HISTAMINE RELEASE FROM RAT NEUTROPHILS AND
MAST CELLS AS INDUCED BY IONOPHORE
— A PHARMACOLOGICAL APPROACH —

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Abstract—A comparative study was carried out on the histamine release from rat neutrophils and mast cells by calcium ionophore A 23 187 (Ionophore). A maximum release of histamine from neutrophils was induced by 10⁻⁶ g/ml Ionophore and that from mast cells was 5 x 10⁻⁶ g/ml. A fairly good correlation was found between the Ca²⁺ incorporation into and the histamine release from both cells. The Ionophore-induced histamine release from both cells was decreased in Ca²⁺-free Tyrode's solution and by pretreatment with 0.05 M EDTA. Effects of different drugs on Ionophore-induced histamine release from neutrophils were similar to those seen in mast cells. Dibutyl cyclic adenosine monophosphate, theophylline, isoproterenol and prostaglandin E₁ had no or only a slight inhibition on the release. The dose dependent inhibition of release was observed with disodium cromoglycate, N-(3',4'-dimethoxyceinnamoyl) anthranilic acid and disodium baicalein phosphate, in experiments using both cells. Colchicine did not inhibit the reaction in these cells, however phosphatidylserine enhanced the reaction. On the other hand, the effect of concanavalin A was different in each type of cells, the release from mast cells was inhibited while the release from neutrophils was potentiated. These findings suggest the similarity of biochemical events in Ionophore-induced histamine release from neutrophils and mast cells.

Mast cells and polymorphonuclear cells (PMN) mediate immunological tissue injury in immediate hypersensitivity reactions. Among PMN, basophils and neutrophils are well established regarding their pathological roles in allergic inflammation. Histamine which is found both in mast cells and in neutrophils (1) is the most important mediator in the increase in vascular permeability and manifestations of allergic reaction. There is evidence that the mechanism regarding antigen-induced release of histamine from sensitized cells or tissues is fundamentally similar to that of secretion (2). Other workers have suggested that the intracellular mechanism of histamine release involves requirements for Ca²⁺, energy, enzymes and cyclic nucleotides modulation (3, 4). A requirement for extracellular Ca²⁺ has been demonstrated in experiments with human leucocytes, guinea pig lung tissue and rat peritoneal mast cells (2–4). Foreman et al. (5) reported that some antibiotics, Ionophore X537A and Ionophore A 23187 increase the permeability of Ca²⁺ into cells with a high selectivity and induce histamine release from normal cells or tissues. These findings suggest that the secretory process is probably triggered through the uptake of Ca²⁺ into cells. To investigate differences between histamine release from mast cells and from neutrophils, Ionophore A 23187 (Ionophore) was employed and effects of drugs on the reaction were studied.
MATERIALS AND METHODS

Compounds: Ionophore A 23187 (Ionophore), kindly donated by Eli Lilly Co. (Indianapolis, Ind. U.S.A.), was dissolved in dimethylsulfoxide and diluted in the concentrations required with Tyrode's solution. Disodium cromoglycate (DSCG) was a gift from Fujisawa Pharmaceutical Industries Ltd. and N-(3',4'-dimethoxyxycinnamoyl)anthranilic acid (N-5') was kindly provided by Kissei Pharmaceutical Industries Ltd. Disodium baicalein phosphate (BPS) was obtained through the courtesy of Takeda Chemical Industries Ltd.. The other agents including dibutyl cyclic adenosine monophosphate (dib-cyclic-AMP), theophilline, isoproterenol, prostagrandin E₁, colchicine, concanavalin A (Con A) and phosphatidylserine (PS) were purchased from the Nakarai Co. Ltd.. All agents were dissolved in Tyrode's solution before use.

Rat peritoneal mast cells and neutrophils: Male Wistar rats weighing 200 to 250 g were used. According to Johnson and Bach (6), mast cells can be obtained from ascites of rats which had been injected with 5 ml of Tyrode's solution containing heparin 1 unit/ml i.p., by centrifuging through 35% Ficoll. The mast cells obtained using this method were washed twice with cold Tyrode's solution under 200 x G at 4°C for 8 min, and finally suspended (10⁶ cells/ml) in the same solution. Neutrophils were obtained from ascites of rats injected with 5 ml of 0.2% sodium caseinate i.p. one day before, and suspended 10⁷ cells/ml in Tyrode's solution. Neutrophils obtained by this method were 98% purity, and contained less than 1% of mast cells.

Histamine release: Reaction was carried out in the tissue culture plate for microtitration (Falcon, U.S.A.). Cell suspension was poured into all wells in a volume of 0.1 ml each. Unless otherwise stated, the cells were preincubated with 0.1 ml of the drug solution (in concentrations required) at 37°C for 20 min. For the control, a vehicle only was added. Ionophore was then added in a volume of 0.1 ml to each well. Twenty minutes later, the plate was cooled in ice to stop the reaction, and centrifugation was carried out at 230 x G for 10 min. Histamine in the supernatant was assayed biologically using isolated guinea pig ileum. Total histamine of mast cells was 3365.7 ± 1490.75 ng/10⁶ cells (5 experiments), whereas that of neutrophils was 138.9 ± 33.49 ng/10⁶ cells (5 experiments). Histamine release was expressed as percentage to the total histamine and was corrected for spontaneous release without Ionophore (about 2%). The assay was not interfered with by Ionophore in any of the concentrations used.

⁴⁵Ca incorporation: ⁴⁵CaCl₂ (New England Nuclear, Boston, Mass., U.S.A.) with a specific activity of 22.4 mCi/mg was used. Ten microcuries of ⁴⁵CaCl₂ was added to 10⁶ cells suspended in 300 µl of Tyrode's solution. Fifty microliters of Ionophore solution was added and the reaction mixture was incubated at 37°C for 20 min. The cell suspension was then filtrated on 25 mm, 8 µm Millipore filter. The filter which was washed three times with 5 ml of Tyrode's solution containing 0.1% bovine serum albumin was dissolved in 0.1 ml glacial acetic acid and the radioactivity was determined in 10 ml Diotal solution using a Beckman Scintillation Spectrometer. Net ⁴⁵Ca uptake was calculated by correcting for background.
RESULTS

Histamine release and $^{45}$Ca incorporation: Histamine was released by exposing to Ionophore both in neutrophils and in mast cells (Fig. 1). With Ionophore in a concentration of $10^{-6}$ g/ml, a maximal release of histamine from neutrophils was observed with approx. 23% release. In contrast, $5 \times 10^{-6}$ g/ml of Ionophore showed a maximum release with approx. 36% in mast cells. Incorporation of $^{45}$Ca showed a fairly good correlation to histamine release in mast cells, whereas in neutrophils, a significant histamine release occurred without incorporation of $^{45}$Ca in a range of $5 \times 10^{-7}$ g/ml to $10^{-6}$ g/ml Ionophore.

Table 1 shows the data of histamine release by $5 \times 10^{-6}$ g/ml Ionophore under various modified Tyrode's solutions. When the reaction was carried out in Ca$^{2+}$-free solution, the release from neutrophils was decreased to 64.1%, and that from mast cells was 41.4%. Both in neutrophils and in mast cells treated with 0.05M EDTA, only about 5% histamine release was observed with Ionophore under Ca$^{2+}$-free Tyrode's solution containing EDTA.

| Composition                  | Neutrophils | Mast cells |
|------------------------------|-------------|------------|
| Tyrode's solution            | 17.3        | 36.2       |
| Mg$^{2+}$-free solution      | 18.3        | 36.2       |
| Ca$^{2+}$-free solution      | 11.1        | 15.0       |
| Ca$^{2+}$-free solution +0.1 mM-Ca$^{2+}$ | 17.5        | 35.5       |
| Ca$^{2+}$-free solution +1 mM-Ca$^{2+}$ | 17.5        | 34.6       |
| Ca$^{2+}$-free solution +0.05 M-EDTA | 1.0         | 2.1        |

Fig. 1. Histamine release from and $^{45}$Ca movement into rat neutrophils and mast cells by Ionophore A 23,187. Each point indicates the mean of 3 experiments. Vertical bars represent standard error. ○: Histamine release, ●: $^{45}$Ca incorporation.
By adding 0.1 or 1 mM Ca\(^{2+}\) into Ca\(^{2+}\)-free solution, Ionophore-induced histamine release was recovered completely. On the other hand, with a Mg\(^{2+}\)-free solution, the reaction was not affected either in neutrophils or in mast cells.

Effect of dib-cyclic-AMP, theophylline, isoproterenol and prostaglandin E\(_1\): As it has been well established that drugs elevating cyclic 3',5'-adenosine monophosphate (cyclic-AMP) levels in mast cells inhibit reaginic antibody-mediated reaction (9, 10), effect of dib-cyclic-AMP, theophylline, isoproterenol and prostaglandin E\(_1\) on Ionophore-induced histamine release from neutrophils and mast cells was examined. When dib-cyclic-AMP in a range of 10\(^{-9}\) to 10\(^{-3}\) g/ml was added, a slight inhibition was found only in 10\(^{-3}\) g/ml in neutrophils. The inhibition of histamine release from mast cells by dib-cyclic-AMP was faintly potent compared with that from neutrophils. Theophylline in concentrations of 10\(^{-4}\) and 10\(^{-3}\) g/ml decreased the histamine release with 10 to 40\% inhibition in both cells. Histamine release was not at all or only slightly affected by treatments of 10\(^{-7}\) to 10\(^{-5}\) g/ml isoproterenol and 10\(^{-7}\) to 10\(^{-5}\) g/ml prostaglandin E\(_1\) in neutrophils. On the other hand, approx. 20\% inhibition was found with 10\(^{-7}\) to 10\(^{-6}\) g/ml prostaglandin E\(_1\) in mast cells, and isoproterenol had no effect (Fig. 2).

Effect of DSCG, N-5' and BPS: Effect of anti-allergic drugs including DSCG, N-5' and BPS on Ionophore-induced histamine release was examined in a range of 10\(^{-5}\) to 10\(^{-3}\) g/ml. Results from three identical experiments are shown in Fig. 3. In the experiment using neutrophils, all the drugs used showed a dose dependent inhibition of histamine release. These drugs also inhibited the release from mast cells. Among them, DSCG showed the most potent inhibition by approx. 80\% with 10\(^{-4}\) g/ml of the agent. Inhibitory activity of N-5' was about half that of DSCG.

Effect of colchicine, PS and Con A: Since in preliminary experiments, a 60 min pre-treatment of colchicine was required for evidence of activity in histamine release from rat peritoneal mast cells or guinea pig lung tissues, the cells were incubated with colchicine for 60 min before addition of Ionophore. As shown in Fig. 4, there was no inhibition of
histamine release by colchicine either in the neutrophils or in the mast cells. PS showed an increase of histamine release except in the case of neutrophils with $10^{-6}$ g/ml of the agent. Con A potentiated histamine release from mast cells induced by Ionophore. Histamine release from neutrophils was not affected by pretreatment with Con A.

**DISCUSSION**

The present observations suggest that the release of histamine from neutrophils by Ionophore involves biochemical mechanisms similar to those seen in the case of mast cells.
Our findings with rat peritoneal mast cells were much the same as those reported by other workers (6-8). In the experiment with neutrophils, over a narrow dose range, Ionophore led to a rapid release of histamine with Ca\(^{2+}\) influx into the cells without the inclusion of trypan blue or degranulation. The release of histamine from neutrophils in Ca\(^{2+}\)-free medium was decreased by half that seen in normal medium. When 0.05 M EDTA was added to Ca\(^{2+}\)-free medium, a complete inhibition was observed. On the contrary, Mg\(^{2+}\)-free medium did not affect Ionophore-induced histamine release. Therefore, histamine release from neutrophils by Ionophore results from incorporation of Ca\(^{2+}\) into the cells and the mode of release is based on a secretory mechanism just as is the case with mast cells (5). Our result does not provide direct evidence for the requirement of Ca\(^{2+}\) in histamine release from neutrophils caused by antigen antibody reaction, but it does suggest the requirement of Ca\(^{2+}\) in the reaction.

The role of Ca\(^{2+}\) in the biochemical sequence of antigen-induced histamine release was discussed by Austen and Orange (9) and Lichtenstein et al. (10). They postulated different mechanisms with respect to the participation of Ca\(^{2+}\). They also reported that agents elevating cyclic AMP levels in the cells act on the first stage which does not require Ca\(^{2+}\). Austen and Orange have, however, reported that cyclic AMP acts on a further step after Ca\(^{2+}\) incorporation. Dib-cyclic-AMP, isoproterenol, theophylline and prostaglandin E\(_1\) which elevate cyclic AMP level in cells, except for a high concentration of theophylline, had little effect on the histamine release from either type of cells, in particular from the neutrophils. Anti-allergic drugs including DSCG, N-5' and BPS inhibited the release of histamine from neutrophils, with a high concentration of the agents. The inhibitory activities were less than those seen from mast cells. The inhibition by these drugs would be the result of interference of the releasing pathway following the Ca\(^{2+}\) influx.

Colchicine did not inhibit the histamine release from these cells, however, this compound did inhibit histamine release from sensitized guinea pig lung and rat peritoneal mast cells by antigen (in preparation). Since this agent is known to disaggregate the microtubules and stimulate a protein kinase following antigen antibody reaction, and inhibition of histamine release would necessarily take place disaggregation of the microtubules may precede the Ca\(^{2+}\) influx. PS potentiated the histamine release from both cells by Ionophore. This result supports the hypothesis of Mongar and Svec (11) that the secretion of histamine after the infusion of granule membrane and plasma membrane is caused by bridging of PS and Ca\(^{2+}\). A different result was found with Con A between mast cells and neutrophils. In the case of mast cells, the histamine release was enhanced by the treatment of Con A, while no effect was observed in the case of neutrophils. This result indicates a difference in the surface structure of the two types of cells, particularly with regard to the population of surface immunoglobulins.

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