The Release and Subsequent Synthesis of Histamine in a Transfected Subclone of Rat Basophilic Leukemia Cells That Expresses Human Muscarinic m1 Receptors

Yoshinori Goto and Kazutaka Maeyama

Department of Pharmacology, Ehime University School of Medicine, Shigenobu, Ehime 791-02, Japan

Received July 6, 1995 Accepted August 3, 1995

ABSTRACT—Effects of carbachol and antigen (dinitrophenylated bovine serum albumin) on histamine release and histidine decarboxylase (HDC, the enzyme synthesizing histamine) activity were studied in 2H3-m1 cells, a subclone of rat basophilic leukemia cells that expresses human muscarinic m1 receptors through transfection with the gene. Carbachol stimulated the release of histamine and the activity of HDC with 30–50% the intensity of the maximal effect of the antigen. Pirenzepine, an m1 antagonist, inhibited these carbachol effects in a dose-dependent manner. The effect of the combination of carbachol and antigen on histamine release showed no additivity. These results indicate that these effects of carbachol are exerted via m1 receptors, and they suggest that the actions of carbachol and antigen on histamine release share a common pathway(s), and the release and synthesis of histamine have a positive relationship like in a feedback system.

Keywords: Histamine release, Histidine decarboxylase, m1 Receptor

Rat basophilic leukemia (RBL-2H3) cells, an analog of mast cells, release histamine in response to antigen when sensitized with IgE. Cascades responsible for the release have been considered to involve the aggregation of IgE receptors (1), tyrosine phosphorylation of phospholipase C (2) via src-related tyrosine kinases (3), hydrolysis of inositol phospholipids (4), and mobilization of calcium and activation of protein kinase C by generated inositol trisphosphate (5), enhanced calcium influx from the cell exterior (6) and produced diacylglycerol (7). The final two messengers then elicit the release of histamine synergistically (8). 2H3-m1 cells, a transfected subclone of RBL-2H3 cells that expresses human muscarinic m1 receptors (9), respond to carbachol, an m1 agonist, as well as antigen (9). The release of histamine occurs in an early phase of stimulation, while the induction of tumor necrosis factor (TNF) (10), hemopoietic growth factors (11) and histidine decarboxylase (HDC) (12), which synthesizes histamine from l-histidine, is manifested in a late phase. Baumgartner et al. (10) demonstrated that TNF was generated de novo upon cell stimulation and then released by a Golgi-dependent mechanism in 2H3 cells. In addition, they showed that TNF was produced in 2H3-m1 cells to a lesser extent via stimulation with carbachol compared to that with antigen, but it was released to a similar extent with these stimulants. HDC, one of the substances produced in the late phase, exists in 2H3 cells at a measurable level, and its activity changes upon cell stimulation (12) as did that of TNF (10). Unlike TNF, however, the characteristics of HDC activity have not been determined in 2H3-m1 cells, although we know from our previous studies (12, 13) that the induction of the enzyme requires the activation of protein kinase C (and also the mobilization of calcium from the cell exterior, especially when stimulated via IgE receptors) in the original 2H3 cells. The present study was hence performed to further clarify the requirements for the induction of the enzyme and to elucidate the mechanism of histamine release in 2H3-m1 cells.

The 2H3-m1 cells were a gift from Dr. M.A. Beaven, NIH (Bethesda, MD, USA). Dinitrophenylated bovine serum albumin (DNP-BSA), consisting of 24 mol dinitrophenol bound per 1 mol BSA, and monoclonal IgE against DNP-BSA were gifts from Dr. H. Metzger, NIH. Carbachol, pirenzepine and phorbol myristate acetate (PMA) were purchased from Sigma Chemical Co. (St. Louis, MO, USA).

The 2H3-m1 cells were cultured in Eagle's minimum
essential medium with 15% fetal calf serum in a flask in a humidified atmosphere (5% CO₂ + 95% air, 37°C). Cells were seeded in 24-well or 6-well culture plates, and they were incubated with 0.2 µg/ml monoclonal IgE for at least 5 hr. These cells were used for the following assays of histamine release and HDC activity as described previously (13).

After sensitizing the cells with IgE in a 24-well plate, the cultures were washed with 0.5 ml PIPES buffer (13) twice, and after the final wash, 0.2 ml of the same buffer was added to each well. Cells were then incubated with carbachol or 20 ng/ml DNP-BSA (antigen) at 37°C for 20 min, and histamine released into the supernatants was measured by HPLC-fluorometry (14). In the case of pirenzepine, pirenzepine treatment for 1 min was done immediately before adding the stimulants. Antigen at 20 ng/ml produced the maximum effect on the release reaction. The release rates were evaluated with the following formulae:

Net release (%) = (responsively released histamine - non-responsively leaked histamine) x 100 / (total histamine - non-responsively leaked histamine)

Spontaneous release (%) = leaked histamine x 100 / total histamine

After incubation of the cells with stimulants for 2 hr in 6-well plates containing 1 ml medium/well in a CO₂ incubator (or 1-min preincubation with pirenzepine before the 2-hr stimulation in the case of pirenzepine), the attached cells were washed twice with 1.25 ml of ice-cold HDC solution (13), which was 0.1 M potassium phosphate buffer, pH 6.8, containing 0.2 mM dithiothreitol, 0.01 mM pyridoxal 5'-phosphate, 1% polyethylene glycol and 100 µg/ml phenylmethyl-sulfonyl fluoride; A 1- to 1.25-ml aliquot of the same solution was placed in each well and then the cells were sonicated (20 sec/well). The homogenate was centrifuged at 12,000 x g for 20 min, and the supernatant was dialyzed in 50 volumes of HDC solution three times. The enzyme-rich fraction was incubated with 0.25 mM L-histidine for 150 min at 37°C in 0.5 ml HDC solution, and the reaction was stopped by adding 20 µl of 6.2 M perchloric acid. After brief centrifugation, the histamine generated in the supernatant was measured by HPLC-fluorometry (14). HDC activity is expressed as picomoles of histamine produced per min per mg protein. Protein was measured with a Bio-Rad protein assay kit (Richmond, CA, USA). All results are expressed as means ± S.D. The two-tailed unpaired t-test was applied as appropriate.

As shown in Fig. 1A, carbachol stimulated the release of histamine from 2H3-m1 cells in a dose-dependent fashion, whereas it did not in the original 2H3 cells (data not shown). The carbachol response reached the maximum at 1 mM, and it was 46% of the maximal response produced by antigen, which was attained at 20 ng/ml.
Cell responsiveness to carbachol was variable in different cell batches, ranging from 30% to 50% of the maximal antigen response (data not shown). It seemed to depend on whether the cells received multiple passages of reseeding or not. For the sake of clarity, data obtained from cells that did not undergo a high number of passages and showed good responsiveness to carbachol were used in the present study.

Figure 1B depicts pirenzepine concentration-inhibition curve for the carbachol-induced release of histamine from 2H3-m1 cells. Pirenzepine, an m₁ antagonist, inhibited the release of histamine induced with 1 mM carbachol in a dose-dependent manner with an IC₅₀ of 20 nM. Spontaneous release of histamine, which leaked out even in the unstimulated state, in the presence of 10 μM pirenzepine (4.4 ± 0.2%) was not different from that in its absence (4.6 ± 0.3%).

Table 1 shows the effects of carbachol, antigen and their combination in the absence and presence of pirenzepine on the release of histamine. The effect of the combination had an intensity similar to that of antigen alone, indicating that these effects were not additive (Table 1A). Again, pirenzepine inhibited the carbachol-induced release of histamine, while it did not inhibit the antigen effect (Table 1B).

Figure 2A shows HDC activities in 2H3-m1 cells. The activity was raised with 1 mM carbachol, 10 nM PMA and 20 ng/ml antigen in this increasing order of intensity. The effect of carbachol was 40–50% of the antigen effect,
being consistent with the percentage in the case of histamine release.

Figure 2B shows the pirenzepine concentration-inhibition curve for the carbachol-induced increase in HDC activity. Pirenzepine blocked this response at concentrations higher than 0.01 μM, and it suppressed completely at 10 μM with an IC₅₀ of 450 nM. The HDC activity in the unstimulated state in the presence of 10 μM pirenzepine (14.9 ± 3.2 pmol/min/mg protein) was similar to that in its absence (15.2 ± 2.6 pmol/min/mg protein).

The original 2H3 cells do not respond to muscarinic agonists, e.g., carbachol, because they have no muscarinic receptor (9). On the other hand, 2H3-m1 cells that express muscarinic m1 receptors can respond to carbachol. Pirenzepine, an m1 antagonist, in turn, dose-dependently inhibited the carbachol-induced reactions such as the release of histamine (Fig. 1B) and the increase of HDC activity (Fig. 2B). These reactions should therefore be considered to occur via the m1 receptor.

The effects of carbachol and antigen on histamine release were not additive (Table 1), and hence these effects are considered to be elicited through a similar mechanism. In other words, mechanisms following the step involving the IgE receptor and m1 receptor share a common pathway(s) to the final reaction of histamine release. This is supported by the results presented by Beaven’s group (9, 15) that carbachol and antigen mobilized calcium by similar mechanisms and activated exocytosis of granules.

Once the original cells are transfected with the m1 receptor gene and express the receptor on their cell surfaces, the transformed cells can respond to muscarinic stimulations (e.g., carbachol). Direct manipulation was only the transfection of the gene, but it enabled cells to elicit the final reactions with the expressed receptor spontaneously coupled to the downstream mechanisms required for the ultimate responses such as the release of histamine and the increase of HDC activity. The reason why such an appropriate coupling occurs spontaneously is yet unknown.

Taking the data reported up to the present into consideration (1–10, 15), mechanisms leading to the release of histamine are estimated as follows: Aggregation of IgE receptors stimulates src-related tyrosine kinases (3). The kinases activate phospholipase C with phosphorylation (2), and consequently, inositol trisphosphate and diacylglycerol increase. The former causes a transient increase in the intracellular concentration of calcium (5), which is followed by a second sustained increase in cytosolic calcium through enhanced calcium influx (6), and the latter (diacylglycerol) raises the activity of protein kinase C (7). Finally these messengers synergistically induce the release of histamine (8). On the other hand, muscarinic m1 receptors primed with agonist signal to their corresponding G protein (Gq/11) (10) which activates phospholipase C without phosphorylation (9, 15). Subsequent processes are as above. Since the induction of HDC via IgE receptors requires the activation of protein kinase C and the mobilization of calcium as described by the current authors (12, 13), the same pathways as above may mainly contribute to the increase of HDC activity as well. This is also supported by the present additional evidence that PMA induced the similar increase in HDC activity in 2H3-m1 cells (Fig. 2A). HDC, however, may respond to a smaller amount of signals following the m1 receptor, compared with the case of histamine release, because the pirenzepine concentration-inhibition curve for HDC activity (Fig. 2B) shifted rightwards in comparison with that for histamine release (Fig. 1B).

This is the first report demonstrating that HDC activity changes in response to the stimulations with carbachol as well as antigen in 2H3-m1 cells and that the increase in the activity are proportional to the changes in histamine release. These observations provide further evidence supporting our idea that the release and synthesis of histamine have close relationships like a feedback system (12, 13).

Acknowledgments

We wish to thank Dr. Michael A. Beaven (NIH, Bethesda, MD, USA) for generously providing RBL-2H3-m1 cells. This work was supported in part by a Grant-in-Aid for Scientific Research (07557011) from the Ministry of Education, Science, Sports and Culture, Japan.

REFERENCES

1. Maeyama K, Hohman RJ, Metzger H and Beaven MA: Quantitative relationships between aggregation of IgE receptors, generation of intracellular signals, and histamine secretion in rat basophilic leukemia (2H3) cells. J Biol Chem 261, 2583–2592 (1986)
2. Park DJ, Min HK and Rhee SG: IgE-induced tyrosine phosphorylation of phospholipase C-rl in rat basophilic leukemia cells. J Biol Chem 266, 24237–24240 (1991)
3. Jouvin M-HE, Adamczewski M, Numerof R, Lotourneur O, Valle A and Kinet J-P: Differential control of the tyrosine kinases Lyn and Syk by the two signaling chains of the high affinity immunoglobulin E receptor. J Biol Chem 269, 5918–5925 (1994)
4. Maeyama K, Hohman RJ, Ali H, Cunha-Melo JR and Beaven MA: Assessment of IgE-receptor function through measurement of hydrolysis of membrane inositol phospholipids. J Immunol 140, 3919–3927 (1988)
5. Streb H, Irvine RF, Berridge MJ and Schulz I: Release of Ca²⁺ from a nonmitochondrial intracellular store in pancreatic acinar cells by inositol-1,4,5-trisphosphate. Nature 306, 67–69 (1983)
6. Beaven MA, Rogers J, Moore JP, Hesketh TR, Smith GA and Metcalfe JC: The mechanism of the calcium signal and correlation with histamine release in 2H3 cells. J Biol Chem 259,
Short Communication

7 Nishizuka Y: The role of protein kinase C in cell surface transduction and tumour promotion. Nature 308, 693–698 (1984)

8 Beaven MA, Guthrie DF, Moore JP, Smith GA, Hesketh TR and Metcalfe JC: Synergistic signals in the mechanism of antigen-induced exocytosis in 2H3 cells: Evidence for an unidentified signal required for histamine release. J Cell Biol 105, 1129–1136 (1987)

9 Jones SVP, Choi OH and Beaven MA: Carbachol induces secretion in a mast cell line (RBL-2H3) transfected with the m1 muscarinic receptor gene. FEBS Lett 289, 47–50 (1991)

10 Baumgartner RA, Yamada K, Deramo VA and Beaven MA: Secretion of TNF from a rat mast cell line is a brefeldin A-sensitive and a calcium/protein kinase C-regulated process. J Immunol 153, 2609–2617 (1994)

11 Wodnar-Filipowicz A, Heusser CH and Moroni C: Production of the haemopoietic growth factors GM-CSF and interleukin-3 by mast cells in response to IgE receptor-mediated activation. Nature 339, 150–152 (1989)

12 Maeyama K, Taguchi Y, Sasaki M, Wada H, Beaven MA and Watanabe T: Induction of histidine decarboxylase of rat basophilic leukemia (2H3) cells stimulated by higher oligomeric IgE or phorbol myristate acetate. Biochem Biophys Res Commun 151, 1402–1407 (1988)

13 Maeyama K, Taguchi Y, Sakurai E, Sasaki M, Yamatodani A and Watanabe T: Effects of inhibitors of protein kinase C on the release and synthesis of histamine in rat basophilic leukemia cells (2H3). Jpn J Pharmacol 58, 291–298 (1992)

14 Yamatodani A, Fukuda H, Iwaeda T, Watanabe T and Wada H: HPLC determination of plasma and brain histamine without previous purification of biological samples: Cation exchange chromatography coupled with post-column derivatization fluorometry. J Chromatogr 344, 115–123 (1985)

15 Choi OH, Lee J-H, Kassessinoff T, Cunha-Melo JR, Jones SVP and Beaven MA: Antigen and carbachol mobilize calcium by similar mechanisms in a transfected mast cell line (RBL-2H3 cells) that expresses m1 muscarinic receptors. J Immunol 151, 5586–5595 (1993)