

Changes in protein kinase C activity during histamine release from activated rat mast cells

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Rat mast cells were challenged with compound 48/80 or calcium ionophore A23187, and protein kinase C activity in the cell pellets and the amount of histamine release into the supernatant were measured. After stopping the reaction, rat mast cells were lysed in a medium which prevents alterations in phosphorylation and dephosphorylation during sample processing. Histamine was significantly released from compound 48/80-stimulated mast cells at 30 s after the stimulation. In parallel with this, protein kinase C activity in the cell pellets increased at 30 s and 1 min and returned to basal value 3 min after the stimulation. When mast cells were incubated with various concentrations of 48/80 for 30 s, the amount of histamine release and protein kinase C activity increased dependently on the concentration of 48/80. Significant histamine release from A23187-stimulated mast cells was found at 1 min after the stimulation. Also protein kinase C activity in the cell pellets increased at 1 min and returned to basal value 5 min after the stimulation. A reduction of cytosolic protein kinase C activity was observed upon 48/80 treatment in a time- and concentration-dependent manner. Further, staurosporine, a potent inhibitor of protein kinase C, inhibited 48/80-induced histamine release in parallel with the inhibition of protein kinase C activity. These findings suggest that transient increase of protein kinase C activity may be involved in the mast cell activation process.

Key words: histamine release; mast cell; protein kinase C.

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Calcium-activated, phospholipid-dependent protein kinase, so-called protein kinase C, was first described in rat brain (9). This is an enzyme which catalyses phosphorylation of serine and threonine residues in proteins and is typically stimulated by Ca^{2+} , diacylglycerol, phospholipid and unaffected by cyclic nucleotides. With its unique activation requirement of protein kinase C, since stimulators of mast cell secretion produce rapid increases in Ca^{2+} uptake (25), phospholipid metabolism (13) and intracellular diacylglycerol concentrations (14), attempts to isolate protein kinase C from mast cells seem to be particularly appropriate, and there is evidence that rat mast cells and cells of the rat basophilic leukemia line (RBL-1) each contain protein kinase C (17).

Evidence that protein kinase C might pro-

mote histamine release from human basophils (23) and rat mast cells (10) is based on the stimulatory effects of tumor-promoting phorbol esters, such as 12-O-tetradecanoyl 13-acetate (TPA), which directly stimulate the enzyme in broken cell systems (2). Staurosporine isolated from cultures of *Streptomyces* sp. (21) has been shown to inhibit protein kinase C activity (26) and histamine release from stimulated mast cells (16). Further, there is accumulating evidence which indicates that protein kinase C can promote mediator release from mast cells (5, 7, 18, 19, 27).

In the present report, changes in protein kinase C activity during histamine release from activated mast cells were studied for the possible involvement of protein kinase C in the process of mediator release.

MATERIAL AND METHODS

Material

[$\gamma^{32}\text{P}$]ATP (4500 Ci/mmol; ICN Pharmaceuticals, Irvine, CA, USA); calcium ionophore A23187 (A23187), compound 48/80 (48/80), bovine serum albumin (BSA), toluidine blue, Trisma base, ethylene glycol-bis-(β -aminoethyl ether)-N,N'-tetraacetic acid (EGTA), phenylmethylsulfonyl fluoride (PMSF), 1,2-diacylglycerol (diolein), phosphatidylserine (PS), calf thymus lysine-rich histone (type III-S) (Sigma Chemical Co., St. Louis, MO, USA); Percoll (Pharmacia Co., Uppsala, Sweden); NCS tissue solubilizer (Amersham Co., Arlington Heights, IL, USA); staurosporine (Kyowa Medix, Ltd., Tokyo, Japan); others (Wako Pure Chemical Industries, Ltd., Osaka, Japan).

Rat mast cell preparations

Rat serosal cells were obtained by peritoneal and pleural lavage of male Sprague-Dawley rats (250–300 g; Shizuoka Laboratory Animals, Shizuoka, Japan) with heparinized (10 units/ml) balanced salt solution (4.0 mM Na_2HPO_4 , 2.7 mM KH_2PO_4 , 150 mM NaCl, 2.7 mM KCl, 0.9 mM CaCl_2 , pH 7.2) with 0.046 % (w/v) BSA (BSSA). Mast cells were purified on a Percoll gradient as described (4). The mast cells were more than 90 % pure by toluidine blue staining and the viability of the mast cells was shown to be more than 98 % in the dye exclusion test.

Cell incubation conditions

Mast cells were washed twice with BSSA and resuspended. $1.5\text{--}2.0 \times 10^6$ mast cells in 0.9 ml of the medium were incubated at 37°C for 1 min and then subjected to stimulation by 48/80 or A23187 in a final volume of 1 ml for the time indicated. Reactions were halted by adding 1 ml ice-cold BSS, rapidly sedimenting the cells at 4°C, removing the supernatant by aspiration for the histamine assay, and the cells were resuspended in a cold buffer solu-

tion (50 mM NaF, 5 mM sodium pyrophosphate, 10 mM EGTA, 2 mM PMSF, 50 mM Tris/HCl, pH 7.3). The cell suspension was subjected to a freezing-thawing procedure using acetone-dry ice twice, and protein kinase C activity in the cell lysates was measured. This procedure was shown to prevent phosphorylation and dephosphorylation that occur after disruption of the cells (3, 10). Experiments were performed in duplicate and data represent mean and standard errors of three different experiments under the same conditions.

Protein kinase C assay

Protein kinase C was assayed by measuring the incorporation of ^{32}P from [$\gamma^{32}\text{P}$]ATP into lysine-rich histone as described (17). The conditions below were shown to be optimal for quantitation based on preliminary studies under various conditions for time, temperature, and so on. The reaction mixture contained 0.5 mM CaCl_2 , 5 mM MgCl_2 , 20 mM Tris/HCl, pH 7.5, 6 $\mu\text{g/ml}$ diolein, 24 $\mu\text{g/ml}$ PS, 200 $\mu\text{g/ml}$ lysine-rich histone, 10 μM [$\gamma^{32}\text{P}$]ATP (diluted to 5×10^7 cpm/ μmol with unlabeled ATP) and the cell lysates in a 250 μl total volume. Lipids were emulsified in 20 mM Tris/HCl, pH 7.5 by sonication for 5 min with a Braunsonic 1510 sonicator at a 75-W setting immediately before addition to the reaction mixture. Controls contained no diolein nor PS and in some instances EGTA was present in more than two-fold excess over Ca^{2+} . After incubation for 3 min at 30°C, the reaction was stopped by the addition of 100 μg BSA and 2 ml of 25 % (w/v) trichloroacetic acid (TCA). Precipitates were washed twice with 3 ml of 25 % TCA, dissolved in 500 μl of NCS tissue solubilizer and radioactivity was measured by liquid scintillation counting.

Histamine assay

The amount of histamine in the supernatant of activated and control cells was measured with fluorometric assay by the method of Shore et al. (24). For the assay of total

amount of histamine, HClO₄ was added to the mast cell suspension at a final concentration of 4% (w/v); the mixture was centrifuged and the supernatant diluted with water for the assay.

Analysis of data

Unless otherwise stated, each point illustrated in each table or figure represents the mean and standard error of the mean (SEM) of three experiments (each done in duplicate; differences between duplicates were < 10 %). Significance was determined by employing the independent Student t-test (two-tailed).

RESULTS

Effect of buffer solution on protein kinase C activity in rat mast cell lysates

A preliminary study was made of the effect of buffer solution on protein kinase C activity in rat mast cell lysates. Unstimulated rat mast cells were lysed either in BSS or cold buffer solution by freezing and thawing, and protein kinase C activity was measured. As shown in Table 1, rat mast cell lysates in BSS showed no significant protein kinase C activity, whereas that in cold buffer solution showed significant enzyme activity. It has been shown that cold buffer solution is good enough to prevent phosphorylation and dephosphorylation that occur after disruption of the cells (3, 10). Rat mast cells were therefore lysed in cold buffer solution in the following experiments.

Table 1
Effect of buffer solution on protein kinase C activity in rat mast cells

	A	B	C
Protein kinase C activity (cpm)	30 ± 5	65 ± 20	404 ± 28*

Each value represents mean±SEM.
A: back ground; B: mast cells in BSS; C: mast cells in cold buffer solution; *statistical significance from the control at P<0.05.

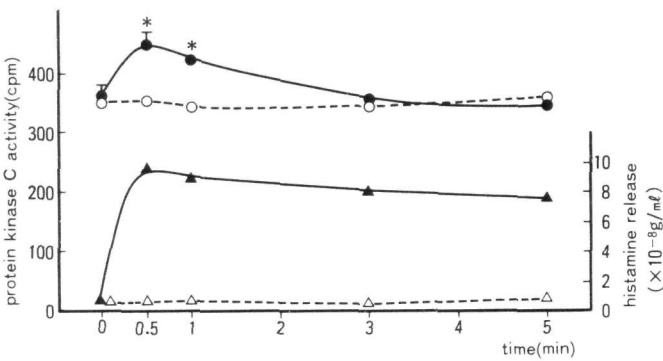


Fig. 1. Time courses of protein kinase C activity in and histamine release from rat mast cells after challenge with 0.5 µg/ml compound 48/80 for 5 min.
●, ○: protein kinase C activity; ▲, △: histamine release; —: with challenge; ----: without challenge. *P<0.05.

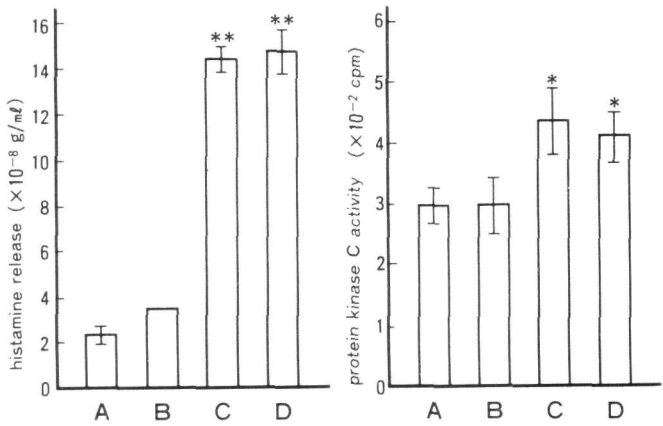


Fig. 2. Compound 48/80-induced changes of protein kinase C activity in and histamine release from rat mast cells. Rat mast cells were incubated for 30 s at 37°C with different concentrations of compound 48/80 (A, 0; B, 0.1; C, 0.5; D, 1.0 µg/ml). Total histamine release was 3.2 × 10⁻⁷ g/ml. *P<0.1, **P<0.01.

Protein kinase C activation in stimulated rat mast cells

Fig. 1 shows that the total protein kinase C activity in rat mast cell lysates increased 1.25-fold within 30 s after the challenge with 0.5 µg/ml 48/80 in parallel with the increase of histamine release into the supernatant. The protein kinase C activity also increased significantly 1 min after the challenge and there were no differences between the activities at 30 s and 1 min after the challenge. When the cell lysates were centrifuged at 100,000 g for 1 h and supernatants and pellets were assayed individually for protein kinase C activity there was a marked change in distribution of the enzyme. In con-

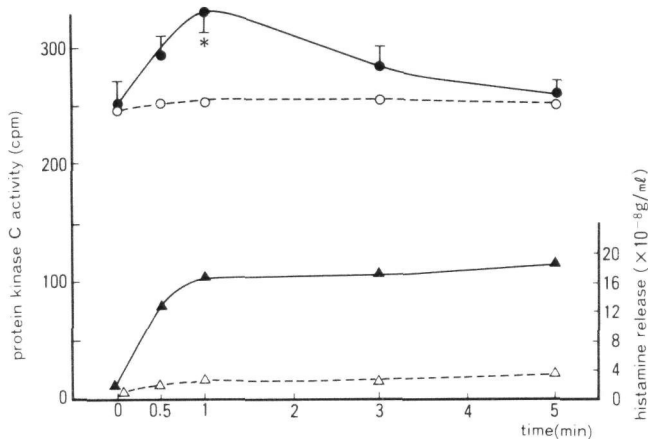


Fig. 3. Time courses of protein kinase C activity in and histamine release from rat mast cells after challenge with 0.3 µg/ml calcium ionophore A23187 for 5 min.

●, ○: protein kinase C activity; ▲, △: histamine release; —: with challenge; ----: without challenge. **P* < 0.05.

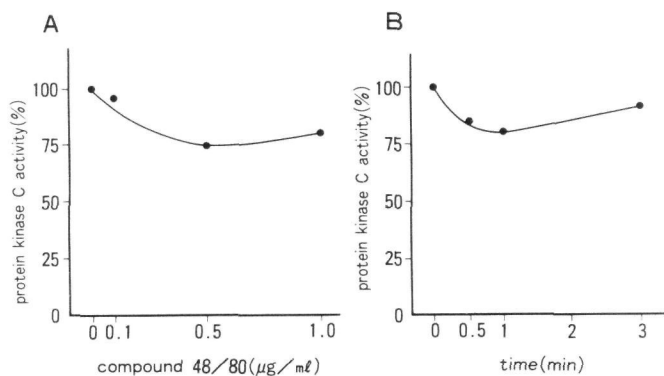


Fig. 4. Effect of compound 48/80 on cytosolic protein kinase C activity in rat mast cells. A: cytosolic protein kinase C activity as a function of compound 48/80 concentration after incubation for 30 s; B: temporal changes in cytosolic protein kinase C activity due to 0.5 µg/ml compound 48/80.

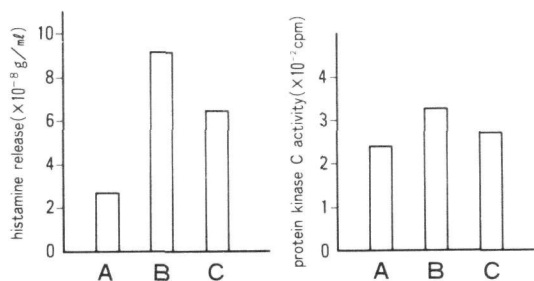


Fig. 5. Effect of staurosporine on compound 48/80-induced changes of protein kinase C activity in and histamine release from rat mast cells. Rat mast cells were incubated for 30 s at 37°C with 0.5 µg/ml compound 48/80 in the absence or presence of 1 µM staurosporine. A: spontaneous; B: control; C: 1 µM staurosporine present.

trast to unstimulated cells, where virtually all of the activity was in the cytosol, after activation the activity was only recovered in the pellets, suggesting that enzyme was changed in its intracellular localization. This redistribution after cell stimulation has been described in other tissues (1, 6, 8). A limited dose-response curve for 48/80-induced changes in protein-kinase C activity is shown in Fig. 2. Comparable stimulation was seen at 0.5 and 1.0 µg/ml with no stimulation at 0.1 µg/ml 48/80. 0.3 µg/ml A23187 also enhanced protein kinase C activity 1 min after the challenge in parallel with the increase of histamine release into the supernatant (Fig. 3). As shown in Fig. 4, 48/80 at concentrations over 0.5 µg/ml induced a detectable loss of cytosolic protein kinase C activity, and this reduction of cytosolic protein kinase C activity was in a time-dependent manner, suggesting translocation of protein kinase C from the cytosol to the membrane.

Effect of staurosporine on protein kinase C activity in and histamine release from stimulated rat mast cells

0.8 ml mast cell suspension were incubated at 37°C for 1 min, and 0.1 ml of staurosporine, a potent inhibitor of protein kinase C, solution (final concentration at 1 µM) was added 5 min prior to the addition of 0.1 ml of 48/80 solution (final concentration at 0.5 µg/ml) and the mixture was further incubated for another 30 s. After stopping the reaction, protein kinase C activity in the cell lysates and amount of histamine in the supernatant were measured under standard conditions. As shown in Fig. 5, staurosporine inhibited the histamine release in parallel with the inhibition of protein kinase C activity.

DISCUSSION

Protein kinase C has been implicated in the transmembrane signaling system in a variety of cell responses to exogenous effectors (20). Substantial evidence has indicated that the tumor promotor-induced activation of cells is followed by movement of the enzyme from cytosol to the membrane fraction (15), suggesting that the

translocation is a prerequisite step in ligand-receptor mediated cell response. Such agonist-induced redistribution of protein kinase C has been reported in many different types of cells (1, 6, 8). White et al. (27) recently showed that in the colony-stimulating factor-dependent mouse mast cell line (PT-18), cross-linking of cell-bound IgE caused a substantial increase in protein kinase C activity in the plasma membrane, which was accompanied by a slight decrease in the enzyme activity in cytosol. They explained these findings by activation and/or modulation of preexisting protein kinase C in membranes. However, less is known about protein kinase C in rat mast cells.

In this study, we investigated changes in protein kinase C activity in activated rat mast cells. Protein kinase C in rat mast cells has been shown to be relatively unstable and its affinity for Ca^{2+} may be changed after cell disruption (17). Heiman & Crews (7) showed that rat mast cells have protein kinase C activity, although not as much as brain tissue or other peritoneal cells, suggesting rat mast cells may contain an endogenous inhibitor of the protein kinase C. Unstimulated rat mast cells lysed in BSS showed no significant protein kinase C activity, whereas the cells lysed in a cold buffer solution contained protease inhibitors, and Ca^{2+} chelators showed the significant enzyme activity. Possible effects of a cold buffer solution can prevent the phosphorylation and dephosphorylation that occur after disruption of the cells (3, 10).

The activation requirements of protein kinase C from rat mast cells suggest its possible involvement in exocytosis from activated mast cells. For optimal mediator release from rat mast cells, Ca^{2+} must be present in the medium, and regardless of the stimulus to release, rapid activation of phospholipid metabolism and accumulation of diacylglycerol is observed (13, 14). While other possible sites of action have to be considered, local anesthetics which inhibit protein kinase C activity also inhibit histamine release from rat mast cells (12). Tumor-promoting phorbol diester has been shown to directly activate the protein kinase C in rat brain (2). The effectiveness of

the phorbol ester in releasing histamine from rat mast cells and human basophils (10, 23) is also consistent with a possible role of protein kinase C in the secretory response. Finally, there is evidence indicating that protein kinase C may be involved in the thrombin- and collagen-induced release of serotonin from platelets (11, 22). In the present study several agents which produce histamine release have been added to intact cells and shown to produce modest but consistent effects on enzyme activity after cell disruption. The changes in protein kinase C activity were rather small compared with baseline. One explanation for this might be insufficient measurement of protein kinase C activity immediately after cell stimulation, because protein kinase C activity in the membrane fraction reached maximum within 30 s of antigen challenge and then gradually declined in mouse mast cell line (PT-18) (27). However, the present results about temporal changes in the cytosolic protein kinase C activity suggested the translocation of protein kinase C from the cytosol to the membrane was completed after 1 min of incubation.

It is unlikely that such a small signal is biologically relevant in the mast cell activation process. About this point, tumor-promoting phorbol diester has been shown to induce a much tighter association of protein kinase C with membranes than a receptor-mediated or a membrane-perturbing stimulant, and protein kinase C activity in the membranes will be clearly increased (19, 27). White et al. (27) also suggested that the receptor-mediated activation may induce a modulation of existing enzyme to a state of higher catalytic activity and less translocation than that by tumor-promoting phorbol diester-mediated activation.

The question still remains whether the activation and/or modulation of protein kinase C is involved in the process of histamine release from activated rat mast cells. The present preliminary experiments showed that staurosporine, a potent inhibitor of protein kinase C (26), inhibited histamine release in parallel with the tendency to inhibit protein

kinase C activity. These results suggest that the modulation and/or activation of protein kinase C may be involved in the process of histamine release from activated rat mast cells. However, further studies are required for the conclusion.

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