

Role of protein kinase C in histamine release from human basophils

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In this study, we investigated the role of calcium and phospholipid-dependent protein kinase (protein kinase C, PKC) in the modulation of histamine release from human basophils. A novel and potent inhibitor of PKC, K-252a, inhibited the release of histamine induced by anti-IgE in a dose-dependent manner with ID_{50} (the dose required for 50% inhibition of histamine release) of 2.2×10^{-8} M. Histamine release stimulated with 12-0-tetradecanoyl-phorbol-13-acetate (TPA) was also suppressed by K-252a with maximal inhibition of $48.0 \pm 9.3\%$ at 10^{-7} M. In contrast, K-252a did not inhibit the release of histamine in response to FMLP and ionophore A23187. Another inhibitor of PKC, H-7, exhibited a dose-dependent inhibition of anti-IgE-induced histamine release with ID_{50} of 8.6×10^{-4} M. H-8 and HA1004, which closely resemble H-7 in chemical structure but are less potent in inhibiting PKC, did not inhibit histamine release stimulated with anti-IgE, but rather enhanced the release at higher concentrations. These results strongly suggest that PKC activation plays a crucial role in the mediation of IgE-mediated histamine release from human basophils.

Key words: H-7; histamine release; K-252a; protein kinase C.

Accepted for publication 4 august 1987.

There is accumulating evidence which indicates that calcium and phospholipid-dependent protein kinase (protein kinase C, PKC) plays an important role in a variety of cellular functions (7).

Histamine release reactions from mast cells and basophils are good *in vitro* model systems for immediate hypersensitivity. It has been reported that exposure of the sensitized PT-18 cells to antigen induces an increase in PKC activity in the plasma membrane (11). Further, the tumor promotor, 12-0-tetradecanoyl-phorbol-13-acetate (TPA), which has the ability to activate PKC, induces the release of histamine from human basophils and rat mast cells (6, 9). These observations suggest that PKC activation plays a role in the mediation of histamine release.

In this study, we investigated the effect of inhibitors of PKC on basophil histamine release.

MATERIAL AND METHODS

Material

Piperazine-N,N'-bis [2-ethanesulfonic acid] (Pipes), formyl-methionyl-leucyl-phenylalanine (FMLP), and 12-0-tetradecanoyl-phorbol-13-acetate (TPA) were purchased from the Sigma Chemical Co., St. Louis, MO., USA. The calcium ionophore A23187 was obtained from Calbiochem-Behring Corp., La Jolla, CA, USA. Anti-human IgE was purchased from Medical Biological Laboratory, Nagoya, Japan. 1-(5-isoquinolinesulfonyl)-2-methylpiperazine dihydrochloride (H-7), N-[2-(methylamino) ethyl]-5-isoquinolinesulfonamide dihydrochloride (H-8), N-(2-guanidinoethyl)-5-isoquinoline-sulfonamide hydrochloride (HA1004) were purchased from 0.1-1.2 Seikagaku Kogyo Co., Ltd., Tokyo, Japan. (8R*, 9S*, 11S*)-(9-hydroxy-9-methoxycarbonyl-8-methyl-2,3,9,10-tetrahydro-8,

11-epoxy-1H, 8H,11H-2,7b,11a-triazadibenzo [a,g]cycloocta[cde]trinden-1-one (k-252a) was kindly donated by Kyowa Hakko Kogyo Co., Ltd., Tokyo, Japan. FMLP, the calcium ionophore A23187, TPA and K-252a were dissolved in dimethylsulfoxide (Kanto Chemical Co., Tokyo, Japan) at 5×10^{-2} M, 1 mg/ml, 1 mg/ml and 10^{-4} M, respectively and stored at -20°C .

Buffer

Pipes A buffer that contains 119 mM NaCl, 5mM KCl, 25 mM Pipes and 0.03% human serum albumin (pH 7.4) was used to wash leukocytes. Pipes A containing 2 mM Ca^{2+} and 0.5 mM Mg^{2+} (Pipes ACM) was used for histamine release reactions.

Preparation of leukocytes

Venous blood was drawn by venipuncture with heparin from patients with bronchial asthma, allergic rhinitis, and atopic dermatitis and from healthy donors, and allowed to sediment at room temperature for 90 min with 2.5 ml of 6% dextran-2.8% glucose solution (6% dextran in saline, Midorijuji Pharmaceutical Co., Osaka, Japan) per 10 ml of blood. The plasma-leukocyte layer was transferred to another tube. After centrifugation at $400 \times g$ at 4°C , leukocytes were obtained and washed with Pipes A containing 4 mM ethylenediaminetetraacetic acid, washed again with Pipes A and finally suspended in an adequate amount of Pipes ACM.

Histamine release

Leukocytes in 0.2 ml of Pipes ACM were preincubated with 0.2 ml of various concentrations of protein kinase inhibitors for 15 min at 37°C . Histamine release reaction was initiated by the addition of 0.1 ml of anti-IgE, ionophore A23187, FMLP, or TPA. Incubation was carried out at 37°C for 45 min. The final cell concentrations of reaction mixture were 1 to 6×10^6 /ml. Final concentrations of anti-IgE, ionophore A23187, FMLP and TPA were 1:1000 (or 1:10000), 0.2 $\mu\text{g}/\text{ml}$, 10^{-4} M and 100 ng/ml respectively. Tubes with cells and buffer alone were included in each experiment to determine spontaneous histamine release (blank).

The tubes were then centrifuged at $400 \times g$ for 15 min at 4°C . Supernatants were stored at 4°C until histamine assay. Aliquots of cells in 0.5 ml of Pipes ACM were lysed with 2% perchloric acid to evaluate the total cellular histamine. Experiments were performed in duplicate or triplicate.

Histamine was determined by an automated fluorometric technique (10). Histamine release in experimental tubes was calculated as a percentage of total cellular histamine after subtracting blanks from all values. Spontaneous histamine release was consistently less than 5%.

Statistical analysis

An analysis of variance with two-way layout was used to analyse the data. Statistical significance was tested by Bonferroni's test.

Table 1
Effect of K-252a on basophil histamine release

Control ^b	% Histamine release	K-252a (M) ^a			
		10^{-10}	10^{-9}	10^{-8}	10^{-7}
		% Inhibition			
Ionophore A23187 (5)	75.4 ± 4.6	-1.5 ± 3.5^d	-3.0 ± 2.6	1.3 ± 3.3	$-12.1 \pm 5.7^{*c}$
FMLP (5)	35.7 ± 5.3	-3.4 ± 3.2	-5.5 ± 2.9	-3.7 ± 2.2	-4.7 ± 3.3
TPA (4)	43.1 ± 9.3	0.2 ± 11.3	0.1 ± 11.4	6.9 ± 14.3	$48.0 \pm 9.3^{**}$

^aHuman leukocytes were preincubated at 37°C with 10^{-10} to 10^{-7} M of K-252a 15 min before stimulation with ionophore A23187, FMLP or TPA for 45 min.

^bHistamine release in the absence of K-252a.

^cSignificant differences from control values were indicated by asterisks; $*P < 0.05$, $**P < 0.01$.

^dData represent mean $\pm$ SEM of numbers of experiments shown in parenthesis.

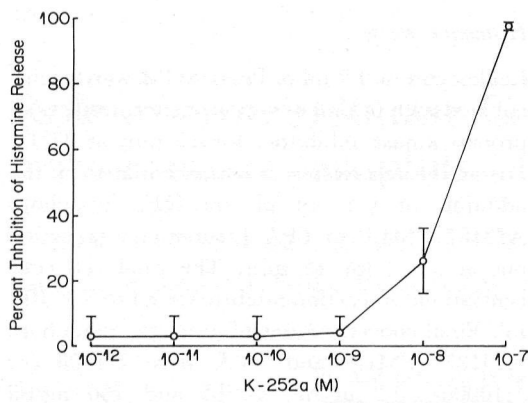


Fig. 1. Effect of K-252a on anti-IgE-induced histamine release from human basophils. Leukocytes were preincubated with 10⁻¹² to 10⁻⁷M of K-252a at 37°C, 15 min before stimulation with anti-IgE for 45 min. Histamine release in the absence of K-252a was 39.2 ± 8.8%. Data represent mean ± SEM of 5 experiments.

RESULTS

Effect of K-252a on histamine release from human basophils

Experiments were performed to investigate the role of PKC in histamine release from human basophils, using an inhibitor of this enzyme. K-252a is a novel and potent inhibitor of PKC and cyclic nucleotide-dependent protein kinases (4, 5).

K-252a inhibited anti-IgE-induced histamine release in a dose-dependent manner with ID₅₀

(the dose required for 50% inhibition of histamine release) of 2.2 × 10⁻⁸M (Fig. 1.) Maximal inhibition was 98.1 ± 0.9 (mean ± SEM) % at 10⁻⁷M (P < 0.01).

As expected, histamine release induced by TPA, which is a potent activator of PKC, was also reduced by K-252a in a dose-dependent fashion (Table 1). Maximal inhibition was 48.0 ± 9.3% at 10⁻⁷M (P < 0.01). In contrast, the release of histamine stimulated by FMLP and ionophore A23187 was not blocked by K-252a at concentrations ranging from 10⁻¹² to 10⁻⁷M (Table 1). K-252a slightly enhanced ionophore A23187-induced histamine release rather than inhibited it (P < 0.05).

Effect of various protein kinase inhibitors on anti-IgE-induced histamine release from human basophils

The results with K-252a appear to indicate that PKC plays an important role in IgE-mediated histamine release from human basophils. However, as K-252a could suppress anti-IgE-induced histamine release via inhibition of cyclic nucleotide-dependent protein kinases, the effect of another inhibitor of PKC (H-7) on histamine release stimulated by anti-IgE was examined. H-7 is a potent inhibitor of protein kinases including PKC (3). As controls, H-8 and HA1004, which closely resemble H-7 in chemical structure but are less potent in inhibiting PKC, were used (1, 3).

H-7 exhibited a dose-dependent inhibition of anti-IgE-induced histamine release with 53.2 ± 12.8% at 10⁻³M (Table 2). ID₅₀ was 8.6 × 10⁻⁴M.

Table 2
Effect of various protein kinase inhibitors of anti-IgE-induced histamine release from human basophils

Inhibitors ^a	Concentrations (M)				
	10 ⁻⁷	10 ⁻⁶	10 ⁻⁵	10 ⁻⁴	10 ⁻³
H-7	-0.8 ± 2.0 ^b	4.7 ± 3.1	6.3 ± 1.7	10.8 ± 9.2	53.2 ± 12.8 ^{**c}
H-8	0.9 ± 0.9	-2.9 ± 2.0	-5.2 ± 4.3	-23.9 ± 9.2 [*]	-38.1 ± 14.2 ^{**}
HA1004	-1.6 ± 6.0	-5.3 ± 1.7	-1.6 ± 3.1	-7.8 ± 1.6	-11.3 ± 1.0 [*]

^aHuman leukocytes were preincubated at 37°C with 10⁻⁷ to 10⁻³M of H-7, H-8 or HA1004 15 min before stimulation with 1:1000 dilution of anti-IgE for 45 min.

Control release in the absence of inhibitors was 50.2 ± 6.4 (mean ± SEM) %.

^bData represent mean ± SEM of 3 experiments.

^cSignificant differences from control values were indicated by asterisks; ^{*}P < 0.05, ^{**}P < 0.01.

In contrast, H-8 and HA1004 did not inhibit this reaction (Table 2). These compounds at higher concentrations augmented histamine release rather than inhibited it (Table 2).

DISCUSSION

The calcium and phospholipid-dependent protein kinase (PKC) has been implicated in the signal transduction in a variety of cellular functions (7). This enzyme has been shown to play a crucial role in the mediation of release from human platelets in response to thrombin reactions, such as serotonin release and collagen, and superoxide anion release from human neutrophils stimulated with TPA (2, 7, 12). Several observations suggest that PKC activation is involved in the activation of mast cells and basophils (6, 9, 11). White et al. have demonstrated antigen-induced increase in protein kinase C activity in the plasma membrane of colony-stimulating factor-dependent mouse mast cell line (PT-18 cells) (11). Histamine release from human basophils and rat mast cells in response to TPA has also been reported (6, 9).

K-252a is a novel and potent inhibitor of PKC, cyclic AMP-dependent protein kinase, and cyclic GMP-dependent protein kinase, with K_i values of 0.025 μM , 0.018 μM and 0.020 μM , respectively (5). K-252a reduced the release of histamine induced by anti-IgE from human basophils. This observation is consistent with a report that this compound inhibits concanavalin A- and immunologically induced histamine release from rat peritoneal mast cells at concentrations ranging from 0.02 μM to 0.6 μM (8). Although our data suggest an important role of PKC in IgE-mediated basophil histamine release, one cannot rule out the possibility that K-252a exerted its inhibitory action through inhibiting cyclic nucleotide-dependent protein kinases. Therefore, we tested another inhibitor of PKC, H-7, for inhibition of histamine release. H-7 caused a dose-dependent inhibition of anti-IgE-induced histamine release. H-7 is not a specific inhibitor of PKC, as is K-252a. It also inhibits cyclic nucleotide-dependent protein kinases to the same extent as that for PKC. However, H-8 and HA1004, which strongly resemble H-7 in chemi-

cal structure but are less potent in inhibiting PKC, did not suppress histamine release, but rather enhanced it at higher concentrations. These observations strongly suggest that PKC plays a crucial role in the mediation of IgE-mediated histamine release from human basophils.

ACKNOWLEDGEMENTS

This work was supported by a grant from Manabe Medical Foundation. We would like to thank Miss Mayumi Asakawa for her excellent technical assistance and Miss Emiko Nagase for her expert secretarial work.

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