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EVIDENCE FOR DE NOVO SYNTHESIS OF PHOSPHATIDYLINOSITOL COUPLED WITH HISTAMINE RELEASE IN ACTIVATED RAT MAST CELLS

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SUMMARY: Phospholipid metabolisms in rat mast cells activated by ionophore A23187 and compound 48/80 were examined with reference to 'phosphatidylinositol (PI) cycle'. The addition of A23187 to [^{3}H]glycerol-prelabeled mast cells induced a marked accumulation of the radioactivity in 1,2-diacylglycerol(DG) and phosphatidic acid(PA) within 10 to 30 sec. A great enhancement of [^{3}H]glycerol incorporation into PA and PI was also detected during histamine release. On the other hand, 48/80 was far less effective than A23187 both in producing 1,2- DG and PA and in accerelating [^{3}H]glycerol incorporation into PA and PI, despite the comparable ability of histamine release. The activity of Ca^{2+} uptake into mast cells, as measured by pulse-labeling with $^{45}Ca^{2+}$, was increased when exposed to both of two agents. These data provide circumstantial evidence that phospholipid metabolisms, mainly de novo PI synthesis, may be a part of the triggering events for Ca^{2+} mobilization and secretory process. The PI metabolism induced by two different stimulants appears to behave in a different manner.

It is generally believed that phospholipid metabolism is markedly enhanced when secretory cells are activated by various stimulating factors (1-7). Although the precise role in secretory process is not yet fully clarified, the metabolism of phosphatidylinositol(PI), known as 'PI cycle', has been reported to be a primary event of receptor-stimulated calcium mobilization and consequent secretory reaction (8). Further evidence in support of this contention was obtained by Serhan et al.(9) and Ohsako & Deguchi (10) who reported that phosphatidic acid(PA), an intermediate of PI-cycle, could serve as a calcium ionophore. We have recently observed that calcium influx due to platelet activation was associated with the production of PA and related to the serotonin release (11).

Mast cells can also rapidly secrete their granules contents(e.g. histamine) in response to a number of different ligands, both IgE-directed and non-IgE-di-

Abbreviations: PI, phosphatidylinositol; PA, phosphatidic acid; DG, diacylglycerol; BSA, bovine serum albumin.

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rected (12). It has been presented by Axelrod and his colleague (13,14) and Ching et al.(15) that mast cells stimulated by IgE shows a transient increase in phospholipid methylation which serves as a triggering event for calcium influx and histamine release. On the other hand, although not as widely studied as phospholipid methylation, 'PI cycle' has been also described to be associated with mast cell activation, for example, increased [^{32}P]phosphate incorporation into PI and PA (16). However, to our knowledge, little information has been available as yet regarding the phospholipid breakdown in activated mast cells. Therefore, we have attempted to study the acute effect of histamine release on phospholipid metabolism in rat mast cells.

In the present communication, rat mast cells are shown to undergo a marked de novo PI synthesis and calcium incorporation in response to A23187 and 48/80, which appear to modulate histamine release by different mechanism.

MATERIALS AND METHODS

Isolation of rat mast cells: Mast cells were obtained from peritoneal cavity of Sprague-Dawley rats and then purified using a BSA density-gradient method according to the procedure of Sullivan et al. (11). Cells were suspended in a buffered salt solution(150 mM NaCl, 3.7mM KCl, 3.0 mM Na_2HPO_4, 3.5 mM KH_2PO_4, 1.0 mM $CaCl_2$, 5.6 mM dextrose, 0.1 % BSA, 0.1 % gelatine and 10 units/ml heparine, pH 6.8) (11). All glasswares were siliconized before used.

Lipid metabolism: Purified mast cells were incubated with [^{3}H]glycerol(300 μCi/10^6 cells) for 60 min at 37°C. After labeling, the labeled cells were washed two times with the above buffered solution and resuspended in the same solution to a final concentration of 2 x 10^5 cells/ml.

Mast cell suspension(10^5 cells/0.5 ml) was activated by the exposure to compound 48/80(2.5 μg) or ionophore A23187(0.25 μg) for indicated time at 37°C. The reaction was stopped by adding 2 ml of chloroform/methanol(1:2, v/v), and lipids were extracted by the method of Bligh & Dyer(17). To observe the incorporation of [^{3}H]glycerol into phospholipids, suspended cells(10^5 cells/0.5 ml) were preincubated for 5 min at 37°C and then subjected to stimulation by two agents in the presence of [^{3}H]glycerol(60 μCi). The phospholipids were separated by two dimensional thin-layer chromatography on Silica gel 60 plates, impregnated by magnesium acetate(2.5 %, w/v), using chloroform/methanol/13.5 N ammonia water(65:35:5.5, v/v) in the 1st dimension, and chloroform/acetone/methanol/acetic acid/ water(3:4:1:1:0.5, v/v) in 2nd dimension (18). The neutral lipids were analyzed on Silica gel G plates containing borate(0.4 M) in a solvent system of chloroform/acetone(96:4, v/v) (19). Spots were identified by comigration with authentic standards. The areas corresponding to individual lipids were scraped into vials and the radioactivity was determined in a liquid scintillation counter (Beckman LS 7500) with toluene/Triton X-100/water/2,5-diphenyloxazole/2,2'-p-phenylene-bis-(5-phenyloxazole)(800 ml: 200 ml: 50 ml: 3.3 g: 0.2 g) (11).

Histamine release: Cell suspension(10^5 cells/0.15 ml) was incubated with a stimulant at 37°C. The reaction was terminated by the addition of 1 ml of ice-cold buffered salt solution containing 10 mM EDTA and then centrifuged at 150 x g for 10 min. Histamine in the resulting supernatant and pellet was measured

fluoremetrically by the modification (12) of the method of Shore et al.(20). The percentage of histamine release was expressed as:

$$\frac{\text{content in medium}}{\text{content in pellet + content in medium}} .$$

Ca^{2+} influx: The activity of Ca^{2+} influx into mast cells was measured by pulse-labeling with $^{45}Ca^{2+}$ as described previously (11). Briefly, cells(10^5 cells/0.1 ml) were exposed to compound 48/80(0.5 μg) or ionophore A23187(0.05 μg) at 37°C for indicated time. At 5 sec before the addition of 0.9 ml of ice-cold 10 mM EDTA, $^{45}Ca^{2+}$(0.8 μCi) was added to reaction mixture. After reaction termination (by adding cold EDTA solution), cells were separated by centrifuging at 4°C. The resulting pellets were washed two times with the cold 10 mM EDTA and the radioactivity was determined in a scintillation counter with toluene/Triton X-100/2,5-diphenyloxazole/2,2'-p-phenylene-bis-(5-phenyloxazole)(1000 ml: 500 ml: 4 g: 0.2 g) (11).

Materials: [2-^{3}H]Glycerol(10 Ci/mmol) and $^{45}CaCl_2$ were obtained from New England Nuclear. Compound 48/80 and ionophore A23187 were obtained from Sigma and Eli Lilly Co., respectively. BSA(fraction V, essentially fatty acid free) was purchased from Sigma. Silica gel 60 plates were obtained from Merck. All other chemicals were of the highest reagent grade.

RESULTS AND DISCUSSION

As shown in Fig.1, stimulation of rat mast cells by compound 48/80 or ionophore A23187 caused a marked release of cellular histamine after a lag of 10 sec.

It is widely accepted that phospholipid (especially PI) metabolism in enhanced when various cells undergo release reaction (1-8). The metabolism of

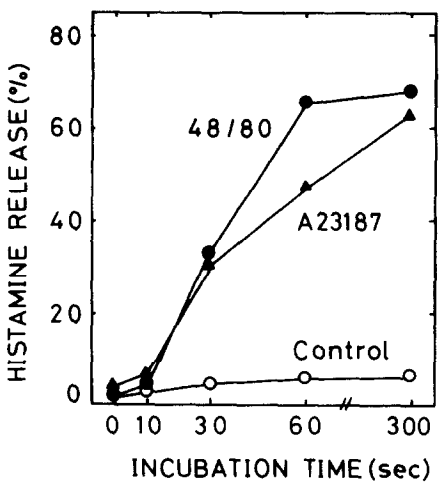

Fig. 1 Time courses of compound 48/80- or ionophore A23187-induced histamine release from rat mast cells. Mast cell suspension (10^5 cells/0.15 ml) was incubated with compound 48/80 (5 μg/ml) or ionophore A23187 (0.5 μg/ml) at 37°C for indicated time. ● , compound 48/80; ▲ , ionophore A23187. Each value is the mean of two experiments performed in duplicate.

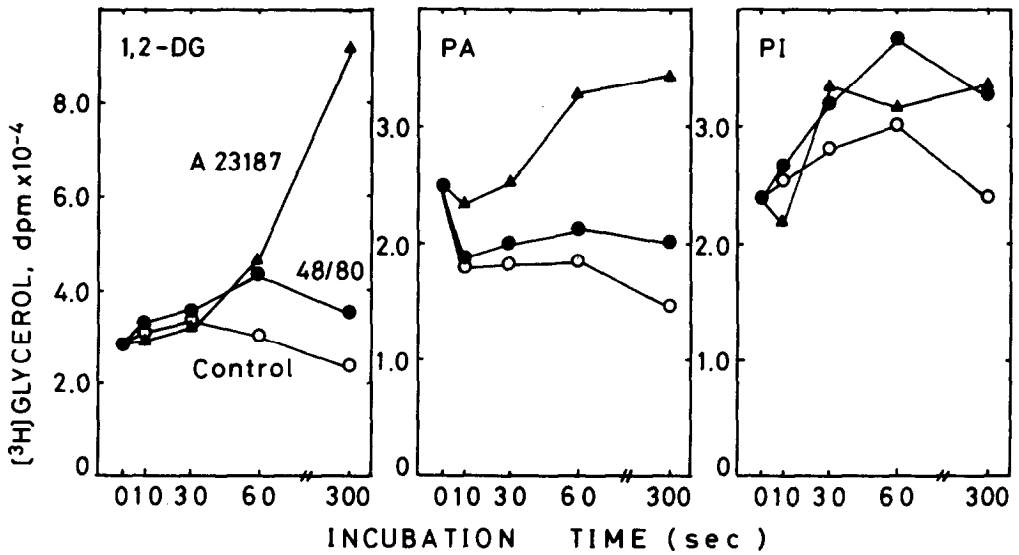

Fig. 2 Effects of compound 48/80 or ionophore A23187 on the changes in radioactivity of 1,2-DG, PA and PI in rat mast cells. Rat mast cells (10^5 cells/0.5 ml) prelabeled with [^{3}H]glycerol were exposed to compound 48/80 (5 μg/ml) or ionophore A23187 (0.5 μg/ml) at 37°C for indicated time. ○, control; ●, compound 48/80; ▲, ionophore A23187. Each value is the mean of two experiments performed in duplicate.

phospholipids induced by mast cell activation is shown in Fig.2 where [^{3}H]glycerol was used to prelabel mast cells. Following activation by A23187, the radioactivity of PA, a key intermediate in both 'PI cycle' and de novo synthesis of phospholipids (21), was increseased from 2.4 x 10^4 dpm to 3.3 x 10^4 dpm within 60 sec and remained at this level up to 5 min. The radioactivity in 1,2-DG was progressively increased from 2.9 x 10^4 dpm to 9.2 x 10^4 dpm. The rate of this rise in 1,2-DG was much greater than that observed in PA and PI. Our previous studies (22) have shown with thrombin-activated platelets that 'PI cycle' is initiated by the rapid hydrolysis of PI to 1,2-DG by a specific phospholipase C, which is shortly followed by phosphorylation to PA by DG kinase. In this context, the present experiments using [^{3}H]glycerol-prelabeled mast cells indicated that after the radioactivity in PA reached a maximum, the radioactivity in 1,2-DG tended to rise rapidly. This prompted us to propose that A23187-activation enhances de novo synthesis of PA and PI. As demonstrated in Table I, A23187 caused an enhanced uptake of [^{3}H]glycerol into PA and PI by 4- and 7-fold, respectively, suggesting an accelerated de novo synthetic pathway of PI. Futher

Table I

Effects of Compound 48/80 and Ionophore A23187 on [^{3}H]Glycerol Incorporation into PA and PI

Stimulants	PA	PI
None	147 (100)	137 (100)
A23187	574 (401)	952 (695)
48/80	193 (135)	215 (157)
	dpm (%)	

Rat mast cells (10^5 cells/0.5 ml) were preincubated for 5 min at 37°C, and then incubated with compound 48/80 (2.5 μg) or ionophore A23187 (0.25 μg) for 60 sec in the presence of [^{3}H]glycerol (60 μCi). Each value is the mean of two experiments preformed in duplicate.

support of stimulated de novo pathway came from enhanced [^{3}H]glycerol incorporation into phosphatidylcholine and phosphatidylethanolamine (data not shown).

On the other hand, compound 48/80 was far less effective than A23187 in accelerating phospholipid metabolism. Although comparable amount of histamine was released (Fig.1), 48/80 induced only 20% and 30% increase of the radioactivity in 1,2-DG and PA, respectively (Fig.2), when compared to the case of A23187 (100%) Furthermore, whereas A23187 led to an enhancement of [^{3}H]glycerol incorporation into PA and PI, a much lower incorporation was observed when 48/80 was used (Table I).

A rise in cytoplasmic Ca^{2+} concentration is generally believed to be one of the earliest and most crucial step in the sequence of secretory cell activation. The Ca^{2+} influx due to histamine release was measured by pulse-labeling with $^{45}Ca^{2+}$ (Fig.3). Upon activation by 48/80, the activity of $^{45}Ca^{2+}$ uptake was markedly increased up to 30 sec and then returned to the initial unactivated level. A23187-stimulation showed a gradual rise in the $^{45}Ca^{2+}$ -uptake.

These results indicate that selective changes in phospholipid metabolism occur during histamine release and these reactions may be an intrinsic part of the biochemical mechanisms related to calcium influx and mediator release. An important role for phospholipid metabolism, especially 'PI cycle', in secretion

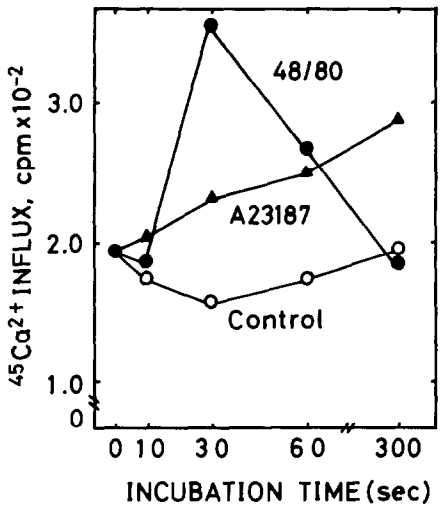

Fig. 3 Effects of compound 48/80 or ionophore A23187 on the activity of $^{45}Ca^{2+}$ uptake into rat mast cells. Rat mast cells suspension was incubated with compound 48/80 (5 μg/ml) or ionophore A23187 (0.5 μg/ml) at 37°C for indicated time. At 5 sec before the addition of ice-cold 10 mM EDTA, $^{45}Ca^{2+}$ (0.8 μCi) was added to reaction mixture. After reaction termination (by adding cold 10 mM EDTA), cells were separated by centrifugation, and then the radioactivity was measured. ○, control; ●, compound 48/80; ▲, ionophore A23187. Each value is the mean of two experiments performed in duplicate.

process has been futher suggested by Kennerly et al.(16) and Cockcroft & Gomperts (23), who demonstrate the coordinate events of the mediators release and the enhanced [^{32}P]phosphate and [^{3}H]inositol incorporation into PI. On the other hand, evidence has been presented that the receptor-activation causes a rapid increase in phospholipid methylation which followed by an influx of Ca^{2+} and the release of histamine in mast cells (13-15) and basophilic leukemia cells (26,27).

The precise role of the rapid PI metabolism in secretory process remains to be elucidated. However, several studies (9,10) have provided evidence that PA serves as a calcium ionophore. Stimulation-induced PA formation is closely associated with the enhancement of Ca^{2+} uptake in platelets (11). Also, as presented by Takai et al. (28), the conversion of PI to DG activates C-kinase which in turn could facilitate secretion reaction. Despite no direct evidence, DG formation may participate in fusion of secretory granule membrane with plasma membrane since DG could also produce a localized increase in membrane fluidity (24,25).

Although any conclusion can not be withdrawn, at the present moment, rapid changes in phospholipid metabolism led us to expect a critical role in Ca^{2+} mobilization and secretory process of mast cells. The results reported in this communication suggest that de novo PI synthesis participates in the modulation of mast cell mediator release.

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