The Seoul Journal of Medicine
Vol. 26, No. 4:331-335, December 1985

A Study on Chemical Mediator of Human Mast Cells†

— Histamine Release from Human Lung and Tonsillar Mast Cells —

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= Abstract =Releasability of histamine, a granule-associated preformed chemical mediator, was observed from enzymatically dispersed human lung and tonsillar mast cells.

IgE-dependent and calcium ionophore A23187 stimulated histamine release from enzymatically dispersed human lung mast cells was increased in dose-dependent fashion. Histamine release reached a maximum in the 1:30 dilution of anti-IgE and at 1.0 μM of calcium ionophore A23187 with human lung mast cells. Above the concentration of secretagogues which caused maximum histamine release, no further release occurred. Almost identical results were obtained with enzymatically dispersed human tonsillar mast cells.

Increasing extracellular calcium concentration from 0.05 to 0.5mM caused a concentration-related increase in histamine release from enzymatically dispersed human lung mast cells.

Key Words: ***Human mast cell, Anti-IgE, Calcium ionophore A23187, Histamine release***

INTRODUCTION

The release of inflammatory mediators from mast cells plays a central role in the pathophysiology of human allergic disorders such as anaphylaxis and bronchial asthma. Type I or immediate hypersensitivity reaction is initiated by a calcium-dependent non-cytotoxic secretion from mast cells of granule-associated preformed mediators and newly generated mediators of inflammation. The secondary recruitment of other cells such as eosinophils and neutrophils by mast cell chemotatic factors contribute further to the ensuing inflammatory tissue reaction (Austen 1979).

Mast cells bear specific receptors for IgE and the reaction of cell-bound IgE molecules, either by multivalent antigen or with divalent anti-IgE antibody induces release of a variety of chemical mediators from the cells (Ishizaka 1981). In recent years, studies on highly purified rat mast cells has advanced our understanding of the early cellular events of coupled activation-secretion, and it has been demonstrated that mast cell IgE-Fc receptor bridging is responsible for triggering mast cells for histamine release (Ishizaka 1981). Since Ca^{++} influx is essential in the initial mast cell activation process, the extracellular calcium concentration may influence the mast cell histamine release.

Most of our knowledge about mast cell activation and secretion has been gained from studies using rat peritoneal mast cells, because they can be readily isolated in a dispersed form and easily purified by differential centrifugation (Holgate *et al.* 1980). However, recent studies have shown that mast cells and basophils from different species and from different sites within the same species may differ both in their structure and function (Church *et al.* 1982).

In this paper histamine releasability from human lung and tonsillar mast cells induced immunologically by anti-IgE and non-immunologically by the calcium ionophore A23187 was investigated. And also the effect of extracellular calcium on histamine release from human lung mast cells was observed.

MATERIALS AND METHODS

Goat anti-human IgE was heat-inactivated by incubation for 1 hour at 56°C. Calcium ionophore A23187, deoxyribonuclease (DNase, bovine pancreas), chymopapain (papaya latex), pronase (*Streptomyces griseus*), human serum albumin and

† This work was supported by 1984 Clinical Research Grant of Seoul National University Hospital.

N-2-hydroxyethyl-piperazine-N -2-ethane sulphonic acid (HEPES) were all obtained from Sigma. Trichloroacetic acid (TCA) and other chemicals were obtained from Junsei Chemical Co. and Kanto Chemical Co.

The composition of the 10mM HEPES buffered salt solution (HBSS) was: NaCl 137mM, KCl 2.7mM, NaH_2PO_4 0.4 mM, $MgCl_2$ 0.5mM, $CaCl_2$ 0.9mM, glucose 5.5mM and HEPES 10mM.

The HBSS was adjusted to pH 7.4 with 2M NaOH and 0.2mg ml^{-1} human serum albumin was added.

Dispersal and challenge of human mast cells:

Human mast cells were dispersed using a modification of methods previously described (Caulfield *et al.* 1980; Church *et al.* 1983; Schulman *et al.* 1982).

Macroscopically normal human lung tissue obtained from surgically resected specimens and surgically removed human tonsils were chopped finely with scissors and digested for 30 minutes at 37°C with pronase (2mg/ml) and chymopapain (0.5mg/ml) in 20mM HEPES-buffered salt solution at pH 7.4. The dispersed cells were separated from tissue fragments by sequential sieving through 800 μm and 60 μm nylon gauze and were rotated with deoxyribonuclease (DNase), 0.02 mg/ml, for 15 to 30 minutes at room temperature to prevent aggregation. Staining of wet preparations with toluidine blue showed mast cells to comprise 1 to 5% total nucleated cells from lung and 0.03 to 0.2% from tonsils.

Before assessment of histamine release, lung and tonsillar mast cells were passively sensitized by incubation for 15 minutes at 37°C with atopic serum. Duplicate aliquots containing 10^5 mast cells in 10 mM HEPES-buffered salt solution at pH 7.4 containing 0.02% human serum albumin (HBSS) were prewarmed to 37°C and challenged by incubation with secretagogues for 15 minutes. Release reactions were stopped by centrifugation at 250 g for 10 minutes at 4°C, and the supernatant was removed, acidified with TCA to a final concentration of 5%, and frozen at -20°C until assay.

Histamine assays:

Histamine was measured by an automated fluorimetric technique, as previously described (Evans *et al.* 1973). Spontaneous histamine release was estimated in duplicate tubes to which no secretagogue was added. Total histamine was measured in parallel duplicate tubes by disintegrating the cells in 5% TCA. Net histamine released by secretagogues is expressed as a percentage of total histamine corrected for spontaneous release.

RESULTS

Histamine release from human mast cells induced by anti-IgE:

Heat inactivated goat anti-human IgE, in dilution of 1:3,000 to 1:10 caused a concentration-related increase in histamine release from enzymatically dispersed human lung mast cells in four experiments. The maximum net histamine release ranged from 7.6 to 26.4% (mean, 19.8%) in the 1:30 dilution of anti-IgE. Above this concentration no further release occurred. Spontaneous histamine release ranged from 5.9 to 11.8% (mean, 8.7%) (Fig. 1.).

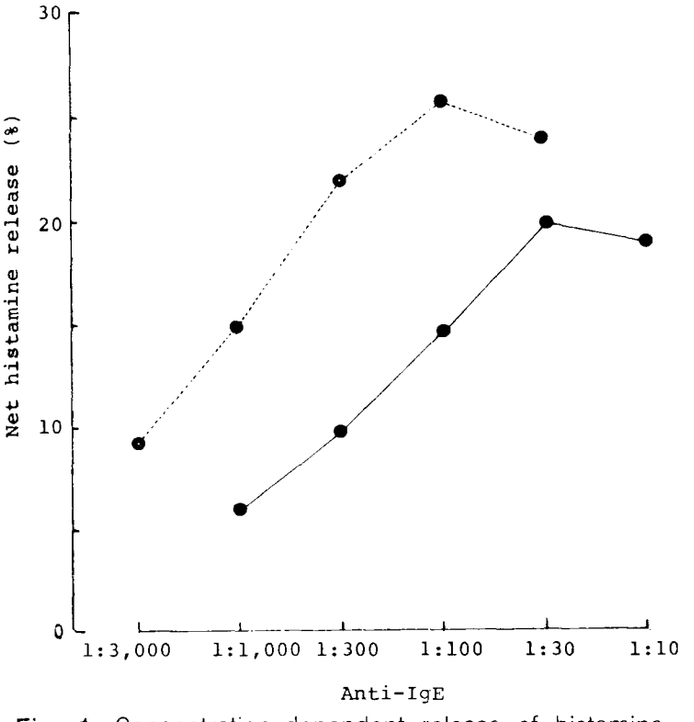

Fig. 1. Concentration-dependent release of histamine from human lung(●) and tonsillar(○) mast cells induced by anti-IgE. In lung mast cells spontaneous histamine release was 8.7% in four experiments. In tonsillar mast cells spontaneous histamine release was 18.4% in three experiments. Results are expressed as mean.

Similar concentration-related increase in histamine release was observed with enzymatically dispersed human tonsillar mast cells in three experiments. The maximum net histamine release ranged from 7.9 to 35.9% (mean, 25.7%) in the 1:100 dilution of anti-IgE. Spontaneous histamine release ranged from 13.5 to 25.0% (mean, 18.4%) (Fig. 1).

Histamine release from human mast cells in-

duced by calcium ionophore A23187:

In four experiments the calcium ionophore A23187 induced a concentration-dependent release of histamine from enzymatically dispersed human lung mast cells that reached a maximum ranged from 38.0 to 59.1% (mean, 51.3%) at 1.0 μM of A23187. Above this concentration no further release occurred (Fig. 2).

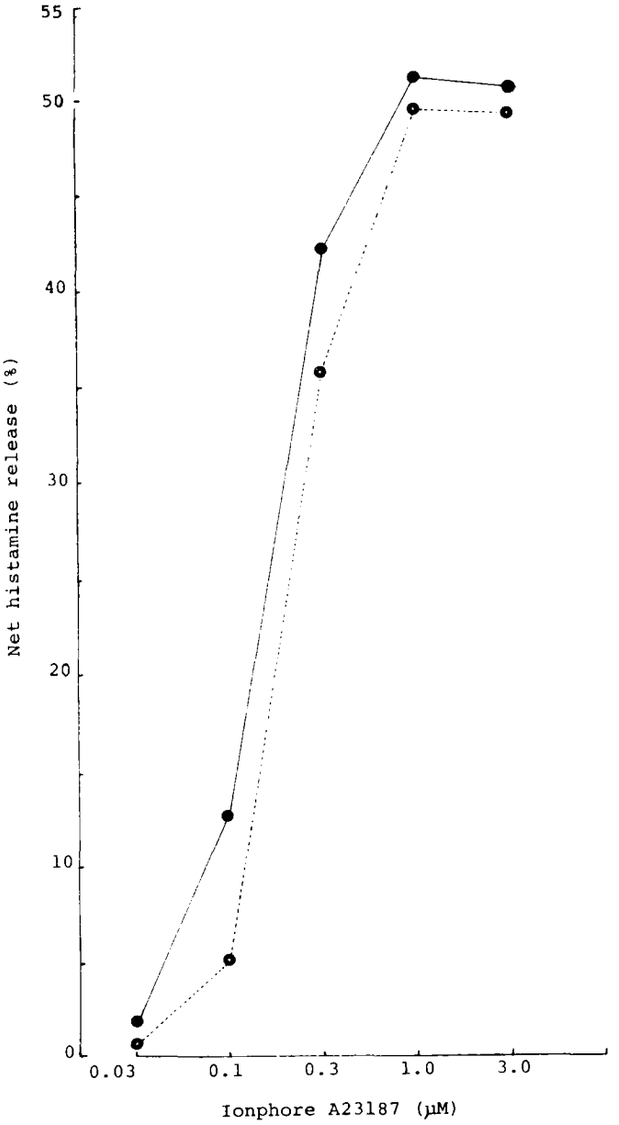

Fig. 2. Concentration-dependent release of histamine from human lung(●) and tonsillar(○) mast cells induced by calcium ionophore A23187. In lung mast cells spontaneous histamine release was 8.7% in four experiments. In tonsillar mast cells spontaneous histamine release was 19.4% in three experiments. Results are expressed as mean.

Similar concentration-related increase in histamine release was observed with enzymatically dispersed human tonsillar mast cells in three experiments that reached a maximum ranged from 44.9% to 56.7% (mean, 49.5%) at 1.0 μM of A23187 (Fig. 2).

Effect of extracellular calcium on histamine release from enzymatically dispersed human lung mast cells:

Experiments were performed to determine the influence of varying extracellular calcium concentration on histamine release from human lung mast cells. Increasing the extracellular calcium concentration from 0.05 to 5 mM resulted in a concentration-related increase from 3.0% to 16.2% in anti-IgE (1:300 dilution) induced histamine release (Fig. 3) and from 3.1% to 33.8% in calcium ionophore A23187 (0.3 μM) induced histamine release (Fig. 4).

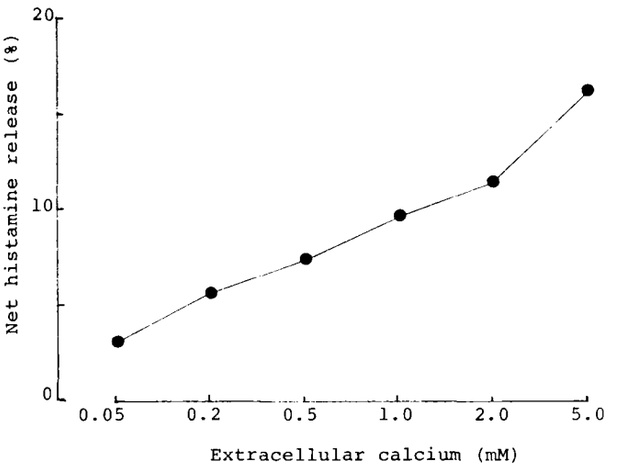

Fig. 3. The effect of extracellular calcium on histamine release from human lung mast cells induced by 1:300 dilution of anti-IgE. Spontaneous histamine release was 6.5% in three experiments. Results are expressed as mean.

DISCUSSION

Tissue mast cells mediate immediate IgE-dependent hypersensitivity reactions through the release of a variety of chemical mediators. The allergic tissue response is initiated by a calcium-dependent non-cytotoxic secretion from mast cells of granule-associated preformed mediators and newly generated mediators of inflammation.

The secondary recruitment of other cells such as eosinophils and neutrophils by mast cell chemotactic factors contribute further to the ensuing allergic inflammatory tissue reaction (Austen 1979).

Mast cells are identified histologically by metachromatic staining of their granules with basic

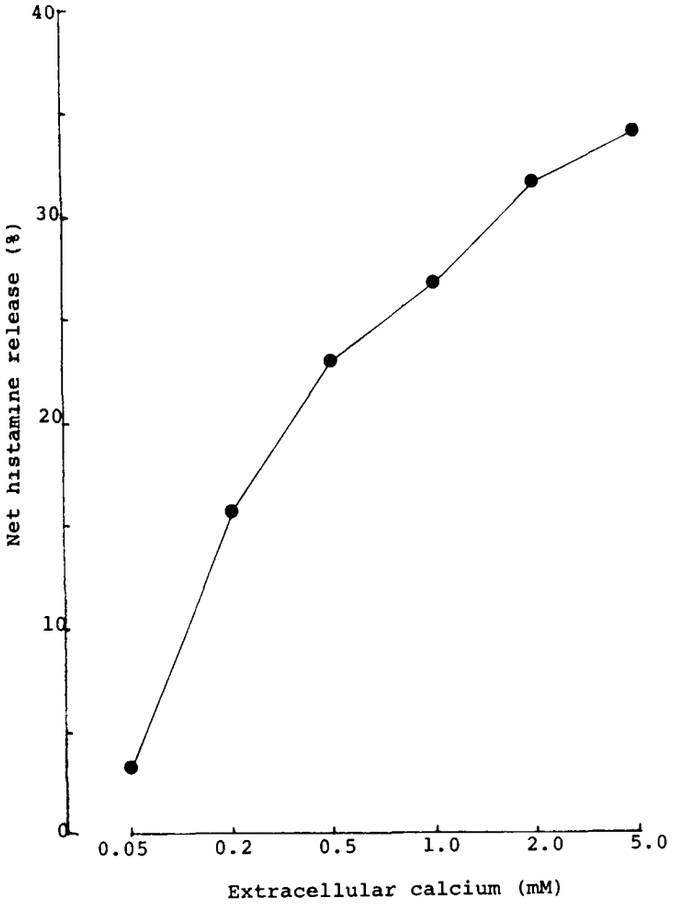

Fig. 4. The effect of extracellular calcium on histamine release from human lung mast cells induced by 0.3 μM of calcium ionophore A23187. Spontaneous histamine releases was 6.5% in three experiments. Results are expressed as mean.

aniline dyes. They are positioned at potential sites of entry of noxious agents such as the perivascular connective tissue, and at serosal and cutaneous surfaces.

Histamine has long been known as a granule marker for mast cell (Hologate and Church 1982). Histamine is synthesized from L-histidine by cytoplasmic histidine decarboxylase and is transported and stored in the secretory granules. Human lung mast cells contain 1.5-5 pg of histamine per cell. The biological effects of released histamine are mediated by two receptor subtypes H_1 and H_2. H_1 receptor mediated biological activities of histamine are vasodilatation, bronchial and intestinal smooth muscle contraction, capillary permeability increment, sensory nerve ending stimulation, bronchial mucus gland secretion increment, chemotaxis for neutrophils and eosinophils (Holgate 1983) which play an important role in acute allergic reaction.

In this experiment, enzymatically dispersed human mast cells have been shown to release a granule-associated preformed chemical mediator, histamine, in concentration-related manner when incubated with either anti-IgE (Fig. 1) or the calcium ionophore A23187 (Fig. 2). The wide diversity in histamine release of human mast cells from different donors indicates that the releasability from human mast cell may depend on individual donor factor as well as degree of sensitization and concentration of secretagogues. It can be said that human lung mast cells and human tonsillar mast cells are closely resemble each other in their secretory response to anti-IgE and calcium ionophore A23187. Like human lung mast cells which are responsible for bronchial asthma, human tonsillar mast cells may be responsible for nasopharyngeal allergic reaction.

Mast cells, like other secretory cells, require an influx of extracellular calcium in order to couple activation to their secretory process (Church *et al.* 1982; Ishizaka 1981; Pearce 1982). In this experiment, the histamine release from enzymatically dispersed human lung mast cells by both anti-IgE and calcium ionophore A23187 depended upon the concentration of extracellular calcium (Fig. 3, Fig. 4), which again confirmed that calcium influx is an obligatory event for mast cell secretion.

These results demonstrate that both human lung and tonsils are rich sources of mast cells which can be dispersed by proteolytic enzyme and can be ideal experimental model to investigate the cellular mechanism of acute allergic reaction.

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= 국문초록 =

인체 비만세포의 화학 매개체에 관한 연구

— 폐 및 편도 비만세포로부터 히스타민 유리 —

서울대학교 의과대학 내과학교실

김 유 영

수술로 적출한 인체의 폐 및 편도조직으로부터 비만세포를 효소로 소화 분리하여 아토피 환자의 혈청으로 수동 감작시킨 후 anti-IgE와 calcium ionophore A23187으로 작동시켜 화학매개체들을 유리시킨후 이들 화학매개체들을 대표할 수 있는 히스타민의 유리농도를 자동 형광분석기를 이용해서 측정, 분석하였다.

결과는 다음과 같다.

1. 폐 비만세포로부터의 히스타민 유리는 anti-IgE와 A23187의 반응량의 증가에 따라 증가하는 용량 반응곡선을 보여 주었는데 anti-IgE는 1 : 30 용액(편도 비만세포의 경우는 1 : 100 용액) A23187은 1.0 μM 용액에서 고평부를 형성하였다.
2. 편도 비만세포로부터의 히스타민 유리도 폐 비만세포와 유사한 반응양상을 보였다.
3. 폐 비만세포로부터의 히스타민 유리는 세포밖의 calcium 농도에 의존적이었다.