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Substance P-related inhibitors of mast cell exocytosis act on G-proteins or on the cell surface

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Abstract

[*p*-Glu^5, D-Trp^{7,9,10}]substance P-(5–11) inhibited mastoparan-stimulated GTPase activity in homogenized rat peritoneal mast cells and decreased histamine secretion induced by mastoparan from streptolysin O-permeabilized mast cells (IC_{50} of about 30 μM), but not from intact cells. In contrast, [D-Pro^4, D-Trp^{7,9,10}]substance P-(4–11) inhibited the secretion from intact cells (IC_{50} of about 10 μM) but had no effect on histamine secretion from permeabilized cells, suggesting that this peptide exerts its inhibitory effect on the plasma membrane, whereas [*p*-Glu^5, D-Trp^{7,9,10}]substance P-(5–11) interacts with G proteins. Pretreatment of mast cells with neuraminidase led to an inhibition of the secretory response to mastoparan and related triggers. This response was restored following cell permeabilization, demonstrating the role of the cell surface on the entry of mastoparan and related triggers and on their ability to reach G proteins sensitive to pertussis toxin and [*p*-Glu^5, D-Trp^{7,9,10}]substance P-(5–11). © 1998 Elsevier Science B.V.

Keywords: Mast cell; G protein; [D-Pro^4, D-Trp^{7,9,10}]substance P-(4–11); [*p*-Glu^5, D-Trp^{7,9,10}]substance P-(5–11); Mastoparan; GTPase activity

1. Introduction

Histamine release from rat peritoneal mast cells has been extensively used as a model of exocytosis (Nakamura and Ui, 1985). The introduction into mast cells of non-hydrolyzable analogs of GTP that persistently activate GTP-binding proteins induces exocytosis (Gomperts and Fernandez, 1985; Aridor et al., 1990). The early events after stimulation of mast cells include the breakdown of phosphoinositides (PIP_2), a rise in the internal Ca^{2+} concentration and the generation of arachidonic acid (Nakamura and Ui, 1985; Penner et al., 1988). A wasp peptide venom, mastoparan, is known to induce histamine release from mast cells (Hirai et al., 1979; Mousli et al., 1989). It was previously shown that mastoparan can interact directly with G proteins and stimulates their GTPase activity (Higashijima et al., 1988). Many of the effects of mastoparan are sensitive to pertussis toxin, which inhibits G_i and G_o by ADP-ribosylation of their α subunit. Based on these studies, it has been proposed that the target of mastoparan in mast cells is a G protein (Mousli et al., 1989) and particularly $G_{\alpha i3}$ (Aridor et al., 1993). The same mechanism has been proposed to explain the stimulation of histamine secretion from mast cells produced by other basic compounds such as substance P, compound 48/80, bradykinin and anaphylatoxin C3a (Mousli et al., 1990a,b, 1992; Bueb et al., 1990). This mechanism has been extended to peptides acting at nanomolar concentrations on rat peritoneal mast cells (Mousli et al., 1995) and on human skin mast cells (Emadi-Khiav et al., 1995).

It has been reported that benzalkonium chloride and a substance P analog, [D-Pro^4, D-Trp^{7,9,10}]substance P-(4–11), can inhibit the secretion of histamine from mast cells stimulated by compound 48/80 (Piotrowski et al., 1984; Piotrowski and Foreman, 1985). More recently Higashijima's group (Mukai et al., 1992) has shown that this peptide and a novel analog [*p*-Glu^5, D-Trp^{7,9,10}]substance P-(5–11) inhibit the mastoparan-promoted GTP hydrolysis by G_i in reconstituted phospholipid vesicles, with [*p*-Glu^5, D-Trp^{7,9,10}]substance P-(5–11) being 10-fold more potent than [D-Pro^4, D-Trp^{7,9,10}]substance P-(4–11). The effect of [*p*-Glu^5, D-Trp^{7,9,10}]substance P-(5–11) on mast cells has not been studied. Another way to block the response of mast cells to mastoparan and related compounds is to hydrolyze sialic acid residues on the cell surface with

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neuraminidase (Foreman and Lichtenstein, 1980; Coleman et al., 1986; Mousli et al., 1989, 1992; Bueb et al., 1990). This observation suggested that preliminary binding of cationic triggers to the cell surface is required for their later interaction with intracellular G proteins. This proposal implied that the permeabilization of mast cells should reverse the inhibitory effect of neuraminidase.

The aim of the present study was to investigate the effect of [D-Pro^4, D-Trp^{7,9,10}]substance P-(4–11) and [*p*-Glu^5, D-Trp^{7,9,10}]substance P-(5–11) on histamine secretion and on GTPase activity in the supernatant of homogenized mast cells, in order to confirm the role of G proteins in the secretory response of mast cells. These peptides do not exhibit amphiphilic properties and thus do not pass through membranes. Thus, cell permeabilization was required to test this putative inhibition. Moreover, we re-examined the requirement of intact sialic acid residues on the cell surface for a potent response of mast cells to triggering peptides.

2. Materials and methods

2.1. Materials

Benzalkonium chloride-C_{14}, type V neuraminidase from *Clostridium perfringens*, compound 48/80 and Ca^{2+} ionophore A23187 were purchased from Sigma Chemical (St. Louis, MO). Mastoparan, neuropeptide Y-(18–36) and substance P were obtained from Neosystem (Strasbourg, France). Substance P analogs were from Bachem Biochimie (Paris, France) and pertussis toxin was from List Biological Laboratory (Campbell, CA). Streptolysin O was from Institut Pasteur (Paris, France). [$^{32}\gamma$P]GTP was purchased from New England Nuclear-DuPont (Boston, MA).

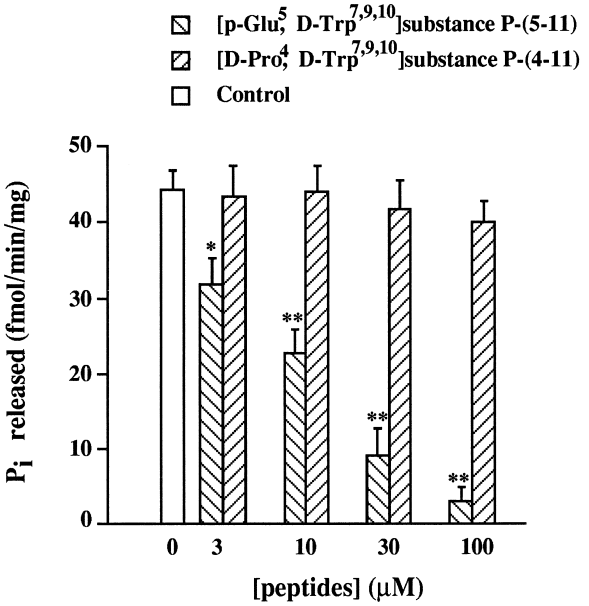

Fig. 1. Effect of substance P analogs on the high-affinity GTPase activity of purified rat peritoneal mast cells. The 12,000× *g* supernatant of homogenized mast cells (10 μg) was incubated at 25°C, for 30 min, in the presence of 100 μM mastoparan with increasing concentrations of [*p*-Glu^5, D-Trp^{7,9,10}]substance P-(5–11) or [D-Pro^4, D-Trp^{7,9,10}]substance P-(4–11). Basal high-affinity GTPase activity = 29.5 ± 3.6 fmol P_i/min/mg protein. Values are means ± S.E.M. of three independent experiments. $^{*}P < 0.05$; $^{**}P < 0.01$.

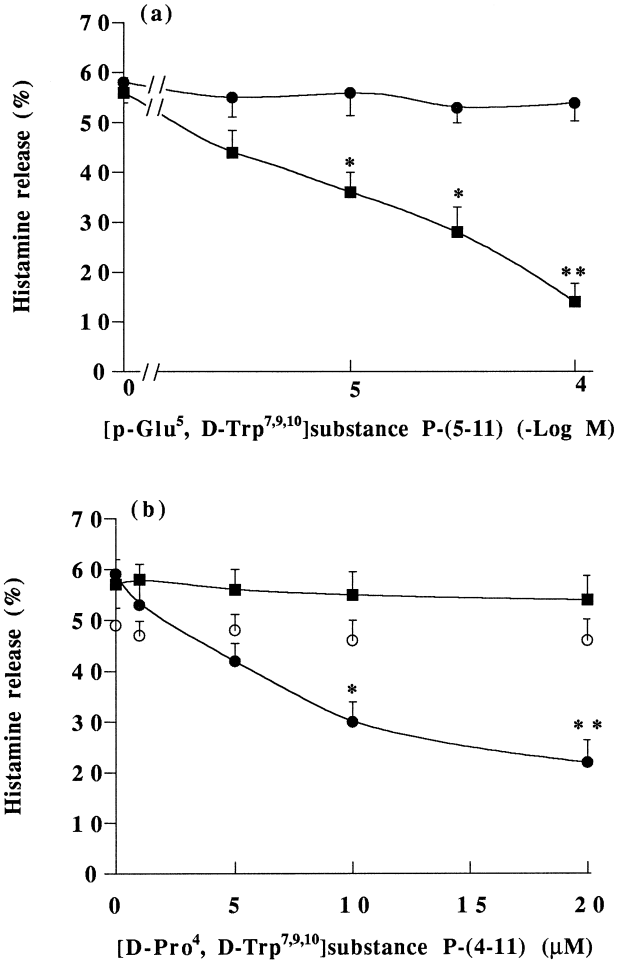

Fig. 2. Effect of substance P analogs on histamine secretion induced by mastoparan from rat peritoneal mast cells. Purified mast cells (3×10^4 cells/assay) were permeabilized with streptolysin O (15 U/ml) (■) for 5 min at 37°C or left intact (●). Cells were then preincubated with increasing concentrations of [*p*-Glu^5, D-Trp^{7,9,10}]substance P-(5–11) (a) or [D-Pro^4, D-Trp^{7,9,10}]substance P-(4–11) (b) for 10 min and stimulated with mastoparan 100 μM for 10 min. In intact cells, the control incubation (○) was performed with 1 μM Ca^{2+} ionophore A23187 in the presence of 1 mM $CaCl_2$. Values are means ± S.E.M. of three independent experiments each in duplicate. $^{*}P < 0.05$; $^{**}P < 0.01$.

2.2. Isolation and treatment of rat peritoneal mast cells

Male Wistar rats (Iffa-Credo, L'Arbresle, France) weighing 300 to 500 g were first stunned and then bled to death. 12 ml of balanced salt solution (HEPES buffer) containing (in mM): NaCl, 137; KCl, 2.7; $CaCl_2$, 0.3; $MgCl_2$, 1.0; NaH_2PO_4, 0.4; glucose, 5.6 and HEPES, 10;

Table 1
Effect of [D-Pro^4, D-Trp^{7,9,10}]substance P-(4–11) and [*p*-Glu^5, D-Trp^{7,9,10}]substance P-(5–11) on histamine secretion induced by different secretagogues from intact mast cells

Compound	Histamine release (% of total content)		
	Control	[D-Pro^4, D-Trp^{7,9,10}] substance P-(4–11)	[*p*-Glu^5, D-Trp^{7,9,10}] substance P-(5–11)
SP (30 μM)	51 ± 4.5	36 ± 3.9	48 ± 3.2
NPY-(18–36) (30 nM)	53 ± 5.2	36 ± 1.0	52 ± 3.6
C3a (10 μM)	50 ± 4.0	35 ± 3.0	49 ± 4.0
Compound 48/80 (0.1 μg/ml)	52 ± 1.0	38 ± 1.0	50 ± 3.8

Purified mast cells were preincubated with [D-Pro^4, D-Trp^{7,9,10}]substance P-(4–11) (20 μM) or [*p*-Glu^5, D-Trp^{7,9,10}]substance P-(5–11) (30 μM) for 10 min at 37°C and the cells were stimulated with indicated concentrations of secretagogues: (SP, substance P; NPY-(18–36), neuropeptide Y-(18–36); C3a, anaphylatoxin C3a). The reaction was stopped 10 min later by addition of ice-cold buffer. Values are means ± S.E.M. of three independent experiments performed in duplicate.

NaOH to pH 7.4, supplemented with 0.1% bovine serum albumin, was injected into the peritoneal cavity. Alternatively, the $CaCl_2$ concentration was raised to 1 mM in experiments performed with the Ca^{2+} ionophore A23187. The peritoneal fluid was collected and centrifuged for 2 min at 180 × *g*. The pellet was resuspended in the same solution and the mast cells were purified on a discontinuous bovine serum albumin gradient (30 and 40% w/v) as described previously (Mousli et al., 1989). The pellet was resuspended in HEPES buffer, and cells were examined under a light microscope for purity (more than 97% was typically observed). The Trypan blue-exclusion test indicated a viability of greater than 95%. Purified mast cells (3×10^4 cells/assay) were permeabilized or left intact and then treated with different reagents before addition of secretagogues as described in the figure legends or in the tables. Reactions were terminated 10 min later by the addition of ice-cold buffer and histamine in the supernatant was assayed fluorometrically, according to the method of Shore et al. (1959) but without the extraction step.

2.3. Mast cell permeabilization

Streptolysin O was prepared as described previously (Vitale et al., 1993). In the experiments with permeabilized cells, the solution used contained: potassium glutamate, 140 mM; glucose, 5 mM; salt of PIPES, 10 mM; MgATP, 5 mM; magnesium acetate, 4.5 mM; EGTA, 0.5 mM and $CaCl_2$, 1 μM; NaOH to pH 7.4. Purified mast cells were permeabilized by exposure to streptolysin O (15 U/ml) for 5 min at 37°C and pre-incubated with substance P analogs before addition of stimulants.

2.4. GTPase activity of mast cells

GTPase activity was determined as described previously (Hilf and Jakobs, 1989). Cells were preincubated for 10 min at 0°C in buffer A containing (in mM): ATP, 1; $MgCl_2$, 2; EDTA, 0.1; dithiothreitol, 1; NaCl, 150; phenylmethanesulphonylfluoride, 0.2; triethanolamine–HCl, 50; pH 7.4. Cells were Potter-homogenized and centrifuged for 15 min at 12,000 × *g*. The supernatant was removed, the volume was adjusted to 2 ml with buffer A and the solution was centrifuged in a Centricon 30 microconcentrator (Amicon, Danvers, MA) to the desired concentration (Bueb et al., 1990). GTPase activity was determined in a reaction mixture containing 10 μg of membrane proteins and 20 μl of drugs in a volume of 80 μl. The reaction was carried out in duplicate, and samples were first pre-incubated for 30 min at 25°C. The reaction was started by addition of 20 μl [$^{32}\gamma$P]GTP (30 Ci/mmol) so as to reach a final concentration of 0.1 μM. GTP hydrolysis was stopped after 15 min of incubation at 25°C by the addition of 0.7 ml of an ice-cold 5% (w/v) charcoal suspension (pH 7.4). The tubes were centrifuged for 15 min at 9000 × *g* at 4°C and 0.4 ml of the supernatant was put in counting vials containing 3.6 ml scintillation liquid to determine the released $^{32}P_i$. High-affinity GTPase activity was calculated by subtracting $^{32}P_i$ released in the presence of 50 μM of unlabelled GTP (low-affinity GTPase activity) from the total $^{32}P_i$ accumulation determined in the presence of 0.1 μM [$^{32}\gamma$P]GTP. Basal high-affinity GTPase activity in the absence of added drugs was 29.5 ± 3.6 fmol P_i/min/mg protein ($n = 3$).

Table 2
Effect of various treatments on histamine release induced by mastoparan (30 μM) from intact and streptolysin O-permeabilized rat peritoneal mast cells. Cells were preincubated with pertussis toxin for 2 h at 37°C, permeabilized or left intact and then stimulated with mastoparan. Benzalkonium chloride was added concomitantly with mastoparan. Values are means ± S.E.M. from three separate experiments

Treatments	Histamine secretion (% of total content)	
	intact cells	permeabilized cells
Control	56 ± 4.1	58 ± 4.0
Pertussis toxin (10 ng/ml)	16 ± 3.0	20 ± 3.6
Benzalkonium chloride (2 μg/ml)	22 ± 3.5	20 ± 2.8

3. Results

3.1. Effect of [p-Glu^5, D-Trp^{7,9,10}]substance P-(5–11) and [D-Pro^4, D-Trp^{7,9,10}]substance P-(4–11) on GTPase activity and histamine release induced by mastoparan

[*p*-Glu^5, D-Trp^{7,9,10}]substance P-(5–11) has been reported as a G protein antagonist (Mukai et al., 1992). Fig. 1 shows that this substance P analog inhibited mastoparan-stimulated high-affinity GTPase activity of the supernatant from homogenized mast cells in a dose-dependent manner with an IC_{50} of about 10 μM. In contrast, [D-Pro^4, D-Trp^{7,9,10}]substance P-(4–11) was ineffective up to 100 μM (Fig. 1). Fig. 2a shows that [p-Glu^5, D-Trp^{7,9,10}]substance P-(5–11) did not modify the response of intact mast cells to mastoparan. We could not exclude that the mast cell membrane is impermeable to this peptide, thus we used streptolysin O-permeabilized rat peritoneal mast cells to study its effect on histamine secretion. In permeabilized cells, preincubation with [*p*-Glu^5, D-Trp^{7,9,10}]substance P-(5–11) for 10 min resulted in a dose-dependent inhibition of histamine secretion induced by mastoparan (Fig. 2a). The half-maximal effect of [p-Glu^5, D-Trp^{7,9,10}]substance P-(5–11) was observed at a concentration of 30 μM. At concentrations less than 3 μM, [*p*-Glu^5, D-Trp^{7,9,10}]substance P-(5–11) inhibited neither GTPase activity nor histamine release induced by mastoparan. In contrast, [D-Pro^4, D-Trp^{7,9,10}]substance P-(4–11) inhibited histamine secretion induced by mastoparan from intact mast cells, as previously shown with other secretagogues (Piotrowski and Foreman, 1985). Histamine release induced by Ca^{2+} ionophore A23187 was not inhibited by [D-Pro^4, D-Trp^{7,9,10}]substance P-(4–11) (Fig. 2b). However, this weak in vitro inhibitor of G proteins (Mukai et al., 1992) was ineffective on permeabilized mast cells (Fig. 2b). We used [D-Pro^4, D-Trp^{7,9,10}]substance P-(4–11) at a concentration of 20 μM because at higher concentrations this peptide causes modest secretion of histamine (Piotrowski et al., 1984 and confirmed by us). Table 1 shows also that [D-Pro^4, D-Trp^{7,9,10}]substance P-(4–11) inhibited histamine release induced by compound 48/80, substance P, anaphylatoxin C3a and neuropeptide Y-(18–36) to the same extent (35–38%), whereas [*p*-Glu^5, D-Trp^{7,9,10}]substance P-(5–11) was ineffective on intact cells, irrespective of the secretagogue used. The concentrations of the different secretagogues in Table 1 were chosen to induce the release of about 50% of the histamine content.

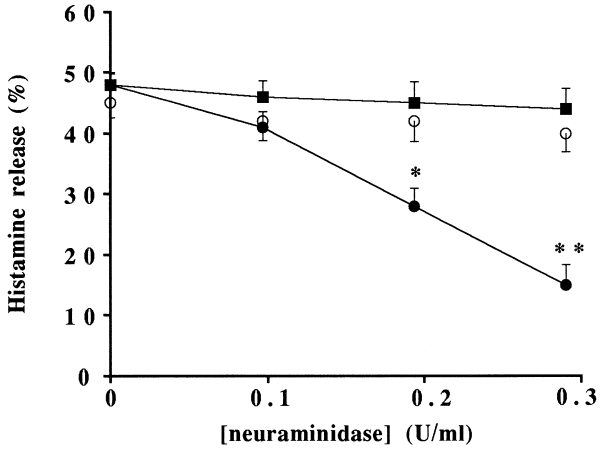

Fig. 3. Effect of neuraminidase pretreatment on histamine secretion induced by mastoparan from intact and permeabilized mast cells. Purified rat peritoneal mast cells were pretreated with increasing concentrations of neuraminidase for 1 h at 37°C and then permeabilized with streptolysin O (15 U/ml) for 5 min (■) or left intact (●). Cells were finally stimulated with 100 μM mastoparan for 10 min. In intact cells, the control incubation (○) was performed with 1 μM Ca^{2+} ionophore A23187 in the presence of 1 mM $CaCl_2$. Results are means ± S.E.M. from three independent experiments; $^{*}P < 0.05$; $^{**}P < 0.01$.

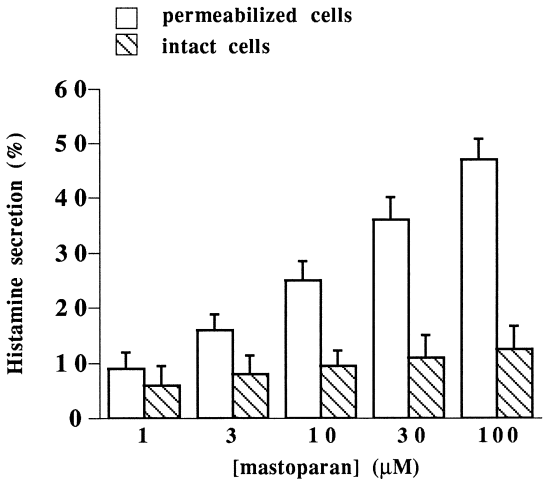

Fig. 4. Effect of permeabilization on mastoparan-induced histamine release from neuraminidase-pretreated cells. Cells were pretreated with neuraminidase (0.3 U/ml) for 1 h at 37°C and then permeabilized or left intact. Cells were finally stimulated with increasing concentrations of mastoparan for 10 min. Values are means ± S.E.M. of three separate experiments carried out in duplicate.

Pretreatment of intact and streptolysin-permeabilized mast cells for 2 h with pertussis toxin (10 ng/ml) had an inhibitory effect on the response to mastoparan (Table 2). In the presence of the G_i protein-selective inhibitor benzalkonium chloride (2 μg/ml) (Higashijima et al., 1990), we also observed an inhibition of secretion from both intact and permeabilized mast cells (Table 2). We observed that benzalkonium chloride used at concentrations higher than 3 μg/ml caused cytotoxic histamine release, as previously shown by Read and Kiefer (1979). [*p*-Glu^5, D-Trp^{7,9,10}]substance P-(5–11), unlike pertussis toxin and benzalkonium chloride, which inhibited histamine release from both intact and permeabilized mast cells (Table 2), inhibited histamine secretion from streptolysin-permeabilized cells only (Fig. 2a). In contrast, [D-Pro^4, D-

Trp^{7,9,10}]substance P-(4–11) had an inhibitory effect on histamine secretion induced by mastoparan from intact cells only with an IC_{50} of about 10 μM (Fig. 2b).

3.2. Effect of streptolysin O-permeabilization on histamine release induced by mastoparan from neuraminidase-pretreated mast cells

Histamine secretion induced by mastoparan was inhibited in a dose-dependent manner by pretreatment of intact mast cells for 1 h with neuraminidase, whereas histamine release induced by Ca^{2+} ionophore A23187 was unaffected (Fig. 3). This inhibition was very similar to that for other cationic secretagogues (Bueb et al., 1990; Mousli et al., 1989), reaching around 70% when mast cells were pretreated with 0.3 U/ml neuraminidase for 1 h (Fig. 3). However, pretreatment with neuraminidase did not have an inhibitory effect on histamine release induced by mastoparan (30 μM) from streptolysin-permeabilized mast cells (Fig. 3). When neuraminidase-pretreated cells were permeabilized with streptolysin O, the dose-dependent secretory response of mast cells to mastoparan was fully restored in comparison to that of non-permeabilized cells (Fig. 4).

4. Discussion

The present experiments were designed to evaluate the effectiveness of [*p*-Glu^5, D-Trp^{7,9,10}]substance P-(5–11) on mast cells as well as other known inhibitors of histamine release induced by peptides. We asked whether the inhibitory effect of substance P-related peptides, [*p*-Glu^5, D-Trp^{7,9,10}]substance P-(5–11) and [D-Pro^4, D-Trp^{7,9,10}]substance P-(4–11), on G proteins described in reconstituted vesicles (Mukai et al., 1992) also occurs in mast cells. In the present study we chose mastoparan as a model of peptides and cationic compounds acting on mast cells, because the effect of this venom peptide on G proteins has been studied extensively. Control experiments were also performed with several other related peptides such as substance P, anaphylatoxin C3a, neuropeptide Y-(18–36) and with compound 48/80 to validate this choice. Mastoparan has been shown to activate heterotrimeric G proteins by a mechanism strikingly similar to that used by receptors (Higashijima et al., 1990).

Mizrahi et al. (1982) proposed [D-Pro^4, D-Trp^{7,9,10}]substance P-(4–11) as a potent antagonist of substance P. It inhibits the hypotensive effect of substance P in anesthetised rats, the vasodilatator effect in the rabbit perfused heart and the relaxation induced by substance P in dog carotid arteries contracted with noradrenaline. Piotrowski et al. (1984) showed that in mast cells [D-Pro^4, D-Trp^{7,9,10}]substance P-(4–11) inhibits histamine release induced by compound 48/80 but causes weak histamine secretion at high concentrations (30 μM or more). Furthermore, this peptide is known to inhibit amylase secretion induced by various structurally unrelated peptides such as substance P, bombesin and cholecystokinin (Zhang et al., 1988), suggesting the possibility of some non-selective mechanism of interaction with the cell surface. [*p*-Glu^5, D-Trp^{7,9,10}]substance P-(5–11) and [D-Pro^4, D-Trp^{7,9,10}]substance P-(4–11) can bind to G proteins and compete with receptors to block its effect on G proteins in reconstituted vesicles (Mukai et al., 1992). Both peptides also inhibited mastoparan-promoted activation of G_i proteins, although [*p*-Glu^5, D-Trp^{7,9,10}]substance P-(5–11) was 10-fold more potent than [D-Pro^4, D-Trp^{7,9,10}]substance P-(4–11). More recently, it has been shown that [*p*-Glu^5, D-Trp^{7,9,10}]substance P-(5–11) antagonizes the effect of mastoparan on catecholamine secretion from streptolysin-permeabilized chromaffin cells (Vitale et al., 1993, 1994). Interestingly, these peptides specifically interact with proteins of the G_i and G_o families (Mukai et al., 1992). In the present study we observed that [*p*-Glu^5, D-Trp^{7,9,10}]substance P-(5–11) (3–100 μM) blocked mastoparan-stimulated GTPase activity in the supernatant of homogenized mast cells. Conversely, [D-Pro^4, D-Trp^{7,9,10}]substance P-(4–11) had no effect up to 100 μM, but this peptide is also less potent in inhibiting mastoparan-promoted G protein activation in reconstituted vesicles (Mukai et al., 1992).

Two other inhibitors of heterotrimeric G proteins were used in intact and permeabilized mast cells. Benzalkonium chloride, a mixture of quaternary benzyldimethylalkylammonium, was found to inhibit histamine secretion induced by substance P, compound 48/80 and other related compounds, but not that caused by antigens (Read and Kiefer, 1979). Benzalkonium chloride causes by itself cytotoxic histamine release at concentrations over 5 μg/ml. Recently it has been reported that other alkylamines, namely tetradecyltrimethylammonium, inhibit not only exocytosis but also GTPase activity in mast cells (Fischer et al., 1993). Benzalkonium chloride selectively inhibits the mastoparan-induced activation of G_i, whereas alone it stimulates GTPase activity of G_o (Higashijima et al., 1990). The results obtained here show that benzalkonium chloride inhibited histamine secretion from both intact and permeabilized mast cells (Table 2).

Pretreatment with pertussis toxin, which inhibits the G proteins of G_i and G_o families by ADP-ribosylation of their α subunit (Gilman, 1987; Mousli et al., 1993), resulted in an inhibition of histamine release induced by mastoparan from both intact and permeabilized mast cells (Table 2). These results show that [*p*-Glu^5, D-Trp^{7,9,10}]substance P-(5–11), unlike pertussis toxin and benzalkonium chloride, inhibits histamine release induced by mastoparan from streptolysin-permeabilized mast cells but has no effect in intact cells. The results indicate also that [*p*-Glu^5, D-Trp^{7,9,10}]substance P-(5–11) is able to block histamine secretion providing that the peptide is introduced directly into the cytoplasm of permeabilized mast cells. The con-

centrations of the peptide used here are comparable to those used in studies of mastoparan-promoted GTP hydrolysis (see above). In contrast, [D-Pro^4, D-Trp^{7,9,10}]substance P-(4–11) inhibited histamine release induced by mastoparan from intact cells but not that caused by Ca^{2+} ionophore A23187 (present results) or by IgE-dependent activation (Piotrowski and Foreman, 1985), indicating that this substance P analog acts by a membrane-dependent mechanism selective for mastoparan-stimulated histamine release. This substance P analog also decreased to the same extent the secretory response of intact mast cells to compound 48/80, neuropeptide Y-(18–36), substance P and anaphylatoxin C3a (Table 1), which are thought to share some common structural properties with mastoparan (Mousli et al., 1990a,b, 1992). These results indicate that this substance P analog at low concentrations interacts at the plasma membrane level to inhibit the initial binding step of cationic secretagogues to the cells. At high concentrations this peptide induces a secretory response, suggesting that it might behave in mast cells rather as a G protein agonist than as an antagonist.

Pretreatment of intact mast cells with neuraminidase (0.1–0.3 U/ml), which hydrolyzes sialic acid residues of the plasma membrane, inhibits histamine secretion induced by mastoparan and other related cationic secretagogues (Coleman et al., 1986; Mousli et al., 1989). Histamine release induced by Ca^{2+} ionophore A23187 (present results) or by IgE-dependent activation (Coleman et al., 1986) was not inhibited by neuraminidase pretreatment. Interestingly, the same selectivity in inhibiting histamine release induced by cationic compounds was observed with [D-Pro^4, D-Trp^{7,9,10}]substance P-(4–11), suggesting again a relationship between the presence of sialic acid residues and the inhibitory action of [D-Pro^4, D-Trp^{7,9,10}]substance P-(4–11). This suggested the crucial role of membrane glycoproteins and/or gangliosides that have sialic acid residues in the initial binding of cationic secretagogues to the mast cell surface. This binding step might induce a secondary structure, allowing the triggers to cross the membrane to reach their intracellular target (G proteins) (Mousli et al., 1989). This proposal implies that the permeabilization of mast cells should overcome the inhibitory effect of neuraminidase. The present data fully support this hypothesis (Fig. 4). Mastoparan is an amphiphilic peptide that penetrates the plasma membrane and activates associated G proteins by forming a membrane-spanning α helix similar in structure to the intracellular loops of G protein-coupled receptors (Higashijima et al., 1988, 1990). It is likely that in neuraminidase-pretreated mast cells mastoparan cannot bind to the cell surface and therefore does not stimulate G proteins. The present findings support the notion that mastoparan stimulates histamine release from neuraminidase-pretreated mast cells by interacting with its intracellular target (G proteins) which is accessible only in streptolysin-permeabilized cells whose plasma membrane is permeable to large molecules.

References

Aridor, M., Traub, L.M., Sagi-Eisenberg, R., 1990. Exocytosis in mast cells by basic secretagogues: Evidence for direct activation of GTP-binding proteins. J. Cell. Biol. 111, 909–917.

Aridor, M., Rajmilevich, G., Beaven, M.A., Sagi-Eisenberg, R., 1993. Activation of exocytosis by the heterotrimeric G protein G_{i3}. Science 262, 1569–1572.

Bueb, J.L., Mousli, M., Bronner, C., Rouot, B., Landry, Y., 1990. Activation of G_i-like proteins, a receptor-independent effect of kinins in mast cells. Mol. Pharmacol. 38, 816–822.

Coleman, J.W., Huang, Q., Stanworth, D.R., 1986. The mast cell response to substance P: Effect of neuraminidase, limulin, and some novel synthetic peptide antagonists. Peptides 7, 171–175.

Emadi-Khiav, B., Mousli, M., Bronner, C., Landry, Y., 1995. Human and rat cutaneous mast cells: Involvement of G protein in the response to peptidergic stimuli. Eur. J. Pharmacol. 272, 97–102.

Fischer, T., Bronner, C., Landry, Y., Mousli, M., 1993. The mechanism of inhibition of alkylamines on the mast-cell peptidergic pathway. Biochim. Biophys. Acta 1176, 305–312.

Foreman, J.C., Lichtenstein, L.M., 1980. Induction of histamine secretion by polycation. Biochim. Biophys. Acta 629, 587–603.

Gilman, A.G., 1987. G proteins: Transducers of receptor-generated signals. Annu. Rev. Biochem. 56, 615–6449.

Gomperts, B.D., Fernandez, J.M., 1985. Techniques for membrane permeabilization. Trends Biochem. Sci. 10, 414–417.

Higashijima, T., Uzu, S., Nakajima, T., Ross, E.M., 1988. Mastoparan, a peptide toxin from wasp venom, mimics receptors by activating GTP-binding regulatory proteins (G proteins). J. Biol. Chem. 263, 6491–6494.

Higashijima, T., Burnier, J., Ross, E.M., 1990. Regulation of G_i and G_o by mastoparan, related amphiphilic peptides and hydrophobic amines. J. Biol. Chem. 265, 14176–14186.

Hilf, G., Jakobs, K.H., 1989. Activation of cardiac G-proteins by muscarinic acetylcholine receptors: Regulation by Mg^{2+} and Na^+ ions. Eur. J. Pharmacol. 172, 155–163.

Hirai, Y., Yasuhara, T., Yoshida, H., Nakajima, T., Fujino, M., Kitada, C., 1979. A new mast cell degranulating peptide mastoparan in the venom of *Vespula lewisii*. Chem. Pharm. Bull. 27, 1942–1944.

Mizrahi, J., Escher, E., Caranikas, S., D'Orleans-Juste, P., Regoli, D., 1982. Substance P antagonists active in vitro and in vivo. Eur. J. Pharmacol. 82, 101–105.

Mousli, M., Bronner, C., Bueb, J.L., Tschirhart, E., Gies, J.P., Landry, Y., 1989. Activation of rat peritoneal mast cells by substance P and mastoparan. J. Pharmacol. Exp. Ther. 250, 329–335.

Mousli, M., Bueb, J.L., Bronner, C., Rouot, B., Landry, Y., 1990a. G protein activation: A receptor-independent mode of action for cationic amphiphilic neuropeptides and venom peptides. Trends Pharmacol. Sci. 11, 358–362.

Mousli, M., Bronner, C., Landry, Y., Bockaert, J., Rouot, B., 1990b. Direct activation of GTP-binding regulatory proteins (G proteins) by substance P and compound 48/80. FEBS Lett. 259, 260–262.

Mousli, M., Hugli, T.E., Landry, Y., Bronner, C., 1992. A mechanism of action for anaphylatoxin C3a stimulation of mast cells. J. Immunol. 148, 2456–2461.

Mousli, M., Hugli, T.E., Landry, Y., Bronner, C., 1993. Peptidergic pathway in human skin and rat peritoneal mast cell activation. Immunopharmacology 27, 1–11.

Mousli, M., Trifilieff, A., Pelton, J.T., Gies, J.P., Landry, Y., 1995. Structural requirements for neuropeptide Y in mast cell and G protein activation. Eur. J. Pharmacol. 289, 125–133.

Mukai, H., Munekata, E., Higashjima, T., 1992. G protein antagonists. J. Biol. Chem. 267, 16237–16243.

Nakamura, T., Ui, M., 1985. Simultaneous inhibitions of inositol phospholipid breakdown, arachidonic acid release, and histamine secretion in mast cells by islet-activating protein, pertussis toxin. J. Biol. Chem. 260, 3584–3593.

Penner, R., Matthews, G., Neher, E., 1988. Regulation of calcium influx by second messengers in rat mast cells. Nature 344, 499–504.

Piotrowski, W., Foreman, J.C., 1985. On the action of substance P, somatostatin, and vasoactive intestinal polypeptide on rat peritoneal mast cells and in human skin. Naunyn-Schmiedebergs Arch. Pharmacol. 331, 364–368.

Piotrowski, W., Devoy, M.A.B., Jordan, C.C., Foreman, J.C., 1984. The substance P receptor on rat mast cells and in human skin. Agents Actions 14, 419–424.

Read, G.W., Kiefer, E.F., 1979. Benzalkonium chloride: A selective inhibitor of histamine release induced by compound 48/80 and other polyamines. J. Pharmacol. Exp. Ther. 211, 711–715.

Shore, P.A., Burkhalter, A., Cohn, V.A., 1959. A method for a fluorimetric assay of histamine in tissues. J. Pharmacol. Exp. Ther. 127, 182–186.

Vitale, N., Mukai, H., Rouot, B., Thiersé, D., Aunis, D., Bader, M.F., 1993. Exocytosis in chromaffin cells. J. Biol. Chem. 268, 14715–14723.

Vitale, N., Aunis, D., Bader, M.F., 1994. Distinct heterotrimeric GTP-binding proteins act in series to control the exocytotic machinery in chromaffin cells. Cell. Mol. Biol. 40, 707–715.

Zhang, L., Mantey, S., Jensen, R.T., Gardner, J.D., 1988. An analog of substance P with broad receptor antagonist activity. Biochim. Biophys. Acta 972, 37–44.