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Direct activation of GTP-binding regulatory proteins (G-proteins) by substance P and compound 48/80

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The neuropeptide substance P and the polyamine compound 48/80, both known to activate mast cell secretory processes, increased the rate of GTPγS binding to G-proteins purified from calf brain (G_o/G_i mixture). The GTPase activity of G-proteins was also increased by substance P and compound 48/80 in a dose-dependent and Mg^{2+}-dependent way. These effects were similar to those of the wasp venom peptide mastoparan, another histamine releaser of rat peritoneal and human skin mast cells. This suggests that the secretory property of compound 48/80 and substance P is not due to a receptor-mediated process but, like mastoparan, results from a direct activation of G-proteins.

G-protein; Substance P; Compound 48/80; Mastoparan; Mast cell

1. INTRODUCTION

Mastoparan, a peptide toxin from wasp venom, has recently been proposed to mimic receptors by activating GTP-binding regulatory proteins (G-proteins) [1]. Mastoparan is a potent histamine releaser from rat peritoneal mast cells [2]. At the cellular level, mastoparan shares common properties with compound 48/80 and also with substance P [3]. These similarities include (i) the kinetics of release and the ionic requirements for mast cell reactivity; (ii) the desensitization of mast cells to these stimuli, following the hydrolysis of sialic acid residues of the cell surface; (iii) the inhibitory effect of pertussis toxin; and (iv) the involvement of phospholipase C activation [3]. Thus, we have proposed a common mechanism for action for these three stimuli of mast cells, related to a common target which might be either a receptor or a G-protein [3]. However, no evidence for selective membrane receptors of these stimuli has been obtained. Effective concentrations of substance P to trigger mast cells (μM) are well above those required to stimulate neurokinin receptors (nM) and the existence of such receptors on mast cells has been extensively questioned [4–6]. In contrast, the role of G-proteins in mast cell exocytosis has been larely documented [3,7–11]. The data presented here show that compound 48/80 and substance P increased both the rate of nucleotide binding and GTPase activity of purified G-proteins.

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Abbreviations: G_o, G_i, G_s and G_t, GTP-binding regulatory proteins; GTPγS, guanosine-5′-*O*-(3-thiotriphosphate); PTX, *Bordetella pertussis* toxin

2. MATERIALS AND METHODS

Purification of a mixed preparation of G_o and G_i was obtained from calf brain membranes by successive elution from DEAE Sephacel (Pharmacia), AcA 34 (LKB) and heptylamine-Sepharose columns as described by Sternweis and Robishaw [12]. This preparation mostly contains G_o [13,14]. All incubations were carried out in buffer A (50 mM Na-Hepes, pH 8, 1 mM EDTA, 1 mM dithiothreitol) containing the concentration of nucleotides and Mg^{2+} indicated in the figure legends. G-proteins were reconstituted into phospholipid vesicles, according to the method of Sternweis [15]. G-proteins (about 120 pmol) in 500 μl of buffer A plus 0.02% Lubrol 12A9 were mixed with 100 μl of buffer A plus 0.84% sodium cholate, 0.05% dioleoyl-phosphatidylcholine, 0.05% bovine brain phosphatidylethanolamine and 0.067% bovine brain phosphatidylserine. The suspension was held at 0°C overnight. The assay of [^{35}S]GTPγS (NEN-Du Pont, Boston, MA) binding was performed according to Ferguson et al. [16], except that 0.1% bovine serum albumin was added to reduce the non-specific binding observed with the higher concentrations of substance P. GTPase activity was determined according to Brandt et al. [17] and Higashijima et al. [18] with [γ-^{32}P]GTP (Amersham, England). Lipids, mastoparan and compound 48/80 were purchased from Sigma (St. Louis, MO), substance P, neurokinin A and neurokinin B from Bachem Feinchemikalien AG (Bubendorf, Switzerland).

3. RESULTS AND DISCUSSION

The effect of the neuropeptide substance P and compound 48/80 on the rate of GTPγS binding was tested using a preparation of G_o/G_i proteins (in which G_o was characterized as the major holoprotein [13,14]). As shown in fig.1, 500 μM of substance P and 100 μg/ml

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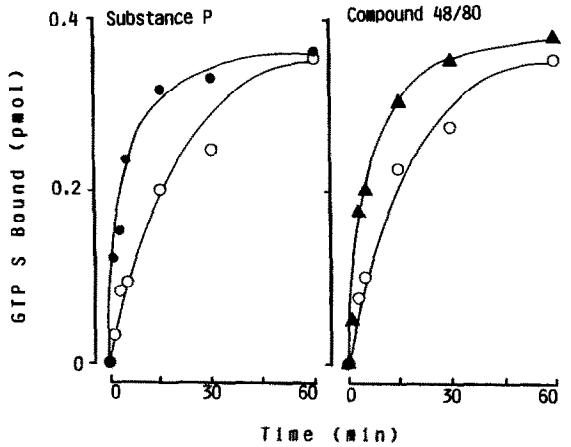

Fig.1. Substance P and compound 48/80 increased the rate of [^{35}S]-GTPγS binding. [^{35}S]GTPγS binding was assayed at 25°C using G-proteins that had been reconstituted into phospholipid vesicles as described under section 2. The assay medium was buffer A that contained 1 mM Mg^{2+} and 0.1 μM [^{35}S]GTPγS either with 500 μM substance P (●) or 100 μg/ml of compound 48/80 (▲) or with no substances (○). At the times shown, 50-μl aliquots containing 0.5 pmol of G-proteins were removed and GTPγS binding was determined by filtration on nitrocellulose filters. Non-specific binding was less than 0.3%. Data shown are from one experiment carried out in duplicate which is representative of the three performed.

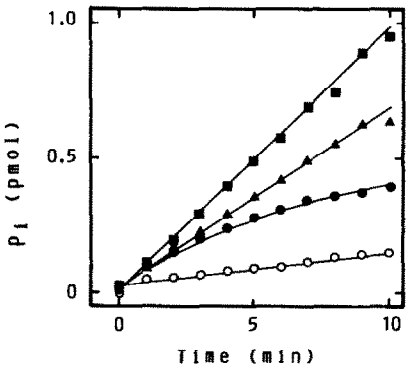

Fig.2. Time-dependent effect of various secretagogues on the GTPase activity. Effects of peptides and compound 48/80 on the GTPase activity were determined on G-proteins reconstituted into phospholipid vesicles. G-proteins (1 pmol/assay) were incubated at 25°C for the times shown in the abscissa in buffer A that contained 0.1 mM of free Mg^{2+} (see table 1), and 0.1 μM of [γ-^{32}P]GTP either without (○) or with 100 μM of mastoparan (■), 300 μM of substance P (●) or 100 μg/ml of compound 48/80 (▲). Activities shown are expressed as molar turnover numbers.

of compound 48/80 stimulated the rate of binding of GTPγS to G-proteins in the presence of 1 mM of Mg^{2+} while the maximal amount of GTPγS binding remained unaltered. Higashijima et al. reported similar results with the amphiphilic tetradecapeptide mastoparan and demonstrated that this effect was due to the enhancement of the dissociation rate of GDP allowing a faster rate of association for the labeled [^{35}S]GTPγS [1].

The mechanism of mastoparan, substance P and compound 48/80 in the activation of rat peritoneal mast cell involves the activation of phospholipase C through a *Bordetella pertussis* toxin-sensitive G-protein [3,7,19]. When examined on the purified calf brain PTX-sensitive G-proteins, all three stimuli of mast cells exocytosis stimulated the GTPase activity in the presence of 0.1 mM Mg^{2+} (fig.2). The presence of Mg^{2+} has been shown to be necessary for the full activation of the G-proteins [18] and, in fact, mastoparan seemed to reduce the Mg^{2+} concentration required for the GTPase stimulation. In the presence of mastoparan, maximal activation could be evidenced at 100 nM of Mg^{2+} while 1 mM was needed in its absence [1]. In agreement with these results, table 1 indicates that basal GTPase activity regularly increased from 0.1 to 10 mM Mg^{2+}, whereas in the presence of substance P and compound 48/80, the GTPase activity was already maximal at 0.1 mM. Also similar to the previously reported effect of mastoparan [1], increasing the Mg^{2+} concentration above 1 mM slightly decreased the activity of G-proteins in the presence of substance P or compound 48/80. These results indicate that substance P and compound 48/80 act similarly to mastoparan on purified G-proteins.

To further validate our hypothesis, the order of potency of various drugs on the GTPase activation was compared with their ability to induce histamine release on mast cells. Substance P belongs to the neurokinin group of peptides bearing the C-terminal sequence -Phe-X-Leu-Met-NH_2. Besides substance P, mammalian neurokinins include neurokinin A and neurokinin B. These three peptides are the endogenous ligands of NK_1, NK_2 and NK_3 receptors, which can be stimulated by nanomolar concentrations of the respective peptides [20]. On mast cells, the effect of substance P is observed from 10^{-6} to 10^{-4} M, while neurokinin A and neurokinin B did not significantly stimulate histamine release at these concentrations [21]. Fig.3 reveals that both neurokinins A and B were unable to modify the GTPase activity of purified G-proteins at 1 mM, whereas substance P was already active at 0.1 mM. This observation strengthens the view that G-

Table 1

Effect of substance P and compound 48/80 on the GTPase activity of G-proteins at varying concentrations of Mg^{2+}

$[Mg^{2+}]$ (mM)	GTPase ($min^{-1} \times 10^3$)		
	Control	Substance P	Compound 48/80
0	0.48	3.93	9.09
0.1	6.89	41.17	70.01
0.3	8.19	42.83	74.79
1	12.63	41.31	78.71
3	23.33	39.85	68.39
10	40.12	38.61	71.69

The activity of G-proteins reconstituted into phospholipid vesicles was assayed as described in the legend of fig.2 with varying concentrations of $MgSO_4$ and EDTA to give the calculated concentration of free Mg^{2+} indicated. Substance P and compound 48/80 were tested at a concentration of 300 μM and 100 μg/ml, respectively. Activities are expressed as molar turnover numbers

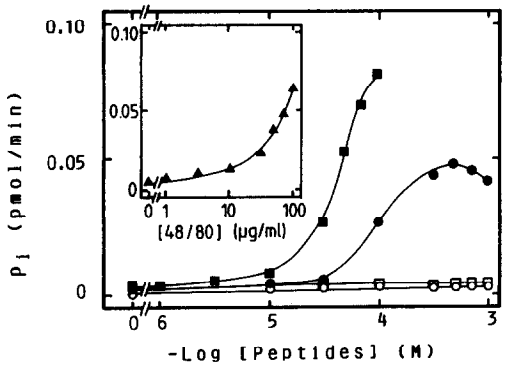

Fig.3. Effect of increasing concentrations of peptides and compound 48/80 on the GTPase activity. The GTPase activity was measued on reconstituted G-proteins (1 pmol) after a 5-min incubation in the presence of 0.1 μM of [γ-^{32}P]GTP as described in the legend to fig.2. The effects of increasing concentrations of mastoparan (■), substance P (●) and the two neurokinins A (□) and B (○) are depicted in this figure. Since compound 48/80 (▲) is a mixture of polymers, its concentration is expressed in μg/ml and its dose-response curve is shown in the inset. Activities are expressed as molar turnover numbers.

proteins might be the target of substance P in rat peritoneal mast cells and in human skin mast cells, considering the effect of peptides on this cellular type [6].

Substance P activates mast cells at slightly higher concentrations than mastoparan [3] and this difference can also be seen in the stimulation of G-proteins (fig.3). It is interesting to note that the maximal GTPase activation by substance P (0.3 mM) was lower than the effect obtained by mastoparan at 0.1 mM. Compound 48/80 is a mixture of polymers obtained from the condensation of *p*-methoxyphenethylamine with formaldehyde and therefore, its concentration cannot be reported on a molar scale (fig.3, inset). Compound 48/80 activated mast cells from 0.01 to 0.5 μg/ml [3,7,22] and purified brain G-proteins from 1 to 100 μg/ml (fig.3). Thus, the secretagogue molecules were more active on intact cells than on their purified putative targets. One might assume that the shift in the active concentrations on the two systems is related to differences in the phospholipid bilayers of reconstituted vesicles or intact cell membranes. Indeed, before these cationic compounds can interact with G-proteins, they need to cross the plasma membrane, possibly through an electrolytic transfer process as proposed for mastoparan [1]. We have found that neuraminidase treatment induced the desensitization of mast cells implying that negative charges of sialic acid residues facilitate the action of these secretagogues [3]. The nature of the G-protein itself could account for the higher potency at the cellular level since the effect of mastoparan has been shown to be greater on G_o and G_i than on G_s and G_t [1]. In this respect, it is clear that, coupled to phospholipase C, mast cells possess a form of soluble PTX-sensitive G-protein which may not be present in the brain [7]. It thus remains possible that this G-protein is more sensitive to substance P or 48/80 than the brain G_o/G_i.

In summary, our results clearly extend the similarities of actions of substance P, compound 48/80 and mastoparan on mast cells to the parallel effect obtained on purified G-proteins. This suggests that the direct activation of a G-protein might be the mechanism of action on mast cells of all these molecules and, for substance P, would be its physiological pathway on mast cells. Although not yet completely understood, mastoparan was proposed to stimulate G-proteins by mimicking part of the intracellular loop of G-protein-coupled receptors [1] and it is likely that the same mechanism occurs for the G-protein stimulation by substance P and compound 48/80.

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