Gi-Mediated Activation of Mitogen-Activated Protein Kinase (MAPK) Pathway by Receptor Mimetic Basic Secretagogues of Connective Tissue-Type Mast Cells: Bifurcation of Arachidonic Acid-Induced Release Upstream of MAPK¹

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Accepted for publication February 2, 1999 This paper is available online at http://www.jpet.org

ABSTRACT

The family of basic secretagogues of connective tissue mast cells act as receptor mimetic agents, which trigger exocytosis by directly activating G proteins. We now demonstrate that pertussis toxin (Ptx)-sensitive Gi proteins, activated by compound 48/80 (c48/80), a potent member of this family, also activate the p42/p44 MAP kinases (MAPKs). This activation was potentiated by the protein tyrosine phosphatase inhibitor vanadate, whereas the tyrphostin AG-18, a competitive inhibitor of protein tyrosine kinases (PTKs); the protein kinase C inhibitors K252a and GF109203X; the phosphatidylinositol-3-kinase (PI-3K) inhibitors wortmannin and LY294002; and EGTA have abolished this activation. These results suggest that c48/80 activated the p42/p44 MAPKs via a mechanism that involves PTKs, protein kinase C, phosphatidylinositol-3-kinase and Ca²⁺ as mediators. Protein tyrosine phosphorylation and activation of the p42/p44 MAPKs were closely correlated with stimulation of arachidonic acid (AA) release by c48/80 but not with histamine secretion. However, whereas PD98059, the inhibitor of the MAPK kinase has abrogated MAPK activation, this inhibitor failed to effect release of AA. We therefore conclude that by activating Ptx-sensitive Gi protein(s), the basic secretagogues of mast cells stimulate multiple signaling pathways, which diverge to regulate the production and release of the different inflammatory mediators. Whereas the signaling pathway responsible for triggering histamine release is PTK independent, the pathway responsible for the stimulation of AA release bifurcates downstream to PTKs but upstream to the activation of MAPKs.

Mast cells are specialized secretory cells that release various inflammatory mediators in response to activation by external stimuli. These mediators include preformed mediators, such as histamine, that are stored in secretory granules, as well as metabolites of arachidonic acid (AA) (i.e., prostaglandins and leukotrienes) and multifunctional cytokines and chemokines, which are produced de novo on activation (Stevens and Austen 1989; Gordon et al., 1990). The major pathways of activating exocytosis in mast cells include the immunological trigger, which involves the aggregation of their high-affinity receptors (FcεRI) for immunoglobulin E (IgE) by corresponding antigens (Segal et al., 1977), and the peptidergic pathway, which is achieved by polycationic compounds, i.e., the basic secretagogues of mast cells. The latter act as receptor mimetic agents, which trigger mast cell exocytosis by directly activating pertussis toxin (Ptx)-sensitive Gi proteins (Aridor and Sagi-Eisenberg 1990; Aridor et al., 1990; Beub et al., 1990; Mouli et al., 1990). This family of mast cell agonists includes positively charged peptides such as Substance P and bradykinin, various amines such as the synthetic compound 48/80 (c48/80), and naturally occurring polyamines (Lagunoff et al., 1983).

The mechanism by which basic secretagogues activate exocytosis and release of the preformed mediators appears to be distinct from that of the immunological trigger. Unlike FcεRI-induced exocytosis, release triggered by basic secretagogues is faster (seconds versus minutes, as in the case of the immunological trigger), does not require the presence of external Ca²⁺, and is inhibited by Ptx (Saito et al., 1987; Aridor et al., 1990). However, despite these marked differences, the biochemical pathways, initiated by the immunological or G protein-mediated trigger, may still merge into a common pathway to stimulate the production and release of the mediators produced de novo. This idea is based on our recent

ABBREVIATIONS: AA, arachidonic acid; IgE, immunoglobulin E; MAPK, mitogen-activated protein kinase; MAPKK, MAPK kinase; PKC, protein kinase C; cPLA₂, cytosolic phospholipase A₂; PI-3K, phosphatidylinositol 3-kinase; PTK, protein tyrosine kinase; Ptx, pertussis toxin; P-Tyr, phosphotyrosine.
studies demonstrating that c48/80 stimulates the activity of an unidentified protein tyrosine kinase (PTK), leading to the enhanced tyrosine phosphorylation of several cellular proteins (Shefer et al., 1998). Activation of this PTK is dependent on the enzymes protein kinase C (PKC) and phosphati-
dylinositol-3-kinase (PI-3K), which also serve as important mediators with the immunological trigger (Sagi-Eisenberg and Pecht 1984; Sagi-Eisenberg et al. 1985; Yano et al., 1993). This PTK signaling pathway does not contribute to histamine release, but it plays a central role in mediating c48/80-induced release of AA (Shefer et al., 1998).

Release of AA triggered by the FcεRI is regulated primarily through the activation of mitogen-activated protein kinase (MAPK) (Zhang et al., 1997). Furthermore, recent studies have indicated that, similar to PTK-linked receptors, activated G proteins also evoke signaling interactions that effect MAPK activation (Koch et al., 1994; Hawes et al., 1995; Lopez-Ilasaca et al., 1997). Therefore, we set out to investigate whether basic secretagogue-activated Gi proteins could activate MAPKs and what role these kinases might play in controlling AA release. Here, we demonstrate that c48/80 activates both p42 and p44 MAPKs by a mechanism that is strictly dependent on the PTK-signaling pathway. Nevertheless, whereas the release of AA is mediated by the PTK-signaling pathway, activation of p42/p44 MAPKs is not involved.

### Experimental Procedures

#### Materials
- The tyrphostin AG-18 was purchased from Calbiochem Corp. (La Jolla, CA). Monoclonal antibodies against P-Tyr (PY20) were obtained from Transduction Laboratories (Lexington, KY), anti-active MAPK antibodies were purchased from Promega (Madison, WI), anti-COOH-terminal MAPK antibodies and anti-
cPLA₂ antibodies were obtained from Santa Cruz Biotechnologies (Santa Cruz, CA), peroxidase-conjugated AffiniPure goat anti-
mouse or anti-rabbit IgGs were purchased from Jackson ImmunoResearch Laboratories (Avondale, PA), [³H]AA (60–100 Ci/mmol) and [³H]myo-inositol (18 Ci/mmol) were obtained from NEN-DuPont (Boston, MA), and the protease inhibitor cocktail Complete was purchased from Boehringer Mannheim (Indianapolis, IN).

#### Isolation and Purification of Mast Cells
- Rat peritoneal mast cells were obtained from Wistar rats by a peritoneal lavage and purified as described previously (Aridor et al., 1990). Briefly, a suspension of washed peritoneal cells was layered over a cushion of 30% Ficoll 400 (Pharmacia Biotech Inc., Piscataway, NJ) in buffered saline and 0.1% BSA and centrifuged at 150g for 15 min. The purity of mast cells recovered from the bottom of the tube was >90%, as assessed by toluidine blue staining.

#### Triggering of Intact Cells
- Purified mast cells (1 × 10⁶ cells/ml) were incubated in Tyrode buffer (137 mM NaCl, 2.7 mM KCl, 20 mM HEPES, 1.0 mM CaCl₂, 5.6 mM glucose, 1 mg/ml BSA, pH 7.4) with or without the indicated stimuli for 20 min. Reactions were terminated by placing the tubes in ice followed by a brief centrifugation (12,000g, ~20 s) at 4°C. Supernatants were collected and used to determine the amount of histamine released. Cell pellets were lysed and used to determine protein tyrosine phosphorylation or MAPK activation.

#### Determination of Protein Tyrosine Phosphorylation
- Cell extracts prepared by addition of a lysis buffer (150 mM sucrose, 80 mM β-glycerophosphate, 2 mM EDTA, 2 mM EGTA, 2 mM sodium orthovanadate, 10 mM sodium pyrophosphate, 1% Triton X-100, 1 mM phenylmethylsulfonyl fluoride, and 40 μM protease inhibitor cocktail) were centrifuged for 15 min at 12,000g. Supernatants were mixed with 5× concentrated Laemmli sample buffer (Laemmli, 1970). Samples were boiled, resolved by SDS-10% polyacrylamide gel electrophoresis (10% PAGE) under reducing conditions and transferred to polyvinylidene difluoride membranes. The membranes were incubated overnight at 4°C with monoclonal antibodies directed against P-Tyr (PY20, 1 μg/ml). Bound antibodies were visualized by enhanced chemiluminescence detection with the use of goat antiserum to mouse coupled to horseradish peroxidase (Jackson ImmunoResearch Laboratories).

#### Determination of MAPK Activation
- Cell extracts, prepared as described above for the determination of protein tyrosine phosphorylation, were resolved by SDS-10% PAGE under reducing conditions and transferred to nitrocellulose membranes. The membranes were incubated overnight at 4°C with polyclonal antibodies directed against the active, phosphorylated form of p42/p44 MAPKs (1:20,000 dilution). Bound antibodies were visualized by enhanced chemiluminescence detection with the use of goat antiserum to rabbit coupled to horseradish peroxidase (Jackson ImmunoResearch Laboratories).

#### Determination of Histamine Release
- The amount of histamine released was determined as previously described (Aridor et al., 1990), with the O-phthalaldehyde fluorimetric method (Shore et al., 1959).

#### Determination of AA Release
- Purified mast cells were suspended in RPMI 1640 medium, supplemented with 10% fetal calf serum at a concentration of 5 × 10⁵ cells/ml, and incubated with 2 to 5 μCi/ml [³H]AA for 2 h at 37°C. The cells were subsequently washed three times in Tyrode buffer, resuspended in Tyrode at 2 × 10⁵ cells/ml, and triggered for 20 min. Reactions were terminated by placing the tubes in ice followed by a brief centrifugation (12,000g, ~20 s) at 4°C. Supernatants were collected and used to determine the amount of radio-labeled AA released by liquid scintillation.

#### Determination of Inositol Phosphate (IP) Release
- Purified mast cells were suspended in low inositol medium (medium 199) containing [³H]myo-inositol (100 μCi) and incubated for 18 h. The cells were then washed twice with Tyrode buffer and triggered for 20 min at 37°C in the presence of 10 mM LiCl in a final volume of 400 μl. The reaction was stopped by addition of 1.5 ml chloroform/methanol (1:2) followed by 0.5 ml of chloroform and 0.5 ml of water. Phases were separated by centrifugation for 5 min at 1000 rpm. The water-soluble fraction was loaded on a Dowex column (format form), and free inositol was washed with 2 × 5 ml of water. IPs were eluted sequentially with 2 × 5 ml 0.1 M formic acid/0.2 M ammonium formate, 2 × 5 ml of 0.1 M formic acid/0.4 M ammonium formate, and 2 × 5 ml of 0.1 M formic acid/1 M ammonium formate. The radioactivity released was quantified by liquid scintillation.
Presentation of Data. The data points are means of duplicate determinations that did not vary by >2%. Similar results were obtained on at least two occasions.

Results

Activation of MAPKs by c48/80 in Rat Peritoneal Mast Cells. As shown in Fig. 1A and consistent with our previous studies (Shefler et al., 1998), incubation of rat peritoneal mast cells with c48/80 in the presence of vanadate (0.1 mM), a potent inhibitor of protein tyrosine phosphatases, stimulated tyrosine phosphorylation of several cellular proteins. The presence of vanadate was absolutely necessary, indicating that protein tyrosine phosphorylation in mast cells was tightly regulated by protein tyrosine phosphatases. Western blot analysis of mast cell lysates with an antibody specific for the activated, phosphorylated forms of the p42/p44 MAPKs revealed that c48/80 also increased the phosphorylation of MAPKs (Fig. 1B, top panel). Moreover, this phosphorylation/activation was enhanced when the cells were incubated with vanadate (Fig. 1B, top panel).

Kinetics of Activation of MAPKs by c48/80. The time course of MAPK activation was investigated. As shown in Fig. 2 (A and C), in the absence of vanadate, a biphasic pattern of phosphorylation was observed, where phosphorylation reached a maximum level at 15 s after addition of c48/80 and gradually declined thereafter. In contrast, in the presence of vanadate, phosphorylation of the kinases was sustained for at least 1 h (Fig. 2, B and C).

Effect of Tyrphostin on MAPK Activation. The tyrphostin AG-18, a competitive inhibitor of PTKs, effectively inhibits the enhancement in tyrosine phosphorylation caused by c48/80 (Shefler et al., 1998). Incubation with AG-18 also completely blocked the increase in MAPK phosphorylation induced by c48/80 in the absence (Fig. 1A) or presence (Fig. 3) of vanadate. Inhibition by AG-18 was dose dependent; half-maximal inhibition (IC50) was achieved at a concentration of 40 μM and maximal inhibition at around 100 μM (Fig. 3B).

These results have therefore implicated the involvement of protein tyrosine phosphorylation in MAPK activation.

Dose Response of c48/80-Induced Activation of MAPKs. Half-maximal stimulation of phosphorylation of p42/p44 MAPKs by c48/80 was achieved at a concentration of 2 μg/ml (Fig. 4). This concentration was 10-fold higher than that required to evoke half-maximal histamine release (e.g., 0.25 μg/ml, Fig. 4B) but similar to that of the enhancement in protein tyrosine phosphorylation (Shefler et al., 1998).

Effect of Ptx on c48/80-Induced Protein Tyrosine Phosphorylation and MAPK Activation. c48/80 triggers histamine release from mast cells by activating directly Ptx-sensitive Gi proteins (Aridor and Sagi-Eisenberg 1990; Aridor et al., 1990; Beub et al., 1990; Mousli et al., 1990; Aridor et al., 1993). As illustrated in Fig. 5, pretreatment with Ptx has also completely abolished the increase in both protein tyrosine phosphorylation (Fig. 5A) and p42/p44 MAPK phosphorylation (Fig. 5B) induced by c48/80, indicating that Gi proteins also mediate the increase in protein tyrosine and MAPK phosphorylation induced by c48/80.

Role of PKC in c48/80-Induced Activation of MAPKs. To investigate how activation of Gi proteins signals to the MAPKs, we examined the involvement of PKC, which serves as an mediator in the mechanism by which c48/80 stimulates protein tyrosine phosphorylation (Shefler et al., 1998). For this purpose, several PKC inhibitors were used. Both K252a and GF109203X, which is considered specific for the α, β, γ, ε, and δ PKC isoforms, have completely abolished MAPK phosphorylation in a dose-dependent manner (Figs. 6 and 7). The IC50 values calculated for the inhibition by K252a and GF109203X were 30 ng/ml and 10 nM, respectively. These values correlated well with the effectiveness of these drugs in inhibiting c48/80-stimulated tyrosine phosphorylation. In contrast, Go 6976, which specifically blocks the activity of the PKC α and β isoforms, has failed to affect the MAPK state of phosphorylation through a wide range of concentrations (up to 100 nM) tested (data not shown).

A.  
B.  

Fig. 1. Stimulation of protein tyrosine phosphorylation (A) and activation of MAPKs (B) by c48/80. A, purified mast cells (2 x 10⁷ cells/ml) were incubated for 15 min at 37°C with buffer or vanadate (0.1 mM) as indicated. Buffer or c48/80 (5 μg/ml) was subsequently added, and cells were incubated an additional 20 min. At end of incubation, cells were sedimented, washed, and lysed. Cell extracts were resolved by SDS/10% PAGE and immunoblotted with anti-P-Tyr antibodies. The 68-kDa protein also detected in nonstimulated cells reflects nonspecific binding of antibodies to residual BSA. B, mast cells were incubated for 15 min with buffer, vanadate (0.1 mM), or AG-18 (100 μM) as indicated. Buffer or c48/80 (5 μg/ml) was subsequently added, and cells were incubated for an additional 30 s. Cell extracts were resolved by SDS/10% PAGE and probed with anti-active MAPK antibodies (top panel). Bottom panel, same blot reprobed with anti-MAPK antibodies against total p42/p44 MAPKs.
Effect of Ca\(^{2+}\) on c48/80-Induced Phosphorylation of MAPKs. The enhancement in protein tyrosine phosphorylation induced by c48/80 is dependent on the presence of external Ca\(^{2+}\) (Shefler et al., 1998). Similarly, phosphorylation of the p42/p44 MAPKs was 10-fold higher in the presence of external Ca\(^{2+}\) than in its absence (Fig. 8).

Effect of Tyrphostin on IP Formation. The dependence of MAPK activation on both protein tyrosine phosphorylation and phospholipase C (PLC)-derived second messengers (Ca\(^{2+}\) and PKC) suggests that the putative PTKs, activated by c48/80, could be located either upstream or downstream from PLC. Therefore, to position this PTK, we studied the effect of AG-18 on c48/80-induced IP formation. In the absence of AG-18, c48/80 stimulated the production of IP fourfold (Table 1). Incubation of the cells with AG-18 before stimulation with c48/80 has partially inhibited (by 50%) this response (Table 1), revealing that c48/80 stimulates inositol phospholipid breakdown by two distinct mechanisms, one of which is dependent on protein tyrosine phosphorylation.

Role of PI-3K in c48/80-Induced Activation of MAPKs. Because PI-3K serves a mediator in c48/80-induced protein tyrosine phosphorylation (Shefler et al., 1998), as well as in G protein-triggered activation of MAPKs (Hawes et al., 1996; Lopez-Ilasaca et al., 1997), we used wortmannin and LY294002, two structurally unrelated inhibitors of PI-3K, to examine whether PI-3K also played a role in the activation of p42/p44 MAPKs. Indeed, both wortmannin (Fig. 9) and LY294002 (Fig. 10) inhibited phosphorylation of MAPKs in a dose-dependent manner, with IC\(_{50}\) values (20 nM and 2 \(\mu\)M, respectively) at which they are considered specific for PI-3K (Figs. 9B and 10B).

Role of p42/p44 MAPKs in Mediating c48/80-Induced AA Release. MAPKs mediate the phosphorylation and activation of the cytosolic form of phospholipase A\(_2\) (cPLA\(_2\)) (Lin et al., 1993) and constitute the major pathway of regulation of AA production in immunologically triggered mast cells (Zhang et al., 1997). Activation of the MAPKs by c48/80 is achieved ahead of the release of AA/eicosanoid metabolites (Fig. 2C), and their dose-response relationships are closely correlated (Fig. 4B), suggesting a possible causal relationship. We therefore investigated the role of MAPKs in c48/80-induced release of AA/eicosanoid metabolites. As illustrated in Fig. 11A, c48/80 has increased the phosphorylation of cPLA\(_2\), as evident by the mobility shift of the band (Fig. 11A). However, whereas incubation with the MAPKK inhibitor PD98059 has completely inhibited the increased phosphorylation of p42/p44 MAPKs (Fig. 11B), neither the phosphorylation of cPLA\(_2\) (Fig. 11A) nor the release of AA/eicosanoid...
metabolites (data not shown) was affected by this inhibitor. To further validate the results obtained with anti-active MAPK antibodies, we also performed in vitro kinase assays on c48/80-activated mast cells. As illustrated in Fig. 11D, the direct kinase assay paralleled the results with the anti-active MAPK antibodies. These results indicate that c48/80-stimulated the activation of MAPKs, but this process did not contribute to the stimulation of cPLA₂ phosphorylation or the production of AA metabolites.
Exocytosis in mast cells is activated in response to either aggregation of the high-affinity receptors for IgE, the \( \text{Fc} \varepsilon \text{RI} \) (Segal et al., 1977), or by direct activation of Ptx-sensitive G proteins by the family of receptor mimetic basic secretagogues (Aridor and Sagi-Eisenberg 1990; Aridor et al., 1990; Beub et al. 1990; Mousli et al. 1990). Cytosolic src-like PTKs play a pivotal role in mediating \( \text{Fc} \varepsilon \text{RI} \)-induced exocytosis (Hamawy et al., 1995), whereas basic secretagogue-induced exocytosis is mediated by direct activation of the heterotrimeric G protein \( \text{G}_i \) (Aridor et al., 1993). Nevertheless, we recently demonstrated (Shefler et al., 1998) that c48/80, a synthetic member of the basic secretagogues, activates an unidentified PTK, resulting in enhanced tyrosine phosphorylation of several cellular substrates. This finding suggests that basic secretagogues induce tyrosine phosphorylation as part of their multiple signaling pathways.

The PTK-signaling pathway played no role in the activation of histamine secretion by basic secretagogues, but it largely contributed to the activation of AA release (Shefler et al., 1998). Moreover, both PKC and PI-3K, two important components in the \( \text{Fc} \varepsilon \text{RI} \)-induced signaling pathway (Sagi-Eisenberg and Pecht 1984; Sagi-Eisenberg et al., 1985; Yano et al., 1993), served as mediators in the mechanism by which basic secretagogues stimulated tyrosine phosphorylation. These results suggest that, although basic secretagogues may use a mechanism distinct from that of the immunological trigger to activate exocytosis and release of preformed mediators such as histamine, part of their signaling pathways may converge with that of the immunological trigger to control the release of de novo-formed mediators such as AA metabolites. Therefore, in this study, we further investigated this hypothesis.

AA is the precursor of prostaglandins and leukotrienes, potent inflammatory mediators, which are produced de novo after mast cell activation by either the immunological or the

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**Discussion**

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**TABLE 1**

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<th>Addition</th>
<th>Experiment 1</th>
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<tr>
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<tr>
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![Fig. 7](image-url) Inhibition of c48/80-induced MAPK activation by GF109203X. A, purified mast cells (2 \( \times \) 10^7 cells/ml) were preincubated for 15 min at 37°C in the presence of vanadate (V, 0.1 mM) and indicated concentrations of GF109203X. Buffer (lane 1) or c48/80 (5 \( \mu \)g/ml, lanes 2–7) was subsequently added for another 20-min incubation. At the end of the incubation, cells were sedimented, and activation of MAPKs was determined as described in Fig. 1B. B, intensity of bands corresponding to pp42 (\( \text{F} \)) and pp44 (\( \text{M} \)) were quantified by densitometry and are presented as percentages of their maximal responses.

![Fig. 8](image-url) Effect of external Ca^{2+} on c48/80-induced activation of MAPKs. Purified mast cells (2 \( \times \) 10^8 cells/ml) were incubated in the presence of vanadate (V, 0.1 mM) and Ca^{2+} (1 mM, lanes 1 and 2) or without Ca^{2+} and with EGTA (0.1 mM, lanes 3 and 4). Buffer (lanes 1 and 3) or c48/80 (5 \( \mu \)g/ml, lanes 2 and 4) was added for another 20 min. Cells were subsequently sedimented, and activation of MAPKs was determined as described in Fig. 1B.

![Fig. 9](image-url) Inhibition of c48/80-induced MAPK activation by wortmannin. A, purified mast cells (2 \( \times \) 10^7 cells/ml) were preincubated for 15 min at 37°C in the presence of vanadate (V, 0.1 mM) and indicated concentrations of wortmannin. At the end of the incubation, buffer (lane 1) or c48/80 (5 \( \mu \)g/ml, lanes 2–6) was added for a 20-min incubation period. At the end of the incubation, cells were sedimented, and activation of MAPKs was determined as described in Fig. 1B. B, intensity of bands corresponding to pp42 (\( \text{F} \)) and pp44 (\( \text{M} \)) were quantified by densitometry and are presented as percentages of their maximal responses.
nonimmunological triggers. cPLA\(_2\) is considered to mediate Fc\(\varepsilon\)RI-induced release of AA (Zhang et al., 1997). This enzyme requires, for activation, both elevation of the intracellular concentration of Ca\(^{2+}\) and a phosphorylation step, which could be catalyzed by the MAPK (Lin et al., 1993). Indeed, Fc\(\varepsilon\)RI-induced release of AA is primarily regulated by the p42 MAPK, which phosphorylates and activates cPLA\(_2\) (Zhang et al., 1997). We now demonstrate that c48/80 also activates both p42 and p44 MAPKs (Figs. 1B and 11D). Activation of both MAPKs is strictly dependent on the PTK signaling pathway. Inhibitors such as the PTK inhibitor, AG-18 (Figs. 1 and 3), the PKC inhibitors K252a and GF109203X (Figs. 6 and 7), and the PI-3K inhibitors wortmannin and LY294002 (Figs. 9 and 10), which block tyrosine phosphorylation, also abolish the activation of MAPKs. Moreover, analyses of the inhibition curves revealed that these inhibitors affected the activation of either p42 or p44 MAPKs in a similar manner, suggesting that both were activated by a similar mechanism, which included PKC, PI-3K, and the unidentified PTK as mediators. Conversely, vanadate and Ca\(^{2+}\), which enhance c48/80-induced tyrosine phosphorylation, also potentiate MAPK activation (Figs. 1B and 8). Notably, although vanadate alone can activate MAPKs in certain cell types (D’Onofrio et al., 1994), vanadate alone has no effect in mast cells.

Both stimulation of tyrosine phosphorylation and activation of the p42/p44 MAPKs were abolished when the cells were pretreated with Ptx (Fig. 5). This finding establishes that, like exocytosis, activation of PTKs and MAPKs was mediated by Ptx-sensitive G proteins, presumably Gi\(_o\) or Gi\(_i\), which are the only Ptx substrates present in rat peritoneal mast cells (Aridor et al., 1993).

Interestingly, G proteins have been reported to activate MAPKs by two distinct mechanisms. Both Gaq and Gao stimulate MAPK via a PKC-dependent mechanism (Faure et al., 1994; van Biesen et al., 1996). In contrast, G\(\beta\)\(y\) subunits activate MAPK by a mechanism that is independent of PKC but involves both PI-3K and PTKs as mediators (Crespo et al., 1994). The mechanism by which the basic secretagogue-activated Gi proteins activate p42/p44 MAPKs in mast cells is, however, different. This mechanism is dependent on PKC and yet involves PI-3K and PTK as mediators. Whether this activation is mediated by both the \(\alpha\)- and the \(\beta\)\(y\)-subunits of the activated G proteins or whether it reflects a novel mechanism is unknown. Note that our findings do indicate that activation of PTKs contributes, at least partly, to the activation of PLC and therefore also to PKC (Table 1). However, our
PKC is a highly specific PKC inhibitor that shows selectivity for more general protein kinase inhibitor. However, GF109203X PLC.

Multiple PTKs located both upstream and downstream from MAPKs in mast cells may therefore involve activation of PTK downstream from PKC. The mechanism by which previous studies (Shefler et al., 1998) located PI-3K and a PTK downstream from PKC. The mechanism by which the basic secretagogue-activated GI proteins activate p42/p44 MAPKs in mast cells may therefore involve activation of multiple PTKs located both upstream and downstream from PLC.

The three PKC inhibitors used in this study exhibit distinct specificities toward various PKC isozymes. K252a is a more general protein kinase inhibitor. However, GF109203X is a highly specific PKC inhibitor that shows selectivity for PKCa, P1, β2, γ, δ, and ε, whereas Go6976 selectively inhibits the α- and β- Ca2+-dependent PKC isozymes (reviewed in Hofmann, 1997). Therefore, the failure of Go6976 to effect activation of MAPKs strongly implicates the Ca2+-independent PKC isozymes, PKCβ or PKCε, in mediating this response.

Despite the fact that release of AA metabolites and activation of p42/p44 MAPKs were closely correlated in their dose-response relationships (Fig. 4B), and MAPK activation preceded AA/eicosanoid metabolite release (Fig. 2C), inhibition of MAPK activation by the MAPKK inhibitor PD98059 failed to suppress the release of AA (Fig. 11). Thus, in marked contrast to the immunological triggered release of AA, which is independent of PKC but requires MAPK activation (Hirasawa et al., 1995), the Gi-mediated trigger of AA release is PKC dependent but does not require activation of the p42/p44 MAPKs. This finding suggests that at a certain point downstream from the activation of PKT by cG8/80, the signaling pathways initiated by Ptx-sensitive GI proteins diverge to activate the MAPK pathway and the release of AA/eicosanoid metabolites.

In conclusion, the results of this study provide direct evidence for the activation of p42/p44 MAPKs by the receptor mimetic basic secretagogues of mast cells. This activation is triggered by Ptx-sensitive GI proteins and involves PKC, PI-3K, and PTK as mediators. This study also demonstrates that by directly activating GI proteins, the basic secretagogues transmit multiple signaling pathways that independently regulate the production or release of the various inflammatory mediators. In the light of the increasing number of diseases associated with the activation of mast cells by nonimmunological triggers, e.g., psychogenic asthma, psoriasis, interstitial cystitis, bowel diseases, migraines, and multiple sclerosis (reviewed in Theoharides, 1996), unveiling of the mechanisms by which basic secretagogues activate mast cells is not only theoretically important, but it also has a significant clinical impact.

Acknowledgments

We thank Dr. Yehiel Zick for very helpful discussions and a critical review of the manuscript.

References


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