0026-895X/90/060816-07$03.00/0

MOLECULAR PHARMACOLOGY, 38:816-822

Activation of G_i-Like Proteins, a Receptor-Independent Effect of Kinins in Mast Cells

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Received February 28, 1990; Accepted September 13, 1990

SUMMARY

The peptide hormones bradykinin and kallidin (Lys-bradykinin), as well as their analogues [des-Arg^9]-bradykinin, a selective B_1 agonist, [des-Arg^9,Leu^8]-bradykinin, a selective B_1 antagonist, and [Thi^{5,8},D-Phe^7]-bradykinin and D-Arg^0-[Hyp^3,D-Phe^7]-bradykinin, two selective B_2 antagonists, induced rapid histamine release from purified rat peritoneal mast cells. In contrast, the N-terminal fragment bradykinin-(1–5) was inactive. These peptides also activate the GTPase activity of GTP-binding proteins (G proteins) (G_o/G_i) purified from calf brain, with an order of potency identical to that observed on mast cells, [Thi^{5,8},D-Phe^7]-bradykinin ≫ kallidin > bradykinin > D-Arg^0-[Hyp^3,D-Phe^7]-bradykinin > [des-Arg^9]-bradykinin > [des-Arg^9,Leu^8]-bradykinin > bradykinin-(1–5). This correlation suggested that G proteins are the targets of kinins in mast cells. Accordingly, the concomitant increase in inositol trisphosphates and release of histamine elicited by kinins were inhibited by pertussis toxin pretreatment of mast cells. The inhibitory effect of benzalkonium chloride showed that the G proteins involved belong to the G_i type. GTPase activity was measured in the supernatant of homogenized mast cells but not in the membranous fraction. This activity was stimulated by kinins and by the venom peptide mastoparan. The potency of peptides was similar to that observed with purified bovine G proteins. Sodium dodecyl sulfate-gel electrophoresis of mast cell supernatant revealed pertussis toxin-induced ADP-ribosylation of two proteins, in the M_r 41,000 and 40,000 range, i.e., similar to purified α-subunits of G_{i_1} and G_{i_2} or G_{i_3} subtypes. The data support the proposal that bradykinin and analogues act like mastoparan, substance P, and compound 48/80, interacting first with sialic acid residues of the cell surface and then with G_i-like proteins, inducing phospholipase C activation and intracellular calcium mobilization.

The peptide hormones bradykinin (Arg-Pro-Pro-Gly-Phe-Ser-Pro-Phe-Arg) and kallidin (Lys-bradykinin) are involved in a variety of pathophysiological conditions such as inflammation, trauma, and allergy. The effects of kinins have been attributed to the stimulation of specific bradykinin receptors, called B_1 and B_2 (1). This classification is based on the order of potency of agonists and on selective B_1 (1) and B_2 (2, 3) antagonists. However, receptor-independent mechanisms have been proposed for kinins in guinea pig trachea (3, 4) and in rat peritoneal mast cells (5–7), because current bradykinin antagonists have a stimulant effect in these models. The myotropic activity of bradykinin on guinea pig trachea was entirely dependent on the activation of the arachidonic cascade but could not be completely explained by B_1 and B_2 receptor stimulation, but occurrence of a receptor different from B_1 and B_2 cannot be excluded (3). The secretory activity of kinins on rat peritoneal mast cells has recently been attributed to the direct activation of G proteins (5–7), i.e., a mechanism similar to that proposed for the wasp venom peptide mastoparan (7–10), the neuropeptide substance P (7, 10–12), and the synthetic polyamine compound 48/80 (7, 10–13). This putative mechanism is based both on the sensitivity to pertussis toxin of stimulated histamine release from mast cells and on the potency of these compounds to stimulate G_i and G_o proteins purified from bovine brain (for review see Ref. 7). However, 100 μM bradykinin failed to stimulate purified G_o and G_i proteins (9). The aim of the present study was to fully characterize the effect of bradykinin and analogues, including agonists and antagonists of current B_1 and B_2 receptors, both on rat mast cells and on bovine brain G proteins and to investigate the properties of mast cell G proteins. These results strengthen the hypothesis that considers the direct activation of G proteins as a receptor-independent mechanism of mast cell activation.

Materials and Methods

Purification of mast cells. Mast cells were purified from male Wistar rats (Iffa Credo, L'Arbresle, France) weighing 300–350 g. The rats were sacrificed by stunning and bleeding. Ten milliliters of bal-

This work was supported by INSERM (CRE 89-5009), by the Fondation pour la Recherche Médicale, and by the Association de Recherche sur la Polyarthrite.

ABBREVIATIONS: G protein, GTP-binding protein; HEPES, 4-(2-hydroxyethyl)-1-piperazineethanesulfonic acid; BSA, bovine serum albumin; SDS, sodium dodecyl sulfate.

anced salt solution, containing (in mM): NaCl, 137; KCl, 2.7; $CaCl_2$, 0.3; $MgCl_2$, 1; NaH_2PO_4, 0.4; glucose, 5.6; and HEPES, 10; adjusted to pH 7.4 with NaOH, supplemented with 0.2% BSA, (Sigma Chemical Co., St. Louis, MO) or 0.05% gelatin (Sigma), were injected intraperitoneally. The peritoneal cavity was opened after gentle abdominal massage for 2 min, and the peritoneal fluid was collected and centrifuged for 2 min at 180 × *g*. The pellet was resuspended in the same buffer and the mast cells were purified using a BSA gradient (40 and 30%, w/v) and centrifugation for 10 min at 220 × *g* (14). The pellet was resuspended and the cells were examined under microscope for purity (>97%).

Inhibition of histamine secretion. The effect of neuraminidase (Boehringer Mannheim GmbH, Germany) or pertussis toxin (Sigma) on histamine release was assessed after preincubation of the mast cells for 1 or 2 hr, respectively, at 37° in balanced salt solution supplemented with 0.2% BSA. The cells were challenged for 5 min with mastoparan, bradykinin, kallidin (Sigma), [des-Arg^9]-bradykinin, [des-Arg^9,Leu^8]-bradykinin (Bachem, Bubendorf, Switzerland), [Thi^{5,8},D-Phe^7]-bradykinin, or D-Arg^0-[Hyp^3,Thi^{5,8},D-Phe^7]-bradykinin (Cambridge Research Chemicals Ltd., England), and the reaction was stopped by the addition of ice-cold buffer to the samples, as described previously (15). The effect of benzalkonium chloride (Sigma), a mixture of quaternary benzyldimethylalkylammonium chlorides, on histamine release was assayed as described by Read and Kiefer (16), allowing benzalkonium chloride and bradykinin, its analogues, or mastoparan to act simultaneously for 10 min at 37° on purified mast cells in balanced buffer supplemented with 0.05% gelatin. Histamine from the supernatants was assayed fluorometrically according to the method of Shore *et al.* (17) without extraction steps, and expressed as a percentage of the total histamine content of the cells. Spontaneous histamine release, in the absence of any stimulus, never exceeded 7%.

Inositol phosphate metabolism. The generation of [^{3}H]inositol phosphates was measured using the method of Berridge *et al.* (18), as detailed previously (10). Briefly, purified mast cells (10^7 cells/ml) were pulse labeled with 100 μCi/ml *myo*-[^{3}H]inositol (New England Nuclear-Du Pont, Boston MA) for 2 hr, with or without pertussis toxin, in HEPES-buffered solution at 37°. The cells were then washed twice and resuspended for 30 min in the buffer containing 10 mM *myo*-inositol. After further washing with HEPES-buffered solution, the mast cells were distributed (4 × 10^5 cells/0.2 ml) and incubated for 5 min at 37° before the drugs were added. The induction was stopped 10 sec later with 5% trichloroacetic acid. Supernatants were removed, washed four times with diethyl ether, neutralized with sodium tetraborate (5.6 mM final) deposited on anion exchange resin columns (AG 1 × 8, 200–400 mesh formate form; Bio-Rad, Richmond, CA), eluted successively, and counted as described by Nakamura and Ui (19) and Mousli *et al.* (10) for rat mast cells. Parallel assays with [^{3}H]inositol-1-phosphate, [^{3}H]inositol-1,4-bisphosphate and [^{3}H]inositol-1,4,5-trisphosphate standards (Amersham, Little Chalfont, England) confirmed the accuracy of the separation protocol (10).

Bovine brain G proteins. GTPase activity was determined according to the methods of Brandt *et al* (20) and Higashijima *et al.* (21), with [γ-^{32}P]GTP (Amersham). A mixture of purified G_o and G_i proteins (G_o/G_i), containing mostly G_o (22, 23), was obtained from calf brain membranes by successive elution from DEAE-Sephacel (Pharmacia, Uppsala, Sweden), AcA34 (LKB, Uppsala, Sweden), and heptylamine-Sepharose columns, as described by Sternweis and Robishaw (24). These G proteins were reconstituted into phospholipid vesicles (25). Briefly, G proteins (about 120 pmol) in 500 μl of buffer A (50 mM Na HEPES, pH 8, 1 mM EDTA, 1 mM dithiothreitol) plus 0.02% Lubrol 12A9 were mixed with 100 μl of buffer A containing 0.84% sodium cholate, 0.05% dioleoylphosphatidylcholine, 0.05% bovine brain phosphatidylethanolamine, and 0.067% bovine brain phosphatidylserine (Sigma). Results were expressed per pmol of G protein.

GTPase activity of mast cells. Cells were preincubated at 0° in Tris buffer (50 mM Tris, 1 mM EDTA, 1 mM dithiothreitol, pH 8), containing 1000 units/ml kallikrein inhibitor (Sigma), for 10 min, Potter-homogenized, and centrifuged for 15 min at 12,000 × *g*. The assays with supernatant were carried out in buffer A (see Bovine brain G proteins) containing 0.3 μM [γ-^{32}P]GTP (Amersham) and indicated concentrations of added Mg^{2+}. Results were expressed per 10^5 mast cells.

SDS-gel electrophoresis of ADP-ribosylated soluble proteins from mast cells. Pertussis toxin-catalyzed ADP-ribosylation was performed as described by Rouot *et al.* (26). Purified mast cells (18 × 10^6) were resuspended for 10 min at 0° in 1 ml of buffer containing (in mM): Tris, 50; EDTA, 1; dithiothreitol, 1; pH 7.5, containing 5 μg/ml soybean trypsin inhibitor, 10^{-4} M phenylmethylsulfonylfluoride (a protease inhibitor), and 400 units/ml of kallikrein inhibitor (Sigma). The suspension was Potter-homogenized and centrifuged for 15 min at 12,000 × *g*. The supernatant was removed, the volume was adjusted to 2 ml with Tris buffer, and the solution was centrifuged in a Centricon 30 microconcentrator (Amicon, Danvers, MA) to the desired concentration. Purified α subunits of G_{i1}, G_{i2}, and G_o (14 μg of protein), mast cell membranes (85 μg), and mast cell supernatant proteins (100 μg) were incubated for 60 min with [^{32}P]NAD (New England Nuclear) in the presence of preactivated pertussis toxin, as previously described (22). The reaction was stopped by the addition of SDS (2%) with 100 μg/ml BSA, and proteins were precipitated with trichloroacetic acid. After centrifugation, the pellets were washed twice with diethyl ether and subjected to *N*-ethylmaleimide treatment and electrophoresis for autoradiography, as previously described (27).

Results

Fig. 1 shows that the wasp venom peptide mastoparan, the B_2 antagonist [Thi,^{5,8},D-Phe^7]-bradykinin, the agonists kallidin and bradykinin, and the B_1 antagonist [des-Arg^9,Leu^8]-bradykinin induced histamine release from rat peritoneal mast cells

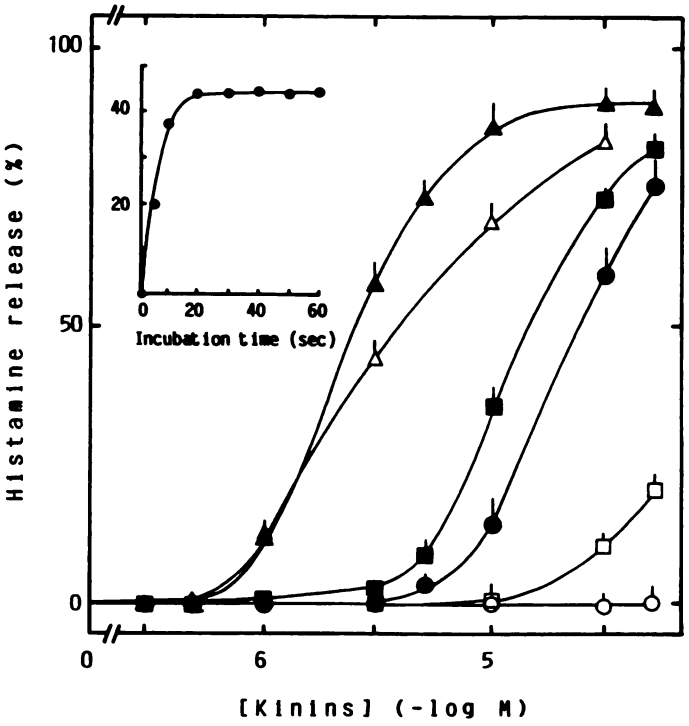

Fig. 1. Effect of mastoparan and kinins on histamine release from rat peritoneal mast cells. Cells were preincubated for 2 hr at 37°, in balanced salt solution supplemented with 0.2% BSA and were stimulated for 5 min with various concentrations of mastoparan (△), [Thi^{5,8},D-Phe^7]-bradykinin (▲), kallidin (■), bradykinin (●), [des-Arg^9,Leu^8]-bradykinin (□), and bradykinin-(1–5) (○). Inset, time course for histamine secretion induced by 2 × 10^{-5} M bradykinin. Values are means ± standard errors of three duplicate experiments.

in a dose-dependent manner. The B_2 antagonist D-Arg^0-[Hyp^3,D-Phe^7]-bradykinin and the B_1 agonist [des-Arg^9]-bradykinin also induced histamine release (Table 1), whereas the N-terminal segment of bradykinin, bradykinin-(1–5), had no effect on mast cells (Fig. 1). Histamine release was complete within 10 sec and reached 88.4 ± 2.9% of the total histamine content when the mast cells were stimulated with 3×10^{-5} M [Thi^{5,8},D-Phe^7]-bradykinin. This B_2 antagonist was more potent than kallidin and bradykinin, with the EC_{50} values being $2.3 \pm 0.5 \times 10^{-6}$, $1.2 \pm 0.9 \times 10^{-5}$, and $2.0 \pm 1.7 \times 10^{-5}$ M, respectively.

Histamine release induced by mastoparan and by the different kinins was inhibited by pretreatment of mast cells for 1 hr with neuraminidase (Table 2), which hydrolyzes the sialic acid residues from the plasma membrane surface (10). This inhibition was very similar for all the stimulants and reached around 85% when mast cells were pretreated with 0.3 milliunits/ml neuraminidase.

The same order of potency was found concerning the ability of kinins to stimulate the GTPase activity of purified bovine G_o/G_i proteins, compared with their histamine-releasing properties, in spite of the higher concentrations necessary at this molecular level (Fig. 2). Kinins that had no or only a slight effect on histamine release, bradykinin-(1–5) and the B_1 antagonist [des-Arg^9,Leu^8]-bradykinin, respectively, did not modify GTPase activity at up to 10^{-3} M (Fig. 2). Mastoparan was much more active in stimulating GTPase activity than [Thi^{5,8},D-Phe^7]-bradykinin, the most potent of the kinins (Fig. 2), although the ability of both peptides to induce histamine release was similar (Fig. 1). The presence of Mg^{2+} was required for the full activation of G proteins by kinins, as shown in Fig. 3.

Incubation of [^{3}H]inositol-labeled rat peritoneal mast cells with bradykinin, kallidin, or [Thi^{5,8},D-Phe^7]-bradykinin induced a transient increase in tris- or tetrakisphosphates (Fig. 4). This increase was maximum within 10 sec. Fig. 4 shows the differences in the inositol tris- and/or tetrakisphosphate levels between pertussis toxin-treated and control rat mast cells after induction with bradykinin, kallidin, or [Thi^{5,8},D-Phe^7]-bradykinin; the increase of the basal levels was greatly reduced by pretreatment of cells with the toxin. Bradykinin-(1–5) neither triggered histamine release (Fig. 1) nor modified phosphoinositide metabolism at up to 10^{-3} M (Fig. 4).

Table 1 shows that pretreatment of the cells for 2 hr with pertussis toxin had an inhibitory effect on histamine release induced by mastoparan and kinins. In contrast, pertussis toxin had no effect on the histamine release induced by the ionophore

TABLE 1

Inhibitory effect of pertussis toxin and benzalkonium chloride on histamine release from rat peritoneal mast cells induced by the bradykinin analogues

Cells were pretreated with pertussis toxin or benzalkonium chloride, as described in Materials and Methods, and stimulated with the indicated concentrations of mastoparan and bradykinin analogues. Values are means ± standard errors of three different experiments performed in duplicate. The corresponding control histamine releases, i.e., without the inhibitors, are shown in parentheses.

	Inhibition of histamine release			
	Pertussis toxin		Benzalkonium chloride	
	1 ng/ml	100 ng/ml	0.5 μg/ml	2 μg/ml
	%			
Mastoparan (3×10^{-6} M)	4.9 ± 0.8 (56.5 ± 2.1)	91.8 ± 0.5	26.2 ± 4.5 (39.5 ± 3.8)	90.5 ± 3.2
[Thi^{5,8},D-Phe^7]-Bradykinin (3×10^{-6} M)	22.4 ± 4.6 (51.5 ± 1.5)	99.3 ± 0.3	4.9 ± 3.4 (77.0 ± 2.0)	96.0 ± 0.7
Kallidin (10^{-5} M)	42.6 ± 1.1 (26.7 ± 2.2)	99.3 ± 0.7	15.8 ± 3.9 (64.7 ± 3.8)	97.1 ± 1.1
Bradykinin (2×10^{-5} M)	21.6 ± 6.6 (39.2 ± 4.5)	98.7 ± 1.3	8.7 ± 1.4 (76.3 ± 4.5)	97.1 ± 1.3
D-Arg^0-[Hyp^3,D-Phe^7]-Bradykinin (2×10^{-5} M)	14.3 ± 3.9 (56.6 ± 3.4)	91.8 ± 3.3	16.4 ± 6.4 (44.7 ± 3.8)	92.0 ± 2.3
[des-Arg^9]-Bradykinin (5×10^{-5} M)	8.6 ± 3.1 (42.0 ± 3.5)	85.5 ± 11.9	87.5 ± 17.7 (17.2 ± 1.1)	93.7 ± 8.8
[des-Arg^9,Leu^8]-Bradykinin (5×10^{-5} M)	34.9 ± 0.7 (28.4 ± 8.1)	85.2 ± 9.9	75.8 ± 19.1 (11.7 ± 1.1)	86.3 ± 14.2

TABLE 2

Inhibitory effect of neuraminidase on histamine release from rat peritoneal mast cells induced by mastoparan and kinins

Cells were pretreated with neuraminidase, as described in Materials and Methods, and triggered with the indicated concentrations of mastoparan and kinins. Values are means ± standard errors of three different experiments performed in duplicate. The corresponding control histamine releases, i.e., without neuraminidase, are shown in parentheses.

	Inhibition of histamine release induced by neuraminidase	
	10^{-4} units/ml	3×10^{-4} units/ml
	%	
Mastoparan (3×10^{-6} M)	28.3 ± 3.1 (63.4 ± 7.8)	88.4 ± 8.9
[Thi^{5,8},D-Phe^7]-Bradykinin (3×10^{-6} M)	21.8 ± 9.3 (53.8 ± 1.9)	88.0 ± 1.6
Kallidin (10^{-5} M)	23.7 ± 5.6 (47.0 ± 4.4)	84.8 ± 3.2
Bradykinin (2×10^{-5} M)	20.8 ± 7.5 (57.3 ± 3.2)	81.8 ± 3.9

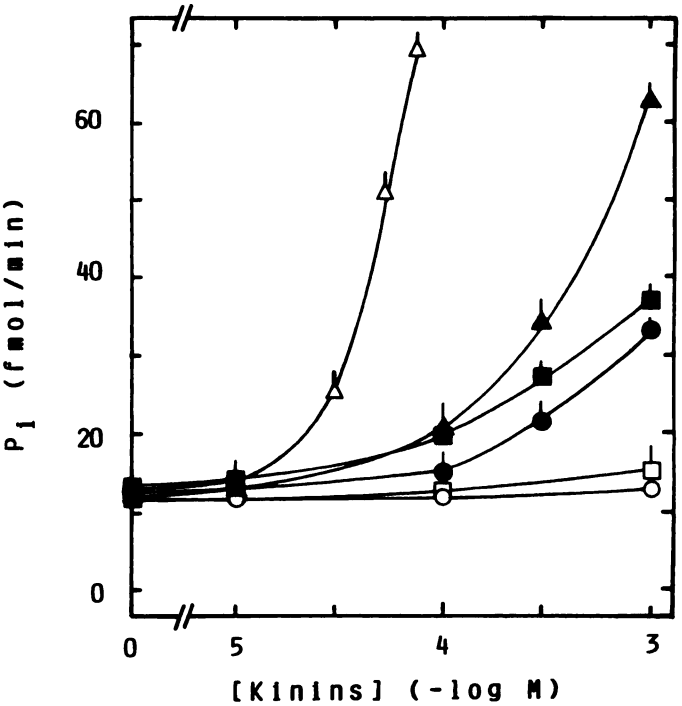

Fig. 2. Effect of mastoparan and kinins on the GTPase activity of G proteins purified from calf brain (G_o/G_i). Reconstituted G proteins were incubated at 25°, for 5 min, with various concentrations of mastoparan (△), [Thi^{5,8},D-Phe^7]-bradykinin (▲), kallidin (■), bradykinin (●), [des-Arg^9,Leu^8]-bradykinin (□), and bradykinin-(1–5) (○), in the presence of 3×10^{-4} M added Mg^{2+}. Values, expressed per pmol of G proteins, are means ± standard errors of three duplicate experiments.

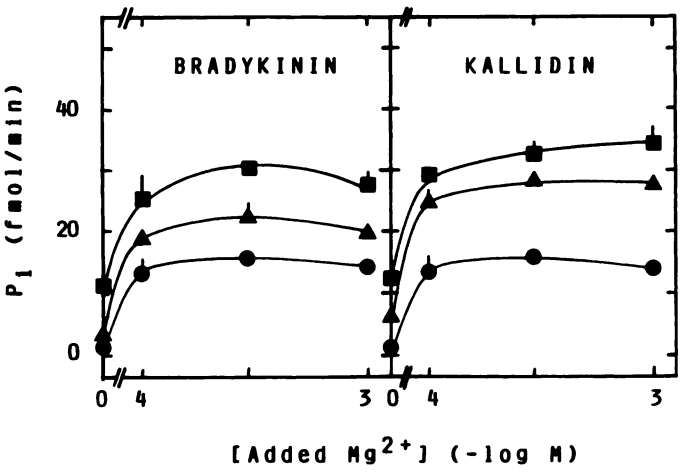

Fig. 3. Effect of bradykinin and kallidin on the GTPase activity of G proteins purified from calf brain (G_o/G_i) at varying Mg^{2+} concentrations. Reconstituted G proteins were incubated at 25°, for 5 min, with various concentrations of $MgSO_4$ (●), in the presence of 5×10^{-4} (▲) or 10^{-3} (■) M bradykinin or kallidin. Values, expressed per pmol of G proteins, are means ± standard errors from three experiments carried out in duplicate.

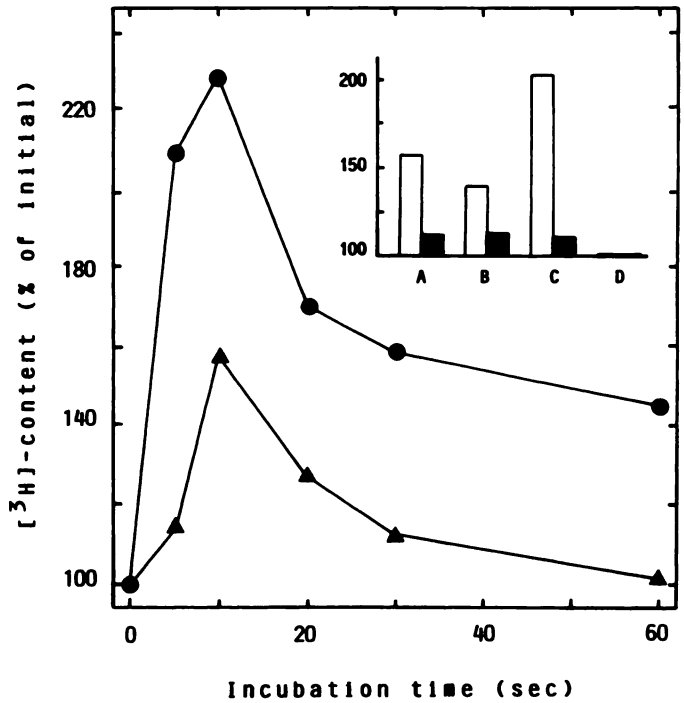

Fig. 4. Generation of inositol tris- and/or tetrakisphosphates in response to stimulation with bradykinin (*main panel*) and various kinins (*inset*). *Main panel*, purified peritoneal rat mast cells were labeled with [^{3}H] inositol and incubated with (▲) or without (●) 300 ng/ml pertussis toxin for 2 hr. The cells were then stimulated with [Thi^{5,8},D-Phe^7]-bradykinin (10^{-5} M), kallidin (3×10^{-5} M), bradykinin (5×10^{-5} M), or bradykinin-(1–5) (10^{-3} M) The ^{3}H contents of inositol tris- and/or tetrakisphosphates are shown as percentages of initial (zero time) values, i.e., immediately before peptides were added. *Inset*, effects on the mast cells of 5 sec stimulation with [Thi^{5,8},D-Phe^7]-bradykinin (A), kallidin (B), bradykinin (C), and bradykinin-(1–5) (D). □, Control; ■, pertussis toxin-treated cells. Data shown are representative of four experiments. The initial values, in dpm/10^6 mast cells, are 110 for treated and 84 for untreated cells.

A23187 (10). These results clearly show that a pertussis toxin-sensitive G protein is involved in the exocytotic process induced by the peptide mastoparan and the kinins. In the presence of benzalkonium chloride (0.1–3 μg/ml), we observed a dose-dependent inhibition of the histamine release induced by mastoparan, bradykinin, kallidin, and [Thi^{5,8},D-Phe^7]-bradykinin (Fig. 5, Table 1). Histamine secretion induced by the B_1-selective agonist [des-Arg^9]-bradykinin and the B_2-selective antagonist D-Arg^0-[Hyp^3,D-Phe^7]-bradykinin was also inhibited by benzalkonium chloride (Table 1). Histamine release was fully inhibited by 3 μg/ml benzalkonium chloride, but higher concentrations were toxic for rat peritoneal mast cells (Ref. 16 and observations not shown). In contrast, benzalkonium chloride failed to inhibit the histamine release induced by 10^{-6} M levels of the ionophore A23187 (Fig. 5).

We also verified the effect of kinins on the GTPase activity of homogenized mast cells. In the presence of Mg^{2+}, mastoparan and the kinins stimulated dose dependently the GTPase activities of mast cell supernatant, with mastoparan being the most active compound (Fig. 6). The peptides exerted the same effect on purified G_o/G_i proteins (Fig. 2) as on the supernatant of mast cells. A difference could be seen in the basal activities;

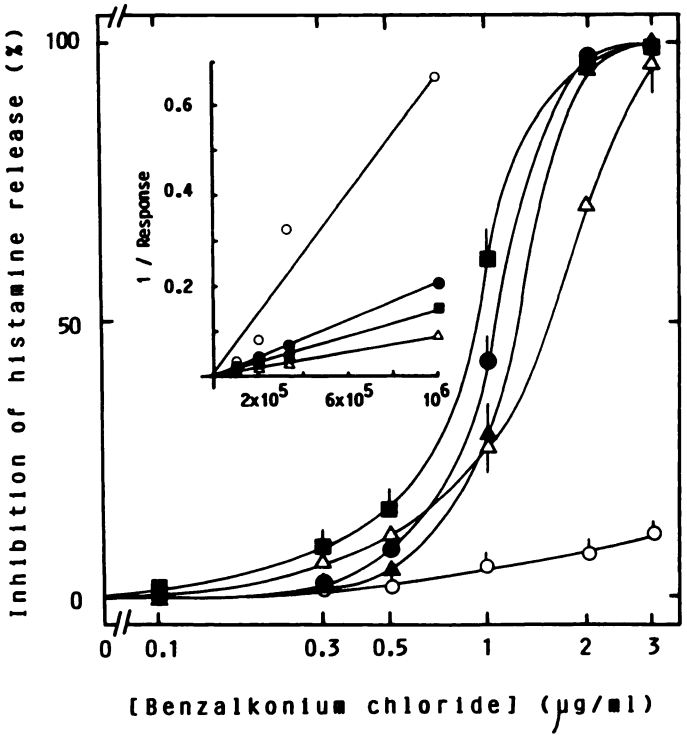

Fig. 5. Inhibitory effect of benzalkonium chloride on histamine release from rat mast cells induced by mastoparan, [Thi^{5,8},D-Phe^7]-bradykinin, kallidin, and bradykinin. *Main panel*, mast cells were treated with various doses of benzalkonium chloride. Concomitantly, histamine release was induced with 5×10^{-6} M mastoparan (△), 3×10^{-6} M [Thi^{5,8},D-Phe^7]-bradykinin (▲), 10^{-5} M kallidin (■), 2×10^{-5} M bradykinin (●), or 10^{-6} M A23187 (○) for 10 min, at 37°, in balanced salt solution supplemented with 0.05% gelatin. Values are expressed as percentages of inhibition of the corresponding controls in the absence of benzalkonium chloride. These controls were 44.8 ± 4.0% of the total histamine content for mastoparan, 77.0 ± 2.0% for [Thi^{5,8},D-Phe^7]-bradykinin, 64.7 ± 3.8% for kallidin, 76.3 ± 4.5% for bradykinin, and 83.2 ± 5.6% for A23187. Values are means ± standard errors of three duplicate experiments. *Inset*, double-inverse plot of the histamine-releasing activity of mastoparan, M^{-1} (△) in the presence of 0.5 (■), 1 (●), or 2 (○) μg/ml benzalkonium chloride.

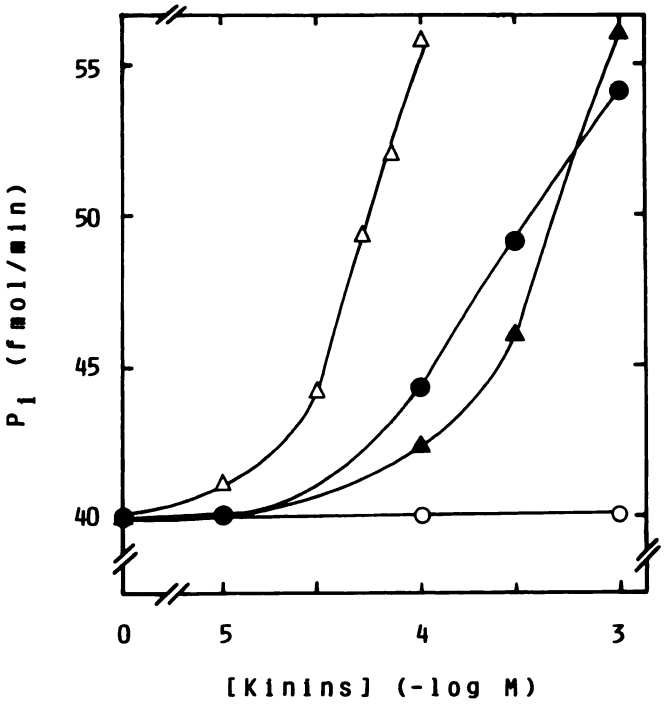

Fig. 6. Effect of mastoparan, [Thi^{5,8},D-Phe^{7}]-bradykinin, and bradykinin on the GTPase activity of the supernatant from purified mast cells. Supernatant of homogenized mast cells (corresponding to 10^5 cells/assay) was incubated at 25°, for 5 min, with 1 mM added $MgSO_4$, in the presence of mastoparan (△), [Thi^{5,8},D-Phe^{7}]-bradykinin (▲), bradykinin (●), or bradykinin-(1–5) (○). Data shown are from one experiment carried out in duplicate, which is representative of the six performed.

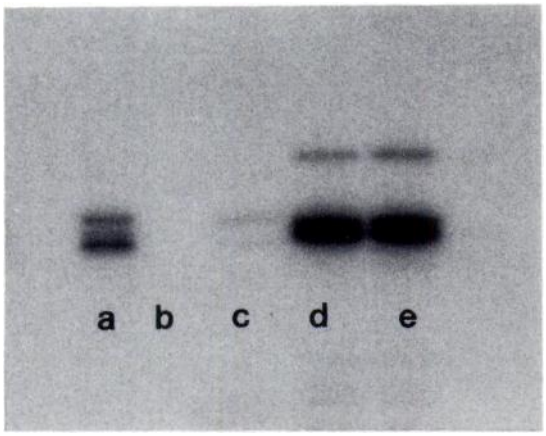

Fig. 7. SDS-gel electrophoresis of [^{32}P]ADP-ribosylated calf brain proteins and mast cells supernatant. Samples of purified calf brain α-subunits of G proteins, of mast cell membranes, and of mast cell supernatant were ADP-ribosylated in the presence of [^{32}P]NAD and pertussis toxin, as described in Materials and Methods, before being loaded on the SDS-gel. *Lane a*, purified calf brain G proteins ($G_{o\alpha}$, M_r 39,000, lower; $G_{i1\alpha}$, M_r 41,000, upper; $G_{i2\alpha}$, M_r 40,000, middle); *lane b*, mast cell membranes; *lane c*, purified β- and γ-subunits from calf brain; *lane d*, β and γ with mast cell supernatant; *lane e*, mast cell supernatant.

mast cell supernatant activity was raised by significant non-specific phosphatase activity, which could not be blocked. Bradykinin-(1–5) had no effect at up to 10^{-3} M.

SDS-gel electrophoresis (Fig. 7) shows that pertussis toxin substrates were observed in the supernatant of mast cells (Fig. 7, *lane e*) but not in the membranous fraction (Fig. 7, *lane b*). Two proteins, ADP-ribosylated by the preactivated pertussis toxin, were identified in the M_r 40,000 and 41,000 range (Fig. 7, *lane e*). The addition of β- and γ-subunits, purified from calf brain G proteins, to mast cell supernatant influenced neither ADP-ribosylation nor migration of these two proteins (Fig. 7, *lane d*). G_o, G_{i1}, and G_{i2} α-subunits purified from calf brain showed M_r 39,000, 41,000, and 40,000, respectively (Fig. 7, *lane a*).

Discussion

Mode of action of kinins in mast cells. The histamine release induced by bradykinin was first attributed to a receptor (28), which was thought to be of the B_2 type (29) because B_1 antagonists [des-Arg^9,Leu^8]-bradykinin and [des-Arg^{10},Leu^9]-kallidin behave as weak agonists and also because the order of potency of agonists, kallidin > bradykinin > [des-Arg^9]-bradykinin, was consistent with that described for B_2 receptors of smooth muscle preparations (1, 2). However, [Thi^{5,8},D-Phe^7]-bradykinin and [Thi^{6,9},D-Phe^8]-kallidin, which have been shown to act as antagonists of B_2 receptors in several systems (2, 30), also induced histamine release (31). These results have ruled out the involvement of B_1 and B_2 receptors (31), and the mode of action of kinins on mast cells remained unexplained. The effect of kinins on mast cells is reminescent of that of other triggers, such as the neuropeptide substance P and the venom peptide mastoparan, recently proposed to act directly on G proteins (7–12). However, until the present results only limited information (6) was available concerning the biochemical events elicited by kinins in mast cells, including a G protein-dependent pathway.

We show here the occurrence of phospholipase C activation and the sensitivity to pertussis toxin of both inositol trisphosphate generation and histamine release. Moreover, the order of potency of kinins on mast cells was identical to their order of potency in stimulating GTPase activity from G proteins (G_o/G_i) purified from bovine brain, [Thi^{5,8},D-Phe^7]-bradykinin > kallidin > bradykinin > [des-Arg^9,Leu^8]-bradykinin > bradykinin-(1–5). This strongly suggested that G proteins are the targets of kinins in mast cells. A direct demonstration of this is provided by the effect of peptides on the GTPase activity of mast cells. Mastoparan and kinins activated, in a Mg^{2+}-dependent manner, the GTPase activity of the supernatant of homogenized mast cells. The order of potency and the maximum efficacy of peptides were quite similar in purified bovine G proteins and in the mast cell supernatant (Figs. 2 and 6).

Structure-activity relationships of kinins in inducing histamine release and in activating G proteins. The knowledge of structure-activity relationships of peptides on mast cells is especially important in the design of clinically useful agonists or antagonists devoid of histamine-releasing activity (5). The structure-activity relationships of these peptides in activating G proteins is interesting because they mimic G protein-coupled receptors and offer promise as models for the receptor-binding domain on the G protein α-subunit (8, 9, 12, 32).

The choice of compounds used in the present study was based on results from Regoli and co-workers (5, 30, 31). The order of potency of kinins in mast cell histamine release has been related to the number of positively charged amino acid residues (29, 31). Bradykinin is a nonapeptide with two arginine residues, one at both the N- and C-termini. Kallidin is a decapeptide that differs from bradykinin only by the presence of an addi-

tional basic residue (lysine) at the N-terminus. Current B_1 and B_2 antagonists also induce histamine release (29, 31); their histamine-releasing activity can be decreased by removal of the positively charged N-terminal amino acid or by *N*-acetylation of the N-terminal arginine (5). [Thi^{5,8},D-Phe^{7}]-bradykinin and [Thi^{6,9},D-Phe^{8}]-kallidin are the most active analogues. Thus, the order of potency and the maximum efficacy are roughly related to the presence and number of positive amino acids. However, amino acid substitution in compounds bearing a similar number of positive charges can lead to peptides that differ in potency by more than 1 log unit. The amino acids Hyp and Thi might lead to conformation changes of the peptides but this has not been thoroughly explored. The present results show that the cellular effects of all the kinins tested are sensitive to pertussis toxin and to benzalkonium chloride, suggesting a similar mode of action.

Under the present experimental conditions, the activation of purified bovine G proteins and the activation of GTPase activity of mast cell supernatant required higher concentrations of kinins than those needed to trigger mast cell exocytosis. A similar difference was previously observed with other active peptides such as mastoparan (8, 9) and neurokinins (11). At the cellular level, the structure of a triggering peptide itself could be involved in its preliminary binding to the cell surface, in the adoption of a membrane-spanning conformation, and in its interaction with G proteins. The signal provided by the activation of G proteins is largely amplified in the cell by subsequent biological events leading to exocytosis. Sialic acid residues of the cell surface are thought to concentrate cationic compounds at the cell surface (7, 10, 33, 34). The present experiments with neuraminidase (Table 2) show that this might also be a feature of the effects of kinins in mast cells, allowing them to reach a high intracellular level. This point requires further study. At the molecular level, the lipid composition of liposomes used to reconstitute purified G proteins also influenced their sensitivity to peptides (8, 9). Thus, a quantitative correlation between the effects of peptides on intact mast cells and those on their purified targets could not be expected. However, the same order of potency of kinin analogues was observed at both the cellular and molecular levels.

Interestingly, the potency of [Thi^{5,8},D-Phe^{7}]-bradykinin is similar to that of mastoparan for stimulation of mast cell secretion (Fig. 1), but it remains less potent than the venom peptide in stimulation of purified bovine brain G proteins (Fig. 2) and GTPase activity of mast cell supernatant (Fig. 6). Thus, analogues of peptides including Thi residues warrant further study. Another apparent discrepancy between the potency to induce histamine release and to activate purified G proteins is provided in the case of angiotensin II. This hormone does activate G proteins (9) but was reported to be inactive on mast cell secretion (29, 35). However, its metabolism by mast cell enzymes cannot be excluded. Within the mastoparan analogues, an increase of either hydrophobicity or hydrophobic moment enhanced their potency and their maximal regulatory activity for purified G proteins (9). The most active compounds were those in which lysine was replaced by either arginine or alanine, but their effects on mast cells have not been determined.

Mast cell G proteins. The introduction of nonhydrolyzable analogues of GTP, which persistently activate G proteins, into mast cells induces histamine release (36, 37). The involvement of two different G proteins has been proposed in mast cells, one coupled to phospholipase C, called G_P, and the other (G_E) directly coupled to exocytosis (38). Both G_P (38) and G_E (13) might be pertussis toxin substrates, but only one ADP-ribosylated protein (M_r 41,000) has been identified by SDS-gel electrophoresis by Nakamura and Ui (19). More recently, Aridor *et al.* (13) reported the presence of a pertussis toxin substrate (or substrates) in the M_r 40,000 range. A protein of a similar molecular weight also reacted on immunoblots with an antiserum that recognizes $G_{i\alpha}$ (13). We show here that exposure of mast cells to pertussis toxin-catalyzed ADP-ribosylation reveals the presence of two substrates in the M_r 40,000 and 41,000 range (Fig. 7). These molecular weights are similar to those of $G_{i1\alpha}$ (M_r 41,000) and $G_{i2\alpha}$ or $G_{i3\alpha}$ (M_r 40,000), purified from bovine brain (24). According to Higashijima *et al.* (9), the sensitivity to benzalkonium chloride of the histamine release induced by kinins (Fig. 5, Table 1) and other cationic amphiphilic peptides (6) also involve G_i-like proteins. Thus, G_P and G_E might belong to the G_i-G protein family. The involvement in mast cells of pertussis toxin-insensitive G proteins cannot be excluded, but such proteins may not be involved in the effect of peptides. Curiously, the pertussis toxin substrates of mast cells have been localized in the soluble fraction of homogenate rather than in membrane fraction (Refs. 13 and 19 and Fig. 7 of present results). It remains unknown whether the protein was originally in the cytosol in intact mast cells or had been liberated from membranes during homogenization. Addition of purified β and γ to mast cell supernatant did not increase ADP-ribosylation by pertussis toxin (Fig. 7), suggesting that the three subunits ($\alpha\ \beta\ \gamma$) were present in this fraction.

In conclusion, the present results support the proposal that G_i-like proteins are the targets of kinins in mast cells. Moreover, the activation of GTPase activity in mast cell supernatant provides the first direct demonstration of a functional correlate to the activation of purified G proteins by mastoparan and other cationic amphiphilic peptides. A more extensive demonstration will require the purification of G proteins from rat peritoneal mast cells.

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