Effect of N-5' on Histamine Release from Rat Peritoneal Exudate Cells Induced by Calcium Ionophore and ATP

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Abstract—We studied the effect of N-(3,4-dimethoxycinnamoyl)anthranilic acid (N-5'), an orally applicable anti-allergic drug, on the histamine release induced by calcium ionophores (A23187 and X537A) and ATP from rat peritoneal exudate cells (PEC). X537A (0.1–33.3 $\mu\text{g/ml}$) induced histamine release in a concentration-related manner, and 2.0 $\mu\text{g/ml}$ of X537A induced release to the same extent as allergic histamine release. Histamine release induced by 2.0 $\mu\text{g/ml}$ of X537A increased with longer incubation time, reaching a peak of about 100% at 120 min. N-5' had no effect on histamine release induced by X537A at the concentrations used (1–1000 μM), but DSCG exhibited significant inhibition at 1–100 μM . A23187 (0.05–0.5 $\mu\text{g/ml}$) induced histamine release in a concentration-related manner, and it seemed that 0.033 $\mu\text{g/ml}$ of A23187 induces the same degree of histamine release as the allergic one. A23187 induced rapid histamine release which attained maximum 1 min after the addition. N-5' exhibited a significant concentration-dependent inhibitory effect on histamine release induced by A23187, and DSCG also exhibited significant inhibition (10 and 1000 μM). N-5' significantly inhibited histamine release induced by 100 μM ATP. These results indicate that N-5' and DSCG effect the histamine release induced by ionophore A23187 and X537A by different manners, and they suggest the possibility that N-5' inhibits some Ca^{++} -dependent processes in histamine release, including the influx of Ca^{++} into cells, which is a trigger of the A23187 and ATP effects.

N-(3,4-dimethoxycinnamoyl)anthranilic acid (N-5') is an anti-allergic drug which specifically inhibits the IgE-mediated reaction, and its characteristic pharmacological action is the inhibition of homologous passive cutaneous anaphylaxis (PCA) (1–3). The inhibition of homologous PCA by N-5' has been shown to be due principally to the suppression of allergic histamine release from mast cells (2), but there are many unclear points regarding the mechanism for inhibition of histamine release. The effect differs from that of disodium cromoglycate (DSCG), and it has been reported that it also differs from the effect of uncouplers such as papaverine

(4, 5).

The focus here is on Ca^{++} which plays a major role in histamine release, and the effects of N-5' on histamine release induced by calcium ionophore A23187 and X537A are compared with those of DSCG. Effect of N-5' on ATP-induced histamine release in which extracellular Ca^{++} is essential (6), as in antigen-induced histamine release from sensitized peritoneal exudate cells, was also examined.

Materials and Methods

1. Preparation of peritoneal exudate cells (PEC) suspension: PEC were prepared as

reported previously (2, 4, 7). The PEC were suspended at a concentration of 5×10^4 mast cells/ml in phosphate buffered saline (PBS) containing 137 mM NaCl, 2.7 mM KCl, 0.9 mM CaCl_2 , 1.0 mM $\text{MgCl}_2 \cdot 6\text{H}_2\text{O}$, 5.6 mM glucose, 5 units/ml of heparin, 5 mM Sorensen phosphate buffer, pH 7.2.

2. Histamine release: The required concentrations of N-5' and DSCG in a volume of 0.4 ml were added to PEC suspended in 2.5 ml of PBS and incubated for 1 min at 37°C , because the inhibition of IgE-mediated histamine release from sensitized rat PEC was most potent with a 1 min pretreatment as described in the previous report (2). Ionophore and ATP solutions (0.1 ml) were then added to bring the total volume to 3 ml. The reaction mixture was then incubated for 10 (ionophore) or 20 min (ATP). The reactions were stopped by chilling in ice, followed by centrifugation at $170 \times g$ for 7.5 min at 4°C . The supernatant was then separated from the precipitate, and the histamine was determined by the method of Shore et al. (8). The percent of histamine release was calculated from the total amount of cellular histamine and the histamine content in the supernatant.

3. Drugs used: The drugs used in this experiment were the following: N-(3,4-dimethoxycinnamoyl)anthranilic acid (N-5', Kissei Pharmaceutical Co., Ltd.), disodium cromoglycate (DCSG, Fujisawa Pharmaceutical Co., Ltd.), heparin (Novo), ionophore A23187 (A23187, Eli Lilly), ionophore X537A (X537A, Hoffman La Roche), ATP 2Na salt (Sigma), and o-phthalaldehyde (Nakarai Chemicals).

N-5' was dissolved in 1% NaHCO_3 aqueous solution and diluted to the required concentration by PBS. Ionophore was dissolved in ethanol and then diluted to the required concentration by PBS. The ethanol concentration was less than 0.1% unless otherwise indicated.

Results

1. Effect on histamine release by X537A:

As shown in Fig. 1, when various concentrations of X537A were added to rat PEC suspension, histamine release began to be seen at the concentration of 0.1 $\mu\text{g/ml}$ of

Fig. 1. Histamine release from rat PEC induced by ionophore X537A. Each point indicates the mean of 4 to 8 experiments, except for 2 experiments at the concentration of 33.3 $\mu\text{g/ml}$. Vertical bars indicate the standard error.

Fig. 2. Time course of histamine release from rat PEC induced by ionophore X537A. Each point indicates the mean of 3 to 8 experiments. Vertical bars indicate the standard error.

X537A, and the percent release was about 100% at the peak concentration of 33.3 $\mu\text{g/ml}$. About 2.0 $\mu\text{g/ml}$ of X537A induced histamine release to the same degree as that induced by antigen from sensitized PEC with rat IgE antiserum in vitro (previously reported [2, 4]). The time course of histamine release induced by X537A at this concentration was examined. In the following experiments, the final ethanol concentration used was 0.1%, a concentration which had no effect on X537A-induced or spontaneous and antigen-induced histamine release (data not shown). As shown in Fig. 2, histamine release induced by X537A increased with longer incubation time, reaching a peak at 120 min.

As shown in Fig. 3, N-5' had no effect on histamine release induced by 2.0 $\mu\text{g/ml}$ of X537A at any concentration used, but DSCG exhibited significant suppressions of 38.8%

Fig. 3. Effect of N-5' and DSCG on histamine release from rat PEC induced by ionophore X537A. Each point indicates the mean of 4 experiments. Vertical bars indicate the standard error. Numbers in parentheses indicate percent inhibition with respect to the control. * and †: Statistical significance from the control at $P<0.05$ and $P<0.02$, respectively, according to Student's *t*-test.

and 38.3% ($P<0.02$) at 10 and 1 μM , respectively. The inhibitory effect of DSCG decreased with the increase in concentration above 10 μM : 27.8% ($P<0.05$) inhibition at 100 μM and virtually no effect at 1,000 μM .

2. Effect on histamine release by A23187:

Figure 4 illustrates the concentration-response curve of histamine release from rat PEC induced by A23187. Histamine release was evident at concentrations exceeding 0.01 $\mu\text{g}/\text{ml}$, and a peak release rate of 78% was attained at about 0.2 $\mu\text{g}/\text{ml}$. It was presumed that 0.02 to 0.05 $\mu\text{g}/\text{ml}$ of A23187 induced the same degree of histamine release as antigen-induced histamine release from sensitized rat PEC, which was previously reported (2, 4) (Fig. 4). Based on these results, the time course of histamine release induced by 0.033 $\mu\text{g}/\text{ml}$ of A23187 was examined. As shown in Fig. 5, A23187 induced a rapid histamine release which attained a maximum 1 min after the addition.

Subsequently, the effects of N-5' and DSCG on A23187-induced histamine release were studied. N-5' had no effect on histamine release induced by A23187 at 1 μM , but

Fig. 4. Histamine release from rat PEC induced by ionophore A23187. Each point indicates the mean of 7 to 8 experiments. Vertical bars indicate the standard error.

Fig. 5. Time course of the histamine release from rat PEC induced by ionophore A23187 (0.033 $\mu\text{g}/\text{ml}$). Each point indicates the mean of 4 experiments. Vertical bars indicate the standard error.

it exhibited a slight inhibitory effect at 10 μM . Its inhibitory effect was significant at 100 μM and 1,000 μM : 28.8% ($P<0.05$) and 78.5% ($P<0.001$), respectively. On the other hand, DSCG exhibited slight inhibition at 1 μM . At 10 and 1,000 μM , it had had a significant inhibitory effects of 39.9% ($P<0.02$) and 72.2% ($P<0.01$), respectively, but no inhibitory effect was evident at 100 μM (Fig. 6).

The effects of N-5' and DSCG on the concentration-response curve for A23187 were studied. As shown in Fig. 7, 100 μM N-5' and 10 μM DSCG exhibited obvious inhibitory effects on histamine release induced by 0.05 and 0.1 $\mu\text{g}/\text{ml}$ of A23187. However, they had no effect on release by 0.01, 0.02 and 0.2 $\mu\text{g}/\text{ml}$, the highest concentration of A23187 used.

Fig. 6. Effect of N-5' and DSCG on histamine release from rat PEC induced by ionophore A23187 ($0.033 \mu\text{g/ml}$). Each point indicates the mean of 6 to 8 experiments. Vertical bars indicate the standard error. Numbers in parentheses indicate percent inhibition with respect to the control. *, ** and †: Statistical significance from the control at $P < 0.05$, $P < 0.02$ and $P < 0.001$, respectively, according to Student's *t*-test.

Fig. 7. Effect of N-5' and DSCG on the concentration-histamine release curve of ionophore A23187. Each point indicates the mean 5 to 8 experiments. Vertical bars indicate the standard error. Numbers in parentheses indicate percent inhibition with respect to the control. * and †: Statistical significance from the control at $P < 0.05$ and $P < 0.02$, respectively, according to Student's *t*-test.

3. Effect on histamine release by ATP:

Slight histamine release was evident with $50 \mu\text{M}$ ATP, and $44.7 \pm 0.34\%$ and $69.9 \pm 0.80\%$ releases were found with 100 and $200 \mu\text{M}$ ATP respectively. Histamine release induced by $100 \mu\text{M}$ ATP involved a comparatively fast reaction for the first 5 min,

Fig. 8. Effect of N-5' on ATP-induced histamine release from rat PEC. Each column indicates the mean of 3 experiments. Vertical bars indicate the standard error. Number in parenthesis indicate percent inhibition with respect to the control. †: Statistical significance from the control at $P < 0.001$ according to Student's *t*-test.

with a slight rise thereafter, up to 20 min after the addition of ATP. Effect of N-5' on the histamine release from rat PEC incubated with $100 \mu\text{M}$ ATP for 20 min was examined. As shown in Fig. 8, pretreatment with $100 \mu\text{M}$ N-5' inhibited histamine release induced by ATP 60.0% ($P < 0.01$).

Discussion

N-5' and of DSCG have different effects on the histamine release induced by the calcium ionophores X537A and A23187. DSCG exhibits inhibitory effects on the release induced by both X537A and A23187, while N-5' exhibits an inhibitory effect, dependent on the concentration, only on histamine release induced by A23187. A23187 has been confirmed to induce rapid histamine release which is dependent on the Ca^{++} in extracellular fluids (6, 9). There had been opposing views by Foreman et al. (10) that histamine release induced by X537A is not dependent on extracellular Ca^{++} and by Cochrane and Douglas, and Kagayama and Douglas (11, 12) that the histamine release is due to exocytosis dependent on Ca^{++} . However, Kazimierzczak et al. (13) reported that histamine release by X537A is dependent

on monovalent cations and not dependent on extracellular Ca^{++} . In addition, there are also reports concerning histamine release and morphological changes in mast cells after treatment with X537A which indicate that cellular swelling is rather important and that the time course of this swelling exhibits a linear relation to histamine release (6, 14, 15). As stated previously, N-5' exhibits a suppressive effect on histamine release induced by A23187, but has no effect on that induced by X537A. In addition, N-5' also exhibits an inhibitory effect on histamine release due to ATP, in which the presence of extracellular Ca^{++} is indispensable (6). From these results, it seems that N-5' inhibits the Ca^{++} -dependent process in histamine release, including the influx of Ca^{++} into cells, which is an essential process of the A23187 and ATP effects. It is also suggested that there are differences in the mechanisms for manifestation of the histamine release induced by the two ionophores used. The significant difference in the chronology of the histamine release induced by the two ionophores used also support this suggestion.

One hundred μM N-5' significantly inhibited only histamine release induced by 0.05 $\mu\text{g/ml}$ A23187. It was established that a high concentration of A23187 induces the release of histamine and lactate dehydrogenase, an enzyme in the cytosol, resulting in the cytolytic action of A23187 (16). N-5' did not inhibit histamine released by a high concentration of A23187. The presumption that N-5' has an inhibitory effect only on the non-cytolytic response but not on the cytolytic one may account for this.

The inhibitory effect of DSCG on X537A-induced histamine release attenuated with increase in its concentration, and 100 μM DSCG scarcely affected A23187-induced histamine release differly from other concentrations tested. We previously reported such dose-independency for the inhibitory effect of DSCG on IgE-mediated or combination of dextran and phosphatidylserine-induced histamine release from rat PEC (4, 7). The reason for this is yet unknown. The inhibitory effect of DSCG on histamine release must be further studied with regards to the mechanisms of histamine release by the various

histamine releasers mentioned above.

Ca^{++} has been reported to be necessary in histamine release mediated by IgE antibody as well as exogenous ATP and ionophore A23187 (6). N-5' exhibits an inhibitory effect on histamine release from rat PEC mediated by IgE antibody, but it has no effect on the release induced by compound 48/80 (4, 7). There have been reports that compound 48/80 can induce histamine release even in Ca^{++} -free media (6, 17, 18). Accordingly, the effect of N-5' in inhibiting histamine release may be due in part to the inhibition of the influx of extracellular Ca^{++} , which is a coupler of stimulus-secretion coupling. However, as direct proof is essential, we are now studying this matter.

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