

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

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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:

$$\text{Net release (\%)} = (\text{responsively released histamine} - \text{non-responsively leaked histamine}) \times 100 / (\text{total histamine} - \text{non-responsively leaked histamine})$$

$$\text{Spontaneous release (\%)} = \text{leaked histamine} \times 100 / \text{total histamine}$$

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 × *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.

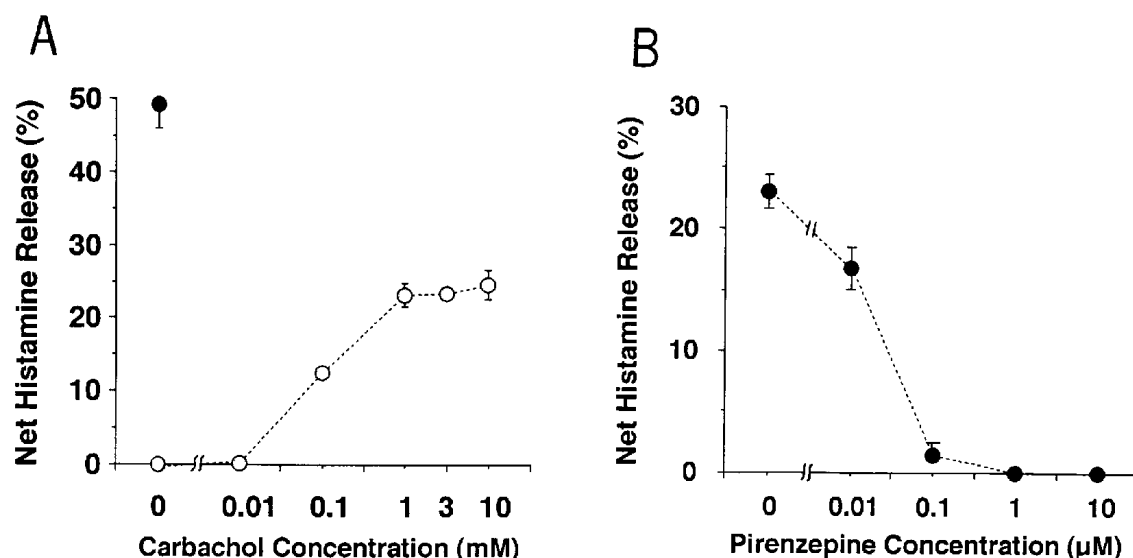


Fig. 1. Histamine release from 2H3-m1 cells. A: Carbachol concentration-response curve for histamine release. Cells were incubated with buffer alone, 20 ng/ml antigen (dinitrophenylated bovine serum albumin) or the indicated concentrations of carbachol at 37°C for 20 min, and the histamine released into supernatant medium was measured. The closed symbol shows the value obtained when the cells are incubated with antigen. Values are means ± S.D., *n* = 3. B: Pirenzepine concentration-inhibition curve for carbachol-induced release of histamine. Cells were incubated with 1 mM carbachol at 37°C for 20 min immediately after pirenzepine was added to give the indicated concentrations, prior to measuring histamine. The IC₅₀ was calculated to be 20 nM. Spontaneous release of histamine, under an unstimulated state, was comparable whether 10 µM pirenzepine was present (4.4 ± 0.2%) or not (4.6 ± 0.3%). Values are means ± S.D., *n* = 3.

Table 1. Effects of carbachol, antigen and their combination in the absence and presence of pirenzepine on histamine release from 2H3-m1 cells

Condition	Net histamine release (%)
A. Without Pirenzepine	
+1 mM Carbachol	22.0±0.14
+20 ng/ml Antigen	46.5±2.43
+ Both	47.0±3.17
B. +10 μM Pirenzepine	
+1 mM Carbachol	-0.13±0.05
+20 ng/ml Antigen	46.9±5.27
+ Both	47.8±1.08

A: Cells were incubated with the indicated stimulants at 37°C for 20 min, and the released histamine was measured. B: Cells were treated with 10 μ M pirenzepine immediately before the incubation with the designated stimulants at 37°C for 20 min, prior to determining released histamine. Spontaneous release of histamine in the absence of 10 μ M pirenzepine (2.6±0.05% of total histamine) was comparable to that in the presence of the agent (2.5±0.1%). Values are means±S.D., n=3. Antigen=dinitrophenylated bovine serum albumin.

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 reseed-ing 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 μ M 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,

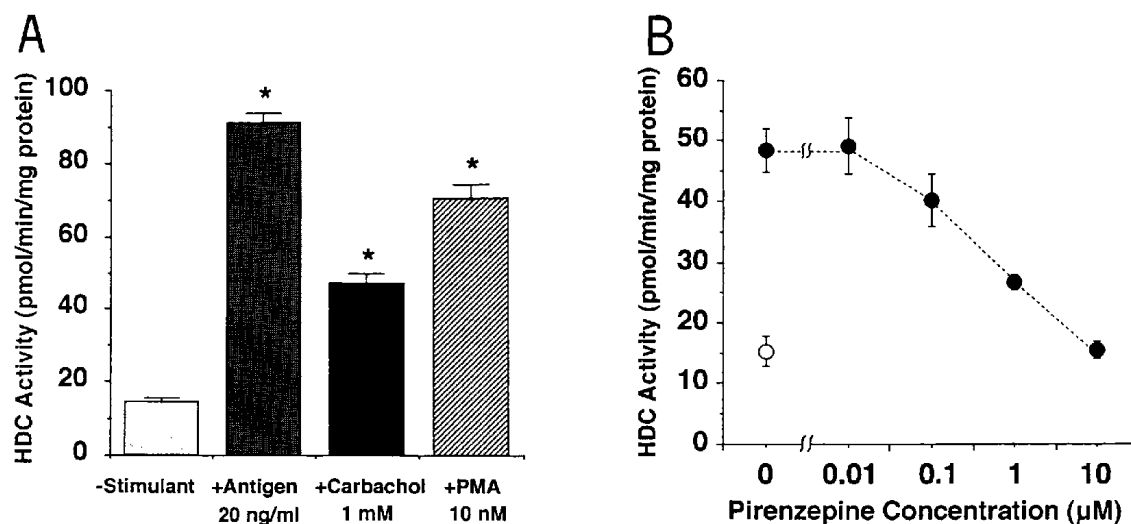


Fig. 2. Histidine decarboxylase (HDC) activity in 2H3-m1 cells. A: HDC activities. Cells were incubated with or without the indicated stimulants at 37°C for 2 hr, and HDC activity was then assayed using cytosol fractions of the cells as the enzyme. Values are means±S.D., n=3. *P<0.01 vs minus stimulant values. Antigen=dinitrophenylated bovine serum albumin. PMA, Phorbol myristate acetate. B: Pirenzepine concentration-inhibition curve for carbachol-induced increase in HDC activity. Cells were incubated with or without 1 mM carbachol at 37°C for 2 hr immediately after being exposed to the indicated concentrations of pirenzepine, followed by the determination of HDC activity. The closed circles denote the value obtained when carbachol was present and the open circle shows the value in its absence. The value obtained when carbachol is absent and 10 μ M pirenzepine is present was 14.9±3.2 pmol/min/mg protein, which was comparable to the value depicted with the open circle (15.2±2.6 pmol/min/mg protein under the plus buffer alone condition). The IC₅₀ was calculated to be 450 nM. Values are means±S.D., n=4.

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 $0.01 \mu\text{M}$, and it suppressed completely at $10 \mu\text{M}$ with an IC_{50} of 450 nM . The HDC activity in the unstimulated state in the presence of $10 \mu\text{M}$ pirenzepine ($14.9 \pm 3.2 \text{ pmol/min/mg protein}$) was similar to that in its absence ($15.2 \pm 2.6 \text{ pmol/min/mg protein}$).

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 correspond-

ing 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).

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