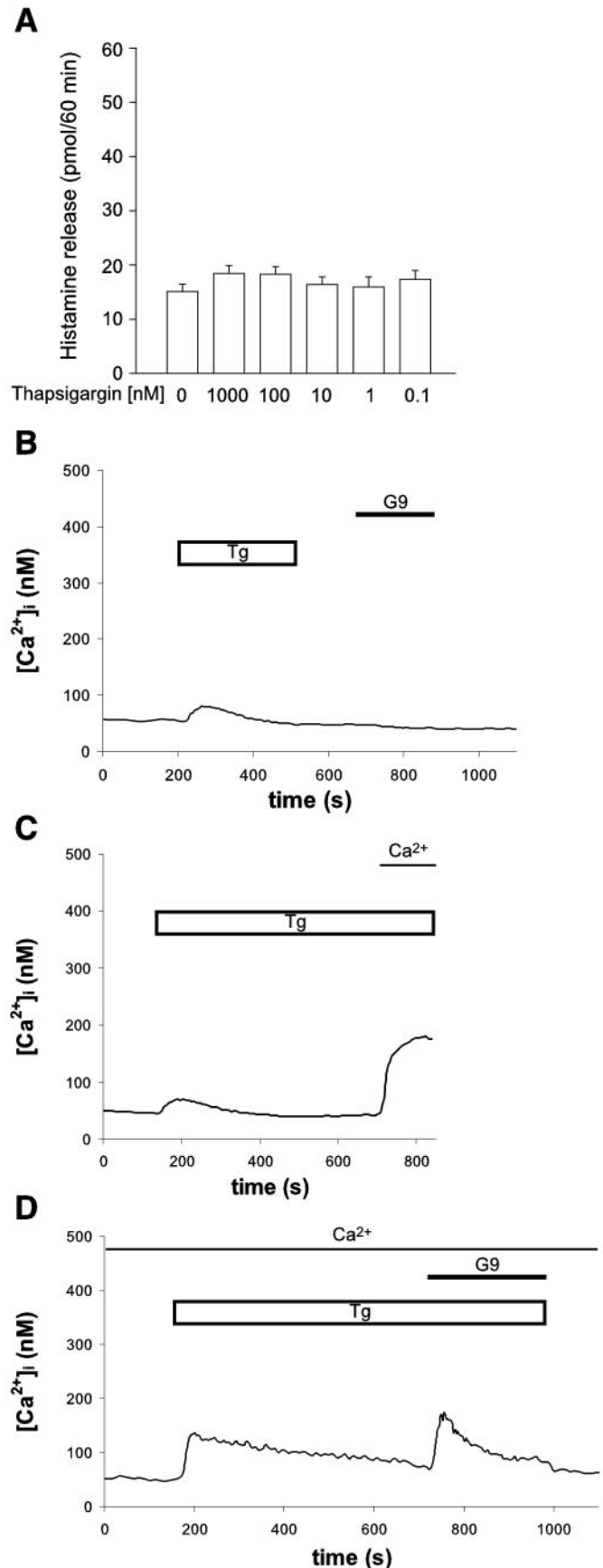
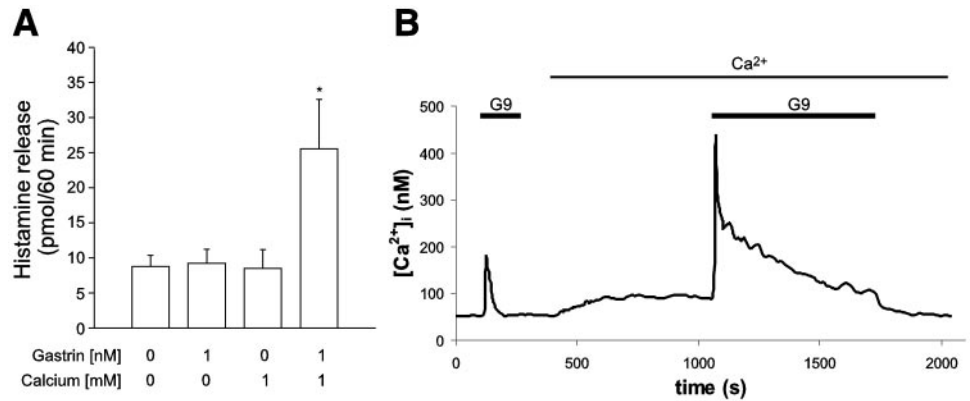


Fig. 2. Importance of calcium release from internal stores. **A**: effect of thapsigargin on histamine release in the presence of extracellular calcium. No histamine secretion was observed after 1-h incubation at various concentrations. **B**: thapsigargin (Tg; $0.5 \mu\text{M}$) effect on $[Ca^{2+}]_i$ in fura 2-loaded ECL cells in the absence of extracellular calcium. Gastrin (1 nM) did not induce a further response in calcium-free medium. **C**: Ca^{2+} add-back protocol. After store mobilization with thapsigargin ($0.5 \mu\text{M}$) and subsequent readdition of extracellular calcium, a prompt increase of $[Ca^{2+}]_i$ was observed because of influx through store-operated calcium channels. **D**: thapsigargin effect on $[Ca^{2+}]_i$ in the presence of extracellular calcium. Addition of gastrin in the continuous presence of thapsigargin produced a biphasic increase in $[Ca^{2+}]_i$.

Fig. 3. Importance of plasmalemmal calcium entry. **A**: gastrin-induced histamine secretion from isolated ECL cells in the absence or presence of calcium ($n = 6$; $*P < 0.05$ vs. basal). Histamine release could only be detected in the presence of extracellular calcium. **B**: gastrin effects on intracellular calcium in the absence or presence of external calcium. Only in the presence of extracellular calcium was gastrin able to generate a biphasic calcium response.

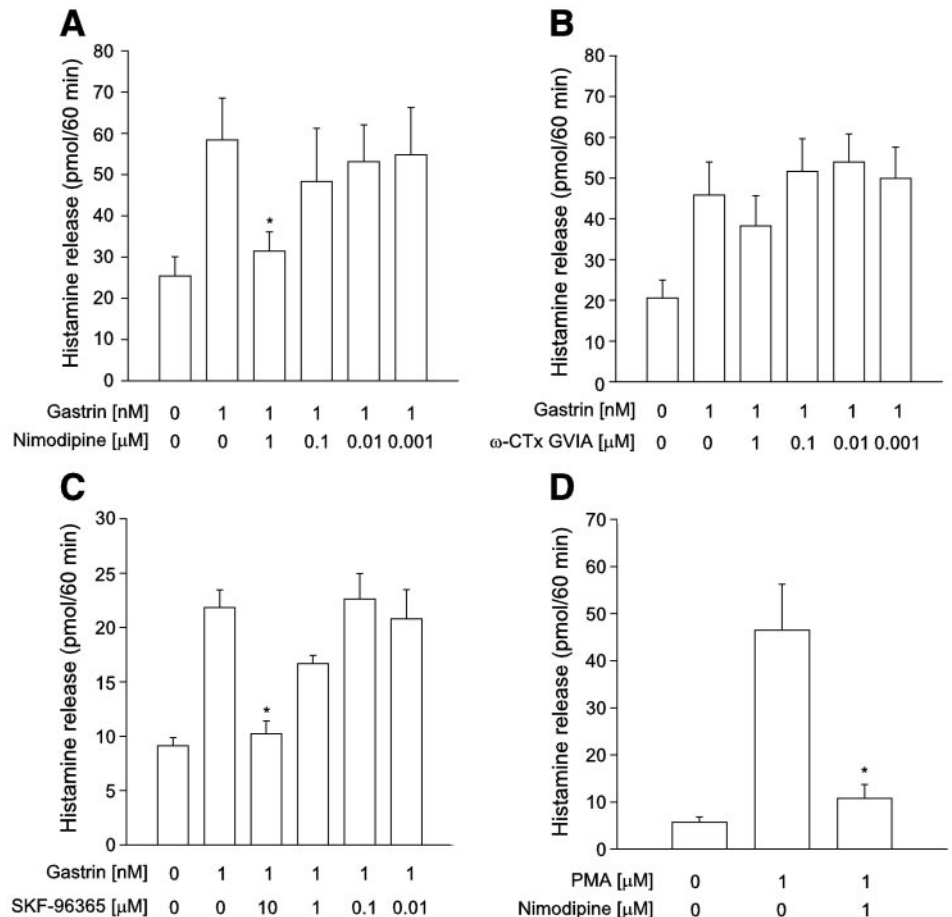


plasma membrane. As depicted in Fig. 3A, gastrin failed to release detectable amounts of histamine in calcium-free medium, indicating the importance of calcium influx from the extracellular space for secretion. Gastrin effects on $[Ca^{2+}]_i$ were also investigated in fura 2-loaded cells. In the absence of extracellular calcium, gastrin induced a small peak but no plateau phase (Fig. 3B), as previously reported (58). This finding is consistent with a previous observation in which the plateau phase was abolished by addition of lanthanum ($10 \mu M$), a nonspecific entry channel blocker (35). After removal of gastrin and addition of extracellular

calcium, a slight increase in basal $[Ca^{2+}]_i$ was observed. Subsequent exposure to gastrin induced a strong biphasic calcium signal that declined to basal level after gastrin washout.

Effects of calcium channel inhibitors on histamine secretion. Several calcium channel blockers were then used to identify the channels involved in plasmalemmal calcium entry. Gastrin stimulated histamine release three- to fivefold after 60 min of incubation. This effect was inhibited by ~70% by the selective L-type calcium channel antagonist nimodipine after 20 min of preincubation (10^{-6} M; Fig. 4A). Similar effects were ob-

Fig. 4. Effects of calcium channel blockers on histamine release. **A**: effect of the L-type calcium channel blocker nimodipine on gastrin-stimulated histamine release (10^{-6} M, $n = 8$; $*P < 0.01$ vs. gastrin stimulation). **B**: effect of the N-type calcium channel blocker ω -conotoxin (CTx) GVIA (10^{-6} M, $n = 6$) on gastrin-induced histamine secretion. **C**: effect of SKF-96365 on gastrin-stimulated histamine release (10^{-5} M, $n = 7$; $*P < 0.0003$ vs. gastrin stimulation). **D**: effect of nimodipine on phorbol 12-myristate 13-acetate (PMA)-induced histamine secretion. PMA (10^{-6} M) induced histamine secretion 3- to 5-fold, and this effect was inhibited by ~80% by nimodipine at 10^{-6} M ($n = 5$; $P < 0.05$ vs. PMA stimulation).



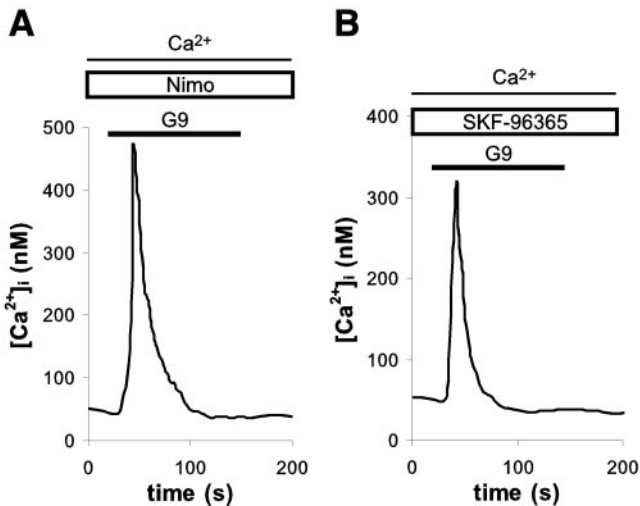


Fig. 5. Effects of calcium channel blockers on calcium signaling. *A*: effect of the L-type calcium channel blocker nimodipine on gastrin-stimulated calcium concentrations in ECL cells. Nimodipine was applied at 10^{-6} M before the addition of gastrin. *B*: effect of the calcium channel blocker SKF-96365 (10^{-6} M) on gastrin-induced changes in intracellular calcium. Both drugs abolished the plateau phase of the calcium signal completely (representative of 8 preparations).

served with nifedipine (10^{-6} M; data not shown). The N-type calcium channel blocker ω -conotoxin GVIA (10^{-6} M) inhibited gastrin-induced histamine secretion only to a small extent after 60-min preincubation. (Fig. 4*B*). SKF-96365 (10^{-5} M), an inhibitor of both voltage- and receptor-operated calcium channels, inhibited gastrin-induced histamine release almost completely after 20-min preincubation (Fig. 4*C*). As shown in Fig. 4*D*, PMA (10^{-6} M, 60 min) induced histamine secretion three- to fivefold, and this effect was inhibited by 60–70% by nimodipine (10^{-6} M).

Effects of calcium channel inhibitors on $[Ca^{2+}]_i$ in fura 2-loaded ECL cells. ECL cells were stimulated with gastrin (1 nM) in the presence of calcium channel blockers, and $[Ca^{2+}]_i$ was determined. Both nimodipine (10^{-6}

M) and SKF-96365 (10^{-5} M) abolished the plateau phase of the gastrin-induced calcium signal (Fig. 5), whereas the initial peak remained almost unchanged.

DISCUSSION

Gastrin, the key stimulus of histamine secretion, binds to G_q -coupled gastrin/cholecystokinin B receptors (16, 17, 30, 34, 54, 55), resulting in a subsequent increase in $[Ca^{2+}]_i$ and exocytosis (13, 15, 29, 38, 50, 55). G_q activates the generation of IP_3 and DAG via phosphatidylinositol-specific phospholipase C in a pertussis toxin-insensitive manner (28). Generation of DAG activates PKC in several cell types. Here, we investigated the role of IP_3 , PKC, and calcium signaling in gastric ECL cells to elucidate the signaling events underlying gastrin-induced histamine release. Figure 6 depicts a possible mechanism based on our results.

We found that gastrin-induced histamine release was completely inhibited by the IP_3R antagonist 2-APB. Moreover, RT-PCR and immunocytochemistry confirmed the presence of these receptors in ECL cells. Our data are in agreement with previous studies with isolated ECL cells from *Mastomys natalensis* (15, 42). These studies determined the production of inositol phosphates in ECL cells in response to gastrin. Our current observations further extend the importance of IP_3 and IP_3R for signal transduction after gastrin receptor activation. Three different receptor subtypes for IP_3 have been identified in rat tissues (reviewed in Refs. 27 and 33). In the digestive tract, IP_3R3 appears to be the predominant form (31). In gastric ECL cells, we found RNAs encoding all three IP_3R subtypes. Because we found specific staining with the IP_3R3 antibody, we conclude that at least this receptor subtype is of functional importance. The role of the other two subtypes remains to be determined. 2-APB also prevented intracellular calcium signaling in response to gastrin. Together, these results show that stimulation

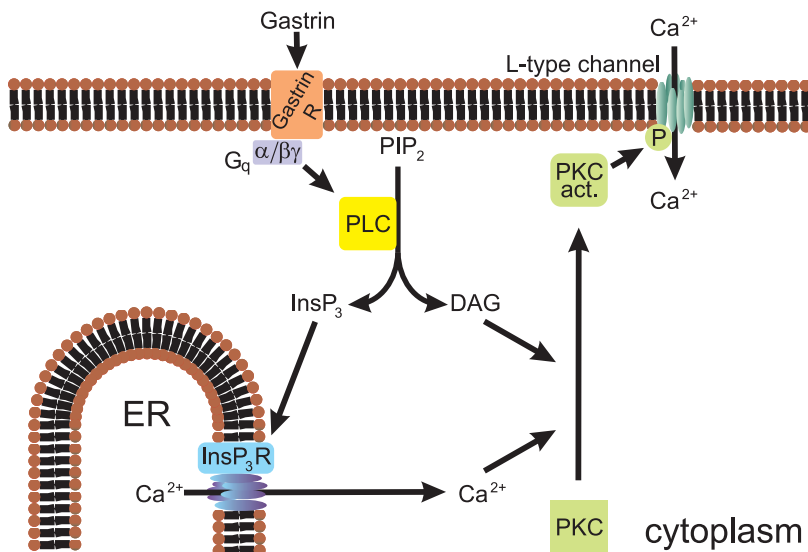


Fig. 6. Hypothetical model of the intracellular signaling cascade after gastrin receptor activation. Binding of gastrin to its receptor activates phospholipase (PLC) through G_q leading to the generation of IP_3 and diacylglycerol (DAG). While IP_3 releases calcium from intracellular stores, DAG activates PKC, yielding phosphorylation and activation of L-type calcium channels. PIP_2 , phosphatidylinositol 4,5-bisphosphate; ER, endoplasmic reticulum.

of IP₃Rs activates a calcium signaling process in ECL cells.

IP₃ usually binds to its receptors on the endoplasmic reticulum, thereby mobilizing calcium that induces store-operated calcium influx. To investigate the contribution of these stores to gastrin-induced histamine release and calcium influx, we also mobilized internal calcium stores with thapsigargin. Thapsigargin addition led to an increase of [Ca²⁺]_i, reflecting mobilization of calcium from the endoplasmic reticulum. However, this increase of [Ca²⁺]_i did not induce histamine secretion. This indicates that calcium release from internal stores and store-operated calcium entry per se is not sufficient for exocytosis. Moreover, gastrin increased [Ca²⁺]_i through calcium influx across the plasma membrane and histamine secretion despite thapsigargin pretreatment. Therefore, another signal transduction pathway appears to be necessary for the activation of plasmalemmal calcium entry besides the release from internal stores.

It must be mentioned that intracellular calcium stores may be slowly depleted by the use of EGTA, causing a reduction in [Ca²⁺]_i and thereby preventing a subsequent effect of thapsigargin in calcium-free media. This effect may not be ruled out. However, thapsigargin induced an increase in [Ca²⁺]_i (Fig. 2D) but did not induce histamine release in calcium-containing medium (Fig. 2A), indicating that gastrin-induced histamine secretion is independent of store-operated calcium entry but dependent on the activation of other calcium channels.

If IP₃R activation, but not store-operated calcium entry, is a requisite for stimulation of histamine secretion in this cell type, it may be speculated that different types or localizations of IP₃R exist that lead to the activation of several calcium signaling processes. Our current data therefore might suggest an effect of IP₃ not only on the endoplasmic reticulum but also on the plasma membrane, indicating IP₃-IP₃R-plasma membrane interaction to allow calcium entry. A location of IP₃R at the plasma membrane was shown in lymphocytes (5, 18), endothelial cells (52), and several other cell types (9, 14, 24). It has also been assumed that a direct interaction of intracellular IP₃Rs with plasmalemmal calcium channels leads to calcium entry across the plasma membrane (for review, see Ref. 41).

Another possibility, supported by our current data, is that besides the activation of IP₃Rs and subsequent release from internal stores, DAG-induced activation of PKC is simultaneously required to activate plasmalemmal calcium entry. Indeed, PMA, a direct activator of PKC, induced histamine release, an effect that was inhibited by nimodipine. This indicates that PKC is involved in histamine release via activation of L-type calcium channels. Similar to these observations, a PMA-induced and dihydropyridine-sensitive calcium current was observed in lymphocytes (46) and in oxygen-sensing glomus cells of the carotid body (49). In hippocampal synaptosomes, PMA-induced VOCC activity was inhibited by PKC inhibitors such as calphostin C or dihydropyridine (4). In contrast, treatment

of chromaffin cells with PMA led to an inhibition of calcium currents through L-type channels (47); however, non-P/Q-type channels might also be negatively regulated by PKC and may interfere with this response. A differential expression of VOCCs in the respective cell type may regulate the PKC-linked activation of these channels. Nevertheless, PKC obviously is crucial for activation of L-type channels, increasing the intracellular calcium level. The generation of DAG appears to be necessary for activation of PKC in ECL cells.

Gastrin did not induce histamine secretion in calcium-free medium, indicating that calcium influx during the plateau phase is essential for exocytosis. Because ECL cells express L- and N-type VOCCs, which are important for secretion in neuronal (2) and chromaffin (10, 20) cells and pancreatic β-cells (19), we studied the effect of selective antagonists of these channels. Both nimodipine and nifedipine inhibited gastrin-induced histamine secretion by ~70% and abolished the plateau phase of the gastrin-induced calcium transient in fura 2-loaded ECL cells. This finding contradicts a previous report, which demonstrated only a small inhibition of histamine secretion by nifedipine (57). The reason for this discrepancy remains obscure because, in our hands, both drugs had a specific effect on secretion and calcium signaling. L-type channel antagonists such as nimodipine are specific inhibitors interacting with the α-subunit of L-type VOCCs at a defined consensus sequence. The concentrations used correspond to the IC₅₀ values observed in other cell types for the inhibition of calcium currents (48, 51, 53).

Previous studies revealed that ECL cells are electrically nonexcitable (21). Nevertheless, our studies provide evidence that L-type channels are functionally important. One explanation for this discrepancy is that L-type channel blockers may affect other calcium channels in a nonspecific manner and the action may be too broad. Nevertheless, the binding of the L-type channel blocker at 0.1–1 μM occurs at a defined consensus sequence that is not found on other channels; the action at this concentration is generally believed to be very specific (48, 51, 53). At higher concentrations (100 μM) L-type channel blockers have been shown to block nicotinic acid-adenine dinucleotide phosphate (NAADP)-induced calcium release (56), but this concentration may reflect nonspecific binding.

It may also be possible that L-type channels become activated in ECL cells at resting potentials without membrane depolarization. A recent study investigated the effects of nimodipine and PKC activators on the changes of quantal acetylcholine release at the frog neuromuscular junction (1). The authors detected that PMA increased the frequency and quantal content of miniature end plate potentials and currents. This effect was completely inhibited by the addition of nimodipine (1 μM). These results suggest that activation of PKC increases quantal acetylcholine release by opening quiescent L-type channels in motor nerve terminals at resting potential and apparently not by depo-

larization. A similar mechanism may also be present in the neuroendocrine ECL cells.

We conclude that, in ECL cells, gastrin-induced histamine secretion involves activation of IP₃Rs and PKC, resulting in calcium entry through dihydropyridine-sensitive channels, presumably L-type channels (see Fig. 6). Our data may provide a unique perspective for the understanding of calcium channel gating mediated by G protein-coupled receptors in nonexcitable cells.

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