The Inhibition Mechanism of Histamine Release by N-(3,4-Dimethoxycinnamoyl) Anthranilic Acid

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Abstract

N-(3,4-Dimethoxycinnamoyl) anthranilic acid (N-5') is an inhibitor of **IgE-mediated** histamine release from mast cells. To elucidate inhibition mechanism, effects of N-5' were examined under various conditions using peritoneal exudate cells and isolated mast cells of rats. N-5' inhibited histamine release induced by antigen, ionophore A 23187, ATP, dextran and phospholipase A₂. But the release induced by compound 48/80 or ionophore X537 A was not inhibited. Influx of Ca⁺⁺ into mast cells and ATP consumption were inhibited. Based on these results, it is presumed that N-5' interferes with the **energy-requiring** system and/or Ca⁺⁺ influx resulting in the inhibition of histamine release.

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From the studies on the anti-allergic actions of principle components of a Chinese herb (*Nandina domestica*), N-(3,4-dimethoxycinnamoyl) anthranilic acid (N-5') was derived from one of the components. N-5' has been already applied orally to prophylactic treatments for asthmatic attacks. Characteristic action of N-5' is the inhibition of allergic histamine release from mast cells [1]. However, the mechanism regarding the inhibition by this agent has not yet been cleared. The present studies were carried out to examine inhibitory effects of N-5' under various conditions and to elucidate inhibition mechanism of N-5'.

Materials and Methods

Peritoneal exudate cells (PEC) were isolated from rats [1] and suspended in phosphate-buffered saline containing 5.6 mM glucose. PEC were sensitized in vitro by incubation with rat IgE anti-serum against DNP-ascaris [1]. In a part of the experiments, isolated mast cells of rats were passively sensitized with this antiserum. Histamine was determined by a fluorometric method [2].

Results and Discussion

Effect on Adenine Nucleotide Levels It has been well known that cAMP plays an important role in IgE-mediated histamine release and

that the energy-requiring process is essential. The relation was investigated between these nucleotides including ATP and the inhibitory effect of N-5'. When DNP-ascaris was added to the sensitized PEC, the histamine release showed a maximum at 0.5–2 min after the antigen challenge. Such a release was inhibited by 100 μM N-5'. Intracellular cAMP and ATP levels decreased in accordance with the time course of histamine release. These decreases in nucleotide levels were also suppressed by N-5'. When sensitized PEC were incubated in the presence of 6–14C-glucose, production of 14CC·2 was accelerated by challenge with the antigen. N-5' suppressed the 14CÜ2 production from glucose to approximately 50%. When glucose was

removed from the incubation medium, histamine release was apparently lower during the absence of glucose than during the presence of glucose. Papaverine, an inhibitor of oxidative phosphorylation, exhibited 85% inhibition of histamine release in the absence of glucose. However, in the presence of glucose, no inhibition was observed. ATP was considered to be supplied through glycolytic pathway. In contrast, N-5' inhibited histamine release to identical percentage with or without glucose. When nonsensitized PEC were incubated with the antigen in the absence of glucose, papaverine decreased ATP level, but no change was observed in the case of N-5'. From these observations, it was assumed that the suppression of the de-

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crease in ATP level by N-5' might be due to the suppression of ATP consumption rather than the inhibition of glucose metabolism.

Thiol-blocking agents such as p-chloromercuri-benzoate and N-ethylmaleimide inhibited degranulation and histamine release from mast cells. These inhibitions were reversed by excess amount of L-cystein as a thiol compound, but the inhibition caused by N-5' as well as DSCG was not reversed by a 10-fold excess of L-cystein. The results indicated the inhibition of histamine release by N-5' was not through blocking of thiol residues of the concerned enzyme system. The activity of crude Na⁺, K⁺-ATPase prepared from rat brain microsomes was inhibited dose-dependently by N-5'. Protein kinase activity in mast cells decreased to approximately 50% 2 min after antigen challenge, when the histamine release was maximum. N-5' inhibited the decrease in the protein kinase activity. Crude preparation of adenylate cyclase of rat liver was inhibited by N-5'. Cyclic AMP phosphodiesterase from rat PEC was also inhibited by N-5'.

Effect on Calcium Influx

Calcium ion has been well established to play a major role in the process of histamine release. The effect of N-5' was examined on the histamine release induced by ionophore and ATP. A 23187 (0.05–0.5 µg/ml) induced histamine release in a concentration-related manner, and N-5' exhibited a significant concentration-dependent inhibitory effect on the histamine release induced by A 23187. Ionophore X537A (0.1–33.3 µg/ml) induced histamine release. N-5' had no effect on the histamine release induced by X537A at the concentrations used (1–1,000 µM), but DSCG exhibited significant inhibition at 1–100 µM. N-5' inhibited histamine release induced by 100 µM ATP. A 23187 has been known to induce histamine release dependent on extracellular Ca⁺⁺ [3], and X537A is Ca⁺⁺-independent [4]. The induction by exogenous ATP absolutely requires Ca⁺⁺ [3]. Compound 48/80 has been reported to be able to induce histamine release in Ca⁺⁺-free medium. N-5' failed to inhibit histamine release by compound 48/80. These observations suggest that the inhibition step by N-5' is a Ca-dependent process, in particular dependent on Ca⁺⁺ influx into cells, which is a trigger of the A 23187 and ATP effects. Actually, Ca⁺⁺ influx was determined by incorporation of ⁴⁵Ca. When the sensitized mast cells were challenged with the antigen, uptake of ⁴⁵Ca was

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Fig. 1. Effect of N-5' (□) on antigen-induced ^{45}Ca influx into sensitized mast cells of rats. The purified mast cells from rats sensitized passively with antiserum were incubated with $^{45}\text{CaCl}_2$ for 5 min after challenge with antigen (DNP-ascaris, 300 ug/ml). ^{45}Ca influx was determined by the method of Foreman et al. [4]. Each bar represents the mean $\pm$ SE of 8–10 observations. Test drugs were administered 1 min prior to challenge with antigen. H = Spontaneous Ca^{++} influx; □ = control; □ = N-5' (concentration-dependent); ■ = antimycin 10^{-5} M. accelerated. N-5' inhibited uptake of ^{45}Ca into the cells dose-dependently (fig. 1).

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