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Human and rat cutaneous mast cells: involvement of a G protein in the response to peptidergic stimuli

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Abstract

Recent evidence suggests that peptides induce the release of mediators from rat peritoneal mast cell by means of a receptor-independent mechanism, possibly involving an interaction with sialic acid residues at the cell surface followed by the activation of a guanine nucleotide binding protein (G protein). We have now examined the potential involvement of sialic acid residues and of G protein stimulation in the activation of both human and rat cutaneous mast cells by neuropeptide Y, its *C*-terminal fragments and the wasp venom peptide, mastoparan. Neuropeptide Y-(18–36) was the most effective histamine releaser of the fragments tested, the order of potency being neuropeptide Y-(18–36) > neuropeptide Y-(22–36) > neuropeptide Y-(1–36). This order of potency suggests that the effects of the peptides are not mediated through classical NPY receptors. The hydrolysis of sialic acid residues by neuraminidase and the inhibition of G proteins by benzalkonium chloride or pertussis toxin significantly inhibited the secretory response of cutaneous mast cells to neuropeptide Y-(18–36) and mastoparan. These results demonstrate that the peptidergic pathway described for the activation of peritoneal rat mast cells is also involved in the response of cutaneous human and rat mast cells to peptides.

Keywords: Mast cell, cutaneous; Neuropeptide Y; Mastoparan; G-protein; Benzalkonium chloride; Pertussis toxin; Neuraminidase

1. Introduction

Histamine release from rat serosal mast cells can be induced by a variety of structurally diverse compounds including synthetic polyamines such as compound 48/80 and natural polyamines (Mousli et al., 1989, 1990; Aridor et al., 1990; Bueb et al., 1992), venom peptides such as mastoparan and the mast cell degranulating peptide (MCD or peptide 401) (Mousli et al., 1991; Fujimoto et al., 1991; Tomita et al., 1991) peptidic hormones such as bradykinin and anaphylatoxins (Bueb et al., 1990; Mousli et al., 1992) and neuropeptides, such as substance P (Mousli et al., 1989) and most recently, neuropeptide Y (Shen et al., 1991; Grundemar and Håkanson, 1991).

It has been proposed that, following initial binding to the membrane, possibly by an ionic interaction with negatively charged surface sialic acid residues (Coleman et al., 1986; Mousli et al., 1989), polycations may intercalate into the membrane by virtue of their hydrophobic moieties. This may permit the interaction of the positively charged domain of the molecule with the carboxy terminus of the G protein and facilitate nucleotide exchange by a mechanism analogous to that of G protein-coupled receptors (Higashijima et al., 1990; Mousli et al., 1994). We proposed that the cellular target of polycationic secretagogues was a G protein of the G_i subtype (Bueb et al., 1990) which is a full agreement with a more recent study in which the G protein G_{i3} has been identified as being of key importance in neuropeptide activation of rat peritoneal mast cells (Aridor et al., 1993). Such a receptor-independent effect of peptides has been described only for rat peritoneal mast cells and its relevancy in the activation of other cells remains unknown. It has been demonstrated that human cutaneous mast cells are unique, compared with those from other human tissue sites, in their ability to release histamine in response to wide variety of non-immunological stimuli (Benyon et al.,

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1987; Tainsh et al., 1992). The heterogeneous nature of the responses of human mast cells to polycationic agents strongly suggests that fundamental differences in cellular membrane structure exist and which may determine the extent and efficiency of the interaction of such secretagogues with the cells. The particular reactivity of the human skin mast cell may be the result of a more fluid or a more negatively charged membrane which might facilitate the non-specific interaction of a diverse array of secretagogues with the cell.

The aim of the present study was to investigate the mechanisms involved in the activation of both rat and human cutaneous mast cells by peptides. We chose mastoparan as a well known direct activator of G proteins (Higashijima et al., 1988 and 1990) and neuropeptide Y which offer the possibility to study fragments with well established selectivities for current receptors (Michel, 1991; Wahlestedt and Reis, 1993).

2. Materials and methods

2.1. Materials

Benzalkonium chloride, bovine serum albumin, antimycin A and 2-deoxyglucose were purchased from Sigma (St. Louis, MO). Mastoparan, neuropeptide Y and neuropeptide Y fragments were obtained from Neosystem (Strasbourg, France), pertussis toxin from List Biological Laboratory (Campbell, CA), neuraminidase and ionophore A23187 from Boehringer-Mannheim (Mannheim, Germany) and Percoll from Pharmacia (Uppsala, Sweden)

2.2. Isolation and purification of cells

Infant foreskins (1–12 years) were obtained following circumcision and were dissected free from underlying fat. Fragments of the tissue, approximately 1 mm^3, were digested with collagenase (Sigma, type 1A, 160 U/ml) and hyaluronidase (Sigma, type 1S, 500 U/ml) in buffer with the following composition (mM): NaCl, 137; glucose, 5.6; KCl, 2.7; $CaCl_2$, 1; NaH_2PO_4, 0.4; Hepes, 10; NaOH to pH 7.4 supplemented with 1.0 mg/ml bovine serum albumin, for 2×90 min at 37°C or longer if after this time substantial tissue remained undigested. Cells were then recovered as previously reported (Ali and Pearce, 1985).

Male Wistar rats (Iffa-Credo, L'Arbresle, France) weighing 200–250 g were killed by stunning and exsanguination. Skin covering the abdomen (4×3 cm) was shaved and cut manually into small fragments with scissors. The tissue fragments were treated as described above for human skin.

Where necessary, mast cells were partially purified on a discontinuous density gradient prepared by overlaying successive bands of 80% and 50% isotonic Percoll. Cells were then recovered as previously reported (Church et al., 1989). Mast cells comprised 4.0 ± 0.6% (rat skin) and 8.2 ± 1.8% (human skin) of the total nucleated cells. The purity of mast cell samples was determined using a Neubauer haemocytometer after staining with Kimura (Kimura et al., 1973) and cell viability was determined using trypan blue. Under these conditions, preparations of up to 55% purity and with greater than 90% viability were obtained (Table 1).

Table 1
Some basic properties of mast cells isolated from human and rat skin ($n = 7$)

	Human	Rat
Mast cells (% total)	8.2 ± 1.8	4.0 ± 0.6
Histamine (pg/mast cell)	1.4 ± 0.2	2.5 ± 0.3
Viable cells (% total)	91.3 ± 1.8	90.5 ± 2.4
Spontaneous histamine release (%)	4.5 ± 0.5	5.4 ± 0.5

2.3. Secretion and inhibition of histamine release

In general, isolated mast cells were left to equilibrate in a water bath (37°C) in the Hepes buffer described above, except that bovine serum albumin was excluded before addition of the releasing agent. The reaction was allowed to proceed for 10 min and was terminated by adding cold Hepes buffer. In experiments to examine the metabolic requirements for release, cells were preincubated (10 min) with antimycin A, to inhibit oxidative phosphorylation, and 2-deoxyglucose to inhibit glycolysis. In other inhibition ex-

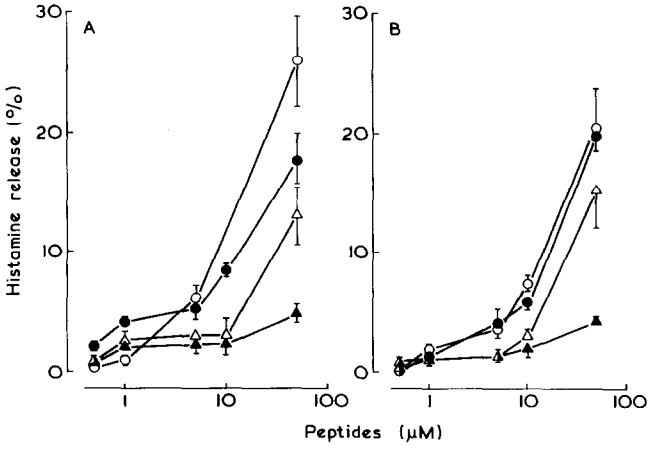

Fig. 1. Histamine release (%) induced by mastoparan (○), neuropeptide Y-(1–36) (▲), neuropeptide Y-(18–36) (●) and neuropeptide Y-(22–36) (△) from rat (A) and human (B) skin mast cells. Mast cells were left to equilibrate at 37°C in the Hepes buffer before addition of the releasing agents. The reaction was allowed to proceed for 10 min and was terminated by addition of cold Hepes buffer. Values are means ± S.E.M. for eight experiments.

periments, cells were preincubated with benzalkonium chloride (10 min), pertussis toxin (120 min) or neuraminidase (30, 60, 120 min) before the induction of secretion.

2.4. Measurement of histamine release

Histamine release was measured using an automated spectrofluorometric assay as described previously (Atkinson et al., 1979). Values for histamine release are expressed as a percentage of the total cellular histamine and are corrected for the spontaneous release of histamine that occurred in the absence of any stimulus. For attenuation experiments, results are given as the percentage inhibition of the control release induced by the stimulus alone. All values are given as means ± S.E.M. for the number (n) of experiments performed.

3. Results

3.1. Histamine release from rat and human skin mast cells

Neuropeptide Y, its C-terminal fragments and mastoparan induced a concentration-dependent release of histamine from rat (Fig. 1A) and human (Fig. 1B) cutaneous mast cells. The release at high concentrations was essentially abolished by preincubating the cells with 1 μM of the metabolic blockers antimycin A and 5 mM 2-deoxyglucose (Fig. 2) hence demonstrating that the induced secretion was non-cytotoxic. Neuropeptide Y-(18–36) was the most effective histamine releaser of the fragments tested, the order of potency being neuropeptide Y-(18–36) > neuropeptide Y-(22–36) > neuropeptide Y-(1–36). Histamine released evoked by neuropeptide Y was significant, using Student's paired t-test, when compared with basal secretion of the amine ($P < 0.01$ in rat and $P < 0.002$ in human cutaneous mast cells). Similar results were obtained when the cells were partially purified (data not shown).

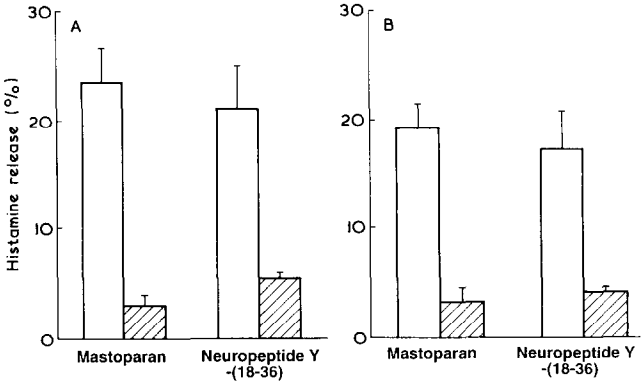

Fig. 2. Effect of neuropeptide Y-(18–36) (50 μM) and mastoparan (50 μM) on mast cells from rat (A) and human (B) skin in the presence (hatched columns) and absence (open columns) of the metabolic inhibitors antimycin A (1 μM) and 2-deoxyglucose (5 mM). Cells were preincubated (10 min) with the inhibitors and then challenged. Secretion was allowed to proceed for a further 10 min. Values are means ± S.E.M. for five experiments.

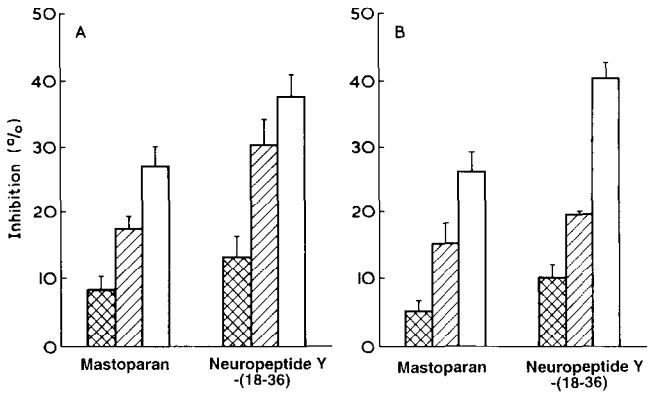

Fig. 3. Effect of neuraminidase on histamine release from rat (A) and human (B) skin mast cells stimulated with mastoparan (control release at 50 μM was 26.0 ± 2.0% and 25.0 ± 1.2%, respectively) and neuropeptide Y-(18–36) (control release at 50 μM was 23.5 ± 1.8% and 22.9 ± 1.0%, respectively). Partially purified (50 ± 4%) mast cells were treated with neuraminidase at concentrations of 2×10^{-4} U/ml (cross-hatched columns), 3×10^{-4} U/ml (hatched columns) and 4×10^{-4} U/ml (open columns) for 60 min. Secretion was then allowed to proceed for a further 10 min. Values are means ± S.E.M. for six experiments.

3.2. Involvement of sialic acid in mast cell activation

The preincubation of partially purified rat (Fig. 3A) and human (Fig. 3B) skin mast cells with the enzyme

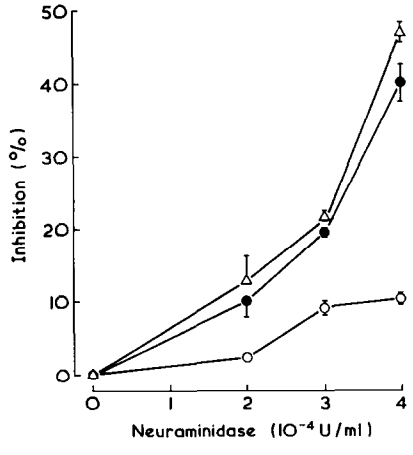

Fig. 4. Effect of neuraminidase at different incubation time periods on human skin mast cells stimulated with neuropeptide Y-(18–36) (control release was 21.0 ± 2.3% at 50 μM). Partially purified (50 ± 4%) skin mast cells were incubated with neuraminidase for 30 min (○), 60 min (●) and 120 min (△) and secretion was then allowed to proceed for a further 10 min period. Values are means ± S.E.M. for four experiments.

Table 2
Inhibitory effect of neuraminidase (3×10^{-4} U/ml) and pertussis toxin (1 μg/ml) on the response of rat and human skin mast cells to 50 μM neuropeptide Y-(18–36) (control releases were 20.3 ± 3.4% and 24.3 ± 2.9%, respectively)

	Incubation time		
	30 min	60 min	120 min
Neuraminidase (human skin mast cells)	9.06 ± 1.5%	35.0 ± 3.0%	34.0 ± 2.5%
Neuraminidase (rat skin mast cells)	11.0 ± 2.9%	30.0 ± 4.1%	32.0 ± 3.2%
Pertussis toxin (human skin mast cells)	2.0 ± 1.0%	10.0 ± 2.0%	60.0 ± 6.0%
Pertussis toxin (rat skin mast cells)	3.0 ± 0.5%	8.0 ± 2.5%	44.0 ± 5.0%

Values show the percentage inhibition of histamine release after different periods of incubation. Values are means ± S.E.M. for four experiments.

neuraminidase, which strips cells of their membrane-associated sialic acid (Coleman et al., 1986; Mousli et al., 1989), was tested. At the highest tested concentration, neuraminidase selectively inhibited the histamine release induced by neuropeptide Y-(18–36) (50 μM) and mastoparan (50 μM) from both rat (by 37.9 ± 3.2% and 27.2 ± 2.6%) and human mast cells (by 40.0 ± 2.5% and 25.9 ± 3.4%), respectively. The inhibition by neuraminidase were time- and dose-dependent (Fig. 4). The liberation of histamine stimulated by calcium ionophore A23187 (1 μM) from human (12.7 ± 1.2%) and rat (19.0 ± 2.3%) cutaneous mast cells was unaffected by the enzyme.

3.3. Involvement of GTP-binding proteins in mast cell activation

A 120 min preincubation with pertussis toxin significantly inhibited histamine release induced by 50 μM neuropeptide Y-(18–36) and 50 μM mastoparan (Fig. 5) in both rat (by 44.5 ± 5.1% and 22.5 ± 4.5%, respectively) and human (by 60.9 ± 6.5%, and 66.0 ± 4.6%, respectively) skin mast cell, respectively. In contrast, the toxin did not affect the liberation of histamine induced by calcium ionophore A23187 (1 μM) either from the human (14.0 ± 2.0%) or rat (17.3 ± 2.5%) cells.

The effect of benzalkonium chloride on histamine release induced by neuropeptide Y-(18–36) and mastoparan from rat and human skin mast cells is shown in Fig. 6. The compound produced a dose-dependent inhibition of release, with or without pretreat-

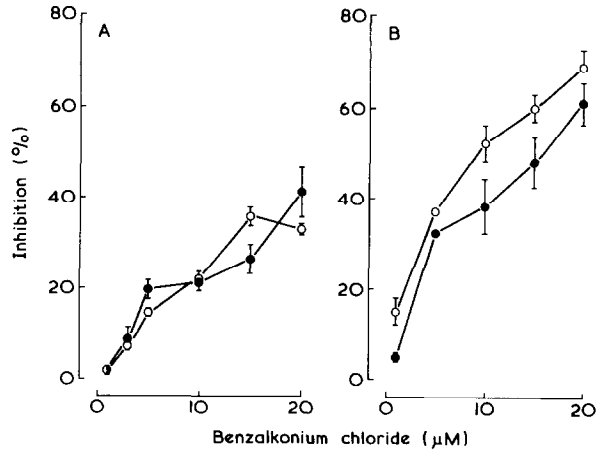

Fig. 6. Effect of benzalkonium chloride on histamine release from partially purified (50 ± 4%) rat (A) and human (B) skin mast cells stimulated with mastoparan (○, control release was 24.0 ± 3.5% and 18.0 ± 1.9%, respectively) and neuropeptide Y-(18–36) (●, control release at 50 μM was 19 ± 3% and 16.5 ± 2.9%, respectively). Mast cells were treated simultaneously with the indicated concentration of peptides and benzalkonium chloride. No effect of benzalkonium chloride was observed on basal release of histamine. Values are means ± S.E.M. for five experiments.

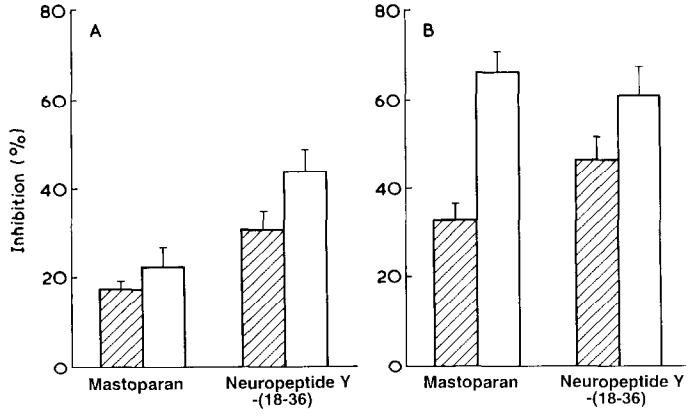

Fig. 5. Effect of pertussis toxin on histamine release from rat (A) and human (B) skin mast cells stimulated with mastoparan (control release at 50 μM was 23.0 ± 0.6% and 16.9 ± 2.1%, respectively) and neuropeptide Y-(18–36) (control release at 50 μM was 18.5 ± 1.2% and 15.2 ± 3.6%, respectively). Partially purified (50 ± 4%) mast cells were treated with pertussis toxin at 0.1 μg/ml (hatched columns) and 1 μg/ml (open columns) for 120 min before addition of peptides and the reaction stopped after a further 10 min. Values are means ± S.E.M. for five experiments.

ment of the mast cells. Concentrations higher than 30 μM were found to be cytotoxic, as reported previously for rat peritoneal mast cells (Read et al., 1982).

4. Discussion

Neuropeptide Y and its *C*-terminal fragments together with the wasp venom toxin mastoparan induced a dose-dependent, non-cytotoxic secretion of histamine from rat and human skin mast cells. However, relatively high concentrations of the peptides were required to elicit a histamine release, when compared to that previously reported in rat peritoneal mast cells (Mousli and Landry, 1994). This difference in mast cell sensitivity to the peptides between rat peritoneal and rat or human skin cells might be due to the dispersion procedure which require enzymatic digestion.

Neuropeptide Y is thought to act on at least three receptor subtypes (Wahlestedt and Reis, 1993). Recent investigations using neuropeptide Y, its *C*-terminal fragments (Mousli and Landry, 1994) and selective receptor agonists (Grundemar and Håkanson, 1991) have been unable to provide evidence for the presence of specific binding sites on the surface of rat peritoneal mast cells. In agreement with results obtained recently in rat peritoneal mast cells (Mousli and Landry, 1994), the rank order of potency of the peptides used in the present study was neuropeptide Y-(18–36) > neuropeptide Y-(22–36) > neuropeptide Y-(1–36). This is in clear contrast to classical neuropeptide Y receptor systems, where the order of potency has been shown to be neuropeptide Y-(1–36) > neuropeptide Y-(18–36) > neuropeptide Y-(22–36). Nuclear magnetic resonance studies of the secondary structure of neuropeptide Y (Saudek and Pelton, 1990) show that the *C*-terminal fragment, from residues 11–36, folds into an amphiphilic α-helix while the *N*-terminal segment assumes no regular structure. Within the neuropeptide Y series used in the present study, neuropeptide Y-(18–36) is the smallest fragment to have a helical conformation and the highest net positive charge. Considering the postulated important role of the α-helical conformation for G protein activation (Higashijima et al., 1988; Tomita et al., 1991; Mousli and Landry, 1993), it is not surprising to find that this fragment is the most active in releasing histamine.

In the present study the treatment of mast cells with neuraminidase, pertussis toxin and benzalkonium chloride inhibited histamine release induced by both neuropeptide Y-(18–36) and mastoparan, but not by calcium ionophore A23187. Recent studies have suggested that alkylamines, including benzalkonium chloride, exert their inhibitory effect selectively via an interaction with mast cell G_i-like proteins (Fischer et al., 1993). Therefore, taken together, these results strongly suggest that neuropeptide Y-(18–36) and mastoparan activate human and rat cutaneous mast cells via an interaction with a pertussis toxin sensitive G proteins of the G_i subtype. The binding to sialic acid residues, present on the cell surface appears to be a pre-requisite for such a peptide/G protein interaction. Thus, the present study clearly shows that the action mechanism of mastoparan, neuropeptide Y and of its fragments in human and cutaneous mast cells follows the well-described peptidergic pathway, i.e. interaction with sialic acid residues followed by the activation of G protein sensitive to pertussis toxin leading to histamine release.

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