Islet-activating protein, pertussis toxin, inhibits Ca^{2+}-induced and guanine nucleotide-dependent releases of histamine and arachidonic acid from rat mast cells

Tsutomu Nakamura and Michio Ui*

Kyoto Research Laboratories, Kaken Pharmaceutical Co., Ltd, Yamashina, Kyoto 607 and **Department of Physiological Chemistry, Faculty of Pharmaceutical Sciences, Hokkaido University, Sapporo 060, Japan*

Received 18 June 1984

Islet-activating protein (IAP) suppressed Ca^{2+}-induced histamine release and phospholipase A_2 activation in Gpp(NH)p-loaded mast cells. Since the guanine nucleotide-binding protein involved in adenylate cyclase inhibition is known to lose its function upon being ADP-ribosylated by IAP, the nucleotide-binding protein is likely to mediate Ca^{2+}-linked biosignalling leading to histamine secretion.

Islet-activating protein *Guanine nucleotide-binding protein* *Ca^{2+}* *Histamine secretion* *Arachidonic acid*

1. INTRODUCTION

It was recently reported that application of micromolar ATP^{4-} renders mast cells permeable to foreign compounds and that the lesions thus generated in the membrane can be resealed within seconds of adding Mg^{2+} to recover functionally intact cells [1]. A non-hydrolyzable GTP analogue such as Gpp(NH)p was introduced into the cytosol of mast cells by this technique. Histamine was secreted from these cells in response to addition of extracellular Ca^{2+}, suggesting that guanine nucleotide-binding regulatory proteins (N) may play a role in Ca^{2+}-dependent regulation of exocytosis [1].

Islet-activating protein (IAP), pertussis toxin, is a specific modifier of the N protein (N_i) that communicates between membrane receptors and the adenylate cyclase catalytic protein in an inhibitory fashion [2]. N_i loses its function as the communicator when its α-subunit (M_r = 41000) is selectively ADP-ribosylated by IAP [3–9]. We now report that less histamine and less arachidonic acid were released in response to Ca^{2+} addition from the Gpp(NH)p-loaded mast cells when the cells had been exposed to IAP. The results show that the IAP substrate with M_r = 41000 may play an important role in Ca^{2+}-mobilizing receptor-mediated histamine secretion from mast cells.

2. MATERIALS AND METHODS

IAP was purified from the 2-day culture supernatant of *Bordetella pertussis* as in [10,11]. Rat peritoneal mast cells (10^6 cells/ml), isolated and purified as in [12], were first exposed to IAP (10 ng/ml or as indicated in fig.3) or its vehicle for 2 h at 37°C in Eagle's minimum essential medium containing 0.2% bovine serum albumin under an atmosphere of 95% O_2/5% CO_2. The medium for this IAP treatment was supplemented with 0.2 μCi/ml of [1-^{14}C]arachidonic acid (52.9 mCi/mmol, New England Nuclear). During this 2 h

* To whom correspondence should be addressed

Abbreviations: Gpp(NH)p, guanyl-5′-yl β,γ-imidodiphosphate; N_s and N_i, the stimulatory and inhibitory guanine nucleotide-binding regulatory components of adenylate cyclase, respectively

Published by Elsevier Science Publishers B. V.

preincubation time, [^{14}C]arachidonic acid was incorporated into cellular phospholipids; about 64% was found in phosphatidylcholine (submitted).

The cells were then washed 3 times with a divalent cation-free solution (137 mM NaCl, 2.7 mM KCl, 20 mM Hepes, 5.6 mM glucose, 1 mg/ml bovine serum albumin, 30 μM EDTA; pH 7.7) and suspended in the same solution (1.5×10^5 cells/40 μl). The cell suspension was incubated first for 5 min as such, then for 5 min after the addition (10 μl) of ATP and Gpp(NH)p to final concentrations of 20 μM (or as indicated in text) and 1 mM (or as indicated in fig.2), respectively, and finally for 5 min after the addition of $MgCl_2$ (2.5 μl) to 2 mM. These incubations were performed at 37°C in air under oscillation. These cells, washed once and resuspended in the divalent cation-free solution, were further incubated for 10 min (or as indicated in fig.1) at 37°C with the addition of Ca^{2+} to make the final concentration 2.5 mM and pH 7.5. The reactions were quenched with Tris-buffered saline and the supernatant obtained by centrifugation was analyzed for histamine and ^{14}C-labeled arachidonic acid and its metabolites as in [12] (submitted).

3. RESULTS

Here, rat peritoneal mast cells were first exposed to IAP for 2 h. This prolonged IAP treatment was essential, because there is a lag time inevitably preceding the onset of IAP action on intact cells (including mast cells [12]) reflecting the time required for the active component of the toxin to traverse the plasma membrane to reach the IAP substrate inside [13–15]. These 2-h preincubated cells were less susceptible to ATP^{4-} than were the fresh cells. The addition of 5 μM ATP^{4-} [with 1 mM Gpp(NH)p] to cell suspensions, followed by Mg^{2+} for the purpose of resealing, resulted in the subsequent secretion of as much histamine as 30% or more of the total cellular content, in response to the addition of 2.5 mM Ca^{2+}, from fresh cells. This confirms the original finding in [1], but only 12.1% histamine was released from 2-h preincubated cells under the same conditions. As the concentration of ATP^{4-} was increased to 10, 20 and 30 μM in the presence of 1 mM Gpp(NH)p, 20.1, 42.6 and 61.2% of cellular histamine was released, respectively, from the 2-h preincubated cells in response to the subsequent addition of Ca^{2+}; the corresponding values were 3.0, 6.5 and 15.0% when Gpp(NH)p was omitted from the ATP^{4-}-containing medium. The following experiments were hence conducted with 2-h preincubated mast cells that were then treated with ATP^{4-} at 20 μM, a concentration evoking a significant effect of Gpp(NH)p on Ca^{2+}-dependent histamine release. The incorporation of [^{14}C]arachidonic acid into phospholipids during this 2 h preincubation period was not affected by the presence of IAP.

Fig.1a illustrates time courses of histamine release from Gpp(NH)p-loaded mast cells in response to the addition of 2.5 mM Ca^{2+}. Histamine was released following a definite lag period of 15–30 s (see inset). The secretion continued at a high rate for 1.5 min and declined rapidly thereafter. This Ca^{2+}-induced histamine secretion was associated with release of ^{14}C-labeled compounds which occurred immediately after Ca^{2+} addition without delay (fig.1b). Thin-layer chromatography on a silica gel plate revealed that roughly 90% of the labeled products was

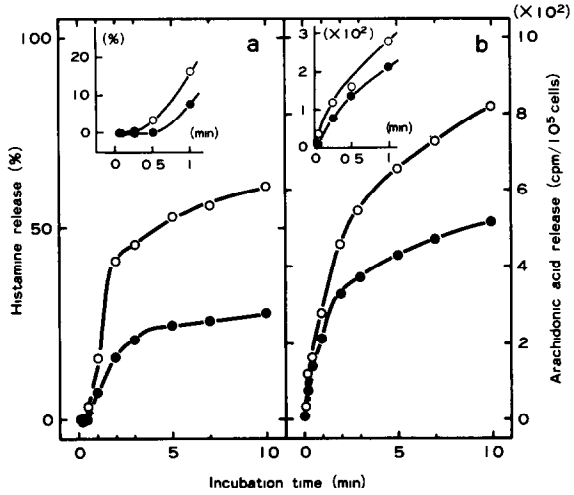

Fig.1. Time courses for Gpp(NH)p-dependent histamine (a) and arachidonic acid (b) release from permeabilized-resealed mast cells in response to Ca^{2+}. Mast cells treated with IAP (●) or its vehicle (○) were loaded with Gpp(NH)p and [1-^{14}C]arachidonic acid as described in section 2. Cells were then incubated for the times indicated after the addition of Ca^{2+}. Histamine release is expressed as % of the total cellular content before incubation. (Inset) Release during the initial short time.

arachidonic acid itself (submitted). Prior treatment of cells with IAP was very effective in inhibiting secretion; the rate of release of histamine or arachidonate from IAP-treated cells was lower than the release from non-treated cells at any time of incubation with Ca^{2+} (fig.1a,b).

Introduction of Gpp(NH)p into the cytosol of mast cells was a prerequisite for the release of histamine and arachidonate upon subsequent addition of extracellular Ca^{2+}; their release was dependent on the concentration of the GTP analogue added to the ATP^{4-}-containing medium (fig.2). There seemed to be an optimum Gpp(NH)p concentration (1–3 mM) for histamine secretion in accordance with [1]. This was also the case with arachidonate release. Less histamine and less arachidonate were released from IAP-treated cells than from non-treated cells at any concentration of Gpp(NH)p, particularly below its optimum concentration.

IAP inhibited Ca^{2+}-induced histamine and arachidonate release from Gpp(NH)p-loaded cells in a concentration-dependent manner (fig.3). The maximal inhibition induced by the highest concentration of IAP was around 50% of the control ([IAP] = 0) value for either histamine or arachidonate release. This inhibition was definitely

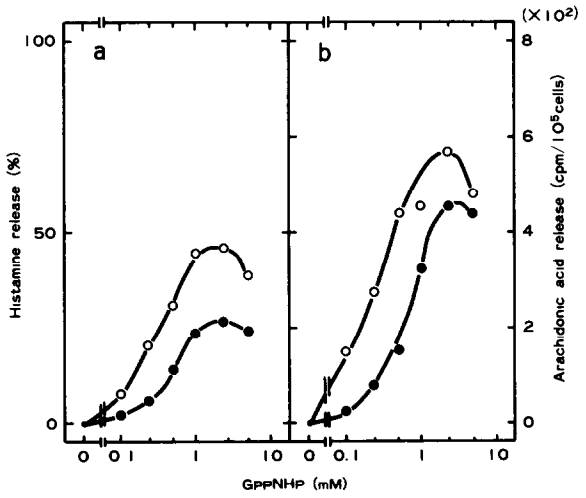

Fig.2. Effect of Gpp(NH)p on Ca^{2+}-induced histamine (a) and arachidonic acid (b) release from permeabilized-resealed mast cells. Experiments were carried out with IAP-treated (●) or non-treated (○) cells as in fig.1 except for changes in the Gpp(NH)p concentration as shown on the abscissa.

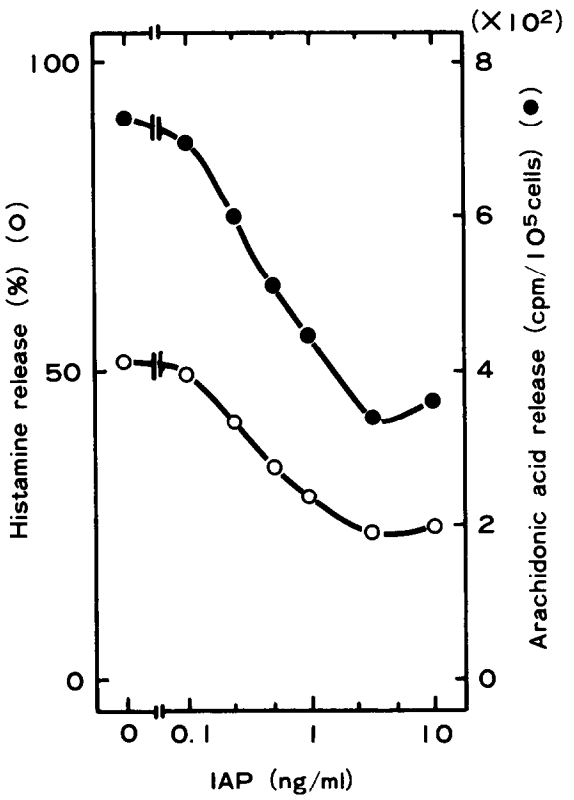

Fig.3. Concentration-dependent inhibition by IAP of Ca^{2+}-induced, Gpp(NH)p-dependent release of histamine and arachidonic acid from permeabilized-resealed mast cells. Experiments were carried out as in fig.1 except for changes in the concentration of IAP as shown on the abscissa.

smaller than the 75–90% inhibition observed for compound 48/80-induced histamine and arachidonate release from intact mast cells ([12], submitted), although the concentration (~0.3 ng/ml) of the toxin required for half-maximal inhibition was of the same order of magnitude for either case.

IAP interacts with the α-subunit of N_i, while cholera toxin catalyzes ADP-ribosylation of the α-subunit of N_s that is involved in receptor-mediated adenylate cyclase activation [2]. In some experiments, IAP was replaced by cholera toxin (1 μg/ml) to compare the effects of both toxins. The cellular content of cyclic AMP was 641, 648 and 2660 fmol/10^6 cells (means of duplicate observations) immediately after Gpp(NH)p loading and Mg^{2+}-induced resealing for control, IAP-treated and cholera toxin-treated cells, respectively. The

Table 1

Effects of inhibitors of phospholipid and arachidonate metabolism on Ca^{2+}-induced and Gpp(NH)p-dependent release of histamine and arachidonic acid from permeabilized-resealed cells

Inhibitor	Histamine release (%)		Arachidonate release (cpm/10^5 cells)	
	$-Ca^{2+}$	$+Ca^{2+}$	$-Ca^{2+}$	$+Ca^{2+}$
None	4.8	23.2	203	588
p-Bromophenacyl bromide (10 μM)	4.9	5.1	227	375
Indomethacin (10 μM)	5.0	23.3	193	593
Nordihydroguaiaretic acid (100 μM)	10.6	11.0	280	791

Inhibitors were added to the media used for 5 min incubation immediately after $MgCl_2$ addition and used for the final 10 min incubation with (or without) 2.5 mM Ca^{2+}. Mast cells not treated with IAP were used. Data are means of duplicate determinations

marked increase in cyclic AMP may reflect cholera toxin-catalyzed ADP-ribosylation of N_s. These cells were further incubated with 2.5 mM Ca^{2+}. Histamine release in response to Ca^{2+} was 35.0, 18.2 and 37.6%, while arachidonate release was 572, 315 and 610 cpm/10^5 cells, from control, IAP-treated and cholera toxin-treated cells, respectively (means of duplicate observations). Thus, the action of IAP in preventing Ca^{2+}-induced histamine and arachidonate release from Gpp(NH)p-loaded mast cells was not mimicked by cholera toxin. The IAP-sensitive α-subunit of N_i, rather than the cholera toxin-sensitive subunit of N_s, appears to play a role in Ca^{2+}-induced histamine release; this role is unlikely to be mediated by cyclic AMP because histamine release was not influenced by cholera toxin-induced accumulation of cyclic AMP.

The effects of inhibitors of arachidonate metabolism were studied, the results being listed in table 1. Ca^{2+}-induced arachidonate release was markedly inhibited by *p*-bromophenacyl bromide, indicating that arachidonate released was a product of phospholipase A_2-catalyzed breakdown of phospholipids. This phospholipase-catalyzed reaction appears to be responsible for histamine secretion, since Ca^{2+} was no longer stimulatory to the secretion in the presence of *p*-bromophenacyl bromide. Indomethacin was without effect; further metabolism of arachidonate to prostaglandins would not be directly related to histamine secretion. Nordihydroguaiaretic acid increased the basal histamine secretion occurring in the absence of extracellular Ca^{2+}, but abolished the action of Ca^{2+} to stimulate the amine secretion despite arachidonate release being normally stimulated by this divalent cation (table 1). Product(s) of a lipoxygenase reaction might play a certain role in exocytosis of histamine granules [16,17].

4. DISCUSSION

Our results confirm the proposal [1] that a class of guanine nucleotide-binding proteins may play a role in the entry of Ca^{2+} into mast cells causing histamine release. Arachidonic acid was also released from the Gpp(NH)p-loaded mast cells in response to Ca^{2+} probably reflecting phospholipase A_2 activation. The nucleotide-binding protein involved appears to be one of the IAP substrates, since Gpp(NH)p-dependent, Ca^{2+}-induced histamine and arachidonic acid release was markedly suppressed by prior treatment of mast cells with the toxin. The pertussis toxin is now known to abolish the functions of nucleotide-binding proteins (N_i [2,5–7] and transducin [18]) as a result of ADP-ribosylation of their α-subunits [3,4,8,9,19].

A protein with the same molecular mass as the α-subunit of N_i was also ADP-ribosylated by IAP in mast cells (submitted).

Exposure of guinea pig neutrophils [20] or mouse 3T3 fibroblasts (submitted) to IAP resulted in marked inhibition of arachidonic acid release from these cells that occurred upon stimulation of membrane receptors by a chemotactic peptide, thrombin or platelet-activating factor. The IAP-induced inhibition was paralleled by ADP-ribosylation of a membrane 41-kDa protein [20]. Arachidonic acid release is a Ca^{2+}-dependent cellular process responsible for histamine secretion from mast cells or basophils [16,17]. Thus, this report suggests that an IAP-susceptible guanine nucleotide-binding protein mediates biosignalling via Ca^{2+}-mobilizing receptors in mast cells. Whether the nucleotide-binding protein is the α-subunit of N_i is a problem for further investigation.

ACKNOWLEDGEMENTS

This work was supported by research grants from the Scientific Research Fund of the Ministry of Education, Science, and Culture, Japan.

REFERENCES

[1] Gomperts, B.D. (1983) Nature 306, 64–66.
[2] Ui, M. (1984) Trends Pharmacol. Sci. 5, in press.
[3] Katada, T. and Ui, M. (1982) Proc. Natl. Acad. Sci. USA 79, 3129–3133.
[4] Katada, T. and Ui, M. (1982) J. Biol. Chem. 257, 7210–7216.
[5] Murayama, T. and Ui, M. (1983) J. Biol. Chem. 258, 3319–3326.
[6] Kurose, H., Katada, T., Amano, T. and Ui, M. (1983) J. Biol. Chem. 258, 4870–4875.
[7] Murayama, T. and Ui, M. (1984) J. Biol. Chem. 259, 761–769.
[8] Katada, T., Bokoch, G.M., Northup, J.K., Ui, M. and Gilman, A.G. (1984) J. Biol. Chem. 259, 3568–3577.
[9] Katada, T., Northup, J.K., Bokoch, G.M., Ui, M. and Gilman, A.G. (1984) J. Biol. Chem. 259, 3578–3585.
[10] Yajima, M., Hosoda, K., Kanbayashi, Y., Nakamura, T., Nogimori, K., Nakase, Y. and Ui, M. (1978) J. Biochem. 83, 295–303.
[11] Yajima, M., Hosoda, K., Kanbayashi, Y., Nakamura, T., Takahashi, I. and Ui, M. (1978) J. Biochem. 83, 305–312.
[12] Nakamura, T. and Ui, M. (1983) Biochem. Pharmacol. 32, 3435–3441.
[13] Katada, T. and Ui, M. (1980) J. Biol. Chem. 255, 9580–9588.
[14] Tamura, M., Nogimori, K., Yajima, M., Ase, K. and Ui, M. (1983) J. Biol. Chem. 258, 6756–6761.
[15] Katada, T., Tamura, M. and Ui, M. (1983) Arch. Biochem. Biophys. 224, 290–298.
[16] Peters, S.P., Siegel, M.I., Kagey-Sobotka, A. and Lichtenstein, L.M. (1981) Nature 292, 455–457.
[17] Peters, S.P., Kagey-Sobotka, A., MacGlashan, D.W., Siegel, M.I. and Lichtenstein, L.M. (1982) J. Immunol. 129, 797–803.
[18] Van Dop, C., Yamanaka, G., Steinberg, F., Sekura, R.D., Manclark, C.R., Stryer, L. and Bourne, H.R. (1984) J. Biol. Chem. 259, 23–26.
[19] Manning, D.R., Fraser, B.A., Kahn, R.A. and Gilman, A.G. (1984) J. Biol. Chem. 259, 749–756.
[20] Okajima, F. and Ui, M. (1984) J. Biol. Chem. 259, in press.