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Inhibition by amiloride and by Na^+-depletion of A23187-stimulated arachidonic acid and histamine release from rat mast cells

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Rat peritoneal mast cells, labelled with [^{14}C]arachidonic acid, released histamine and [^{14}C]arachidonic acid upon the addition of A23187. The release induced by low concentrations of A23187 was suppressed by removal of extracellular Na^+ and by addition of the Na^+/H^+ exchange inhibitor, amiloride. Addition of protein kinase C inhibitors resulted in only a modest decrease of [^{14}C]arachidonic acid and histamine release. [^{14}C]Arachidonic acid was hydrolyzed from both phosphatidylcholine and triacylglycerol. Amiloride inhibited only the hydrolysis from phosphatidylcholine. It is suggested that an Na^+/H^+ exchange is stimulated secondary to an A23187-induced increase in intracellular calcium concentration. Increased Na^+/H^+ exchange seems to facilitate the activation by intracellular calcium ions of phospholipase A_2 as well as of histamine secretion.

Amiloride; Arachidonic acid; Na^+/H^+ exchange; Phospholipase A_2; Histamine; (Mast cell)

1. INTRODUCTION

Rat mast cells respond to several stimuli including calcium ionophores, resulting in non-cytotoxic histamine release. Positive correlation between histamine release and the level of phospholipase A_2 in rat mast cells has been suggested [1]. Increasing evidence suggests that phospholipid metabolism, cyclic AMP formation and increased intracellular calcium concentration play important roles in the modulation of mediator release from mast cells [2]. However, the specific biochemical mechanisms involved in the transmission of an external signal and in the process of secretion still remain to be determined.

In many cells an Na^+/H^+ exchange is stimulated by a variety of hormones and mitogens as an early event of the stimulus-response coupling [3,4]. Different mechanisms have been proposed for activation of Na^+/H^+ exchange, e.g. that an increase in the cytoplasmic calcium concentration is responsible for stimulation of the Na^+/H^+ exchanger [5,6], ant that protein kinase C activation causes stimulation of this antiport [7,8]. The role of the Na^+/H^+ exchange in the activation process of mast cells has not been studied previously.

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Abbreviations: H-7, 1-(5-isoquinolinylsulphonyl)-2-methylpiperazine; Hepes, 4-(2-hydroxyethyl)-1-piperazineethanesulphonic acid; [^{14}C]AA, [^{14}C]arachidonic acid; PC, phosphatidylcholine; PE, phosphatidylethanolamine; PS, phosphatidylserine; PI, phosphatidylinositol; PA, phosphatidic acid; TG, triacylglycerol; DG, diacylglycerol; MG, monoacylglycerol; PGD_2, prostaglandin D_2; AA, arachidonic acid

2. MATERIALS AND METHODS

2.1. *Materials*

Ficoll 400 was from Pharmacia Fine Chemicals (Uppsala, Sweden), [1-^{14}C]arachidonic acid (58.3 mCi/mmol) from Amersham International (England). A23187, amiloride hydrochloride, H-7, *o*-phthaldialdehyde and bovine serum albumin were from Sigma Chemical Co. (St. Louis, MO). Lipids were from Serdary Research Laboratories (London, Ontario).

2.2. *Mast cell purification and labelling*

Mast cells from the peritoneal cavity of male Sprague-Dawley

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rats (250–350 g) were purified by gradient centrifugation as described previously [9]. Purified mast cells (6×10^6/ml) were incubated with [^{14}C]AA (1 μCi/ml) at 37°C for 60 min and washed three times to remove free [^{14}C]AA. The cells took up about 26% of the [^{14}C]AA. Cells were allowed to equilibrate at 37°C for 20 min.

2.3. *Measurement of [^{14}C]AA release*

Cells, suspended in buffered salt solution, were dispensed to polystyrene tubes in aliquots of 0.5 ml (2×10^5 cells). The release reaction was initiated by the addition of A23187 solubilized in 5 μl of 50% dimethyl sulfoxide. Incubations were carried out for 10 min at 37°C. Release was terminated by the addition of 0.5 ml of ice-cold buffered salt solution. The samples were centrifuged at $800 \times g$ for 10 min at 4°C. Total release of [^{14}C]AA and its metabolites was determined by liquid scintillation counting of aliquots of the supernatant. The secretion of [^{14}C]AA was determined relative to the total cell-associated [^{14}C]AA (about 12600 cpm/2×10^5 cells). The correction for non-cell-associated [^{14}C]AA was carried out by subtraction of control values. The ^{14}C-activity of the supernatant after cell stimulation comprises both [^{14}C]AA and to a minor degree [^{14}C]prostaglandin D_2 as previously reported [9], but will be designated as [^{14}C]AA in the following.

2.4. *Inhibition of Na^+/H^+ exchange*

Two procedures were employed to block the Na^+/H^+ exchange. In one, the cells were preincubated 5 min with 1.0 mM amiloride. In the other, an Na^+-free buffer was used in the stimulations. After incubation with [^{14}C]AA the cells were washed three times with Na^+-free buffer (5 mM KCl, 1 mM $MgSO_4$, 10 mM Hepes, 1 mM $CaCl_2$, 5 mM glucose, 1 mg/ml albumin and 140 mM choline chloride, pH 7.5). The cells were suspended in the Na^+-free buffer or an Na^+-containing buffer (the buffer above with 140 mM NaCl instead of choline chloride) and incubated for 15 min at 37°C before stimulation with A23187. The concentration of Na^+ in the extracellular fluid of the cells incubated in Na^+-free buffer was lower than 0.3 mM as determined by flame emission.

2.5. *Lipid metabolism*

The cell suspensions were extracted twice with 3 vols of chloroform/methanol (2:1, v/v). The lipids were separated by TLC using two consecutive developments: chloroform/methanol/acetic acid/water (85:45:8:2, v/v) for a distance of 11 cm and subsequently for 16 cm in hexane/diethyl ether/acetic acid (70:30:2, v/v). The spots were visualized [9] and scraped into vials for determination of radioactivity.

2.6. *Histamine assay*

Histamine and [^{14}C]AA release was measured in the same preparations of cells. Histamine release was determined by estimation of residual histamine in the cell sediment [10]. The release of histamine was expressed as a percentage of the total histamine content in the cells, thereby correcting for spontaneous histamine release, which was 1–10%.

3. RESULTS AND DISCUSSION

Incubation of prelabelled mast cells with A23187 resulted in release of [^{14}C]AA and of histamine (fig.1). Treatment of the cells with the Na^+/H^+ exchange inhibitor amiloride resulted in inhibition of [^{14}C]AA release as well as histamine release at low A23187 concentrations (0.5–1.0 μM) (fig.1). The amiloride inhibition of the release reaction was dose-dependent up to 1.0 mM (not shown). Stimulation of [^{14}C]AA and histamine release triggered by 0.5 μM A23187 was maximally decreased by 49% and 72%, respectively, in the presence of 1.0 mM amiloride. The inhibition observed by removal of extracellular Na^+ (fig.1) was of the same order of magnitude as that observed by the amiloride addition. Treatment of the cells with Na^+-free buffer reduced the responsiveness of the cells. Amiloride has effects other than inhibition of Na^+/H^+ exchange, e.g. inhibition of protein kinase C [11], but our results with the Na^+-free buffer indicate that the observed inhibition by amiloride may be due to its effect on Na^+/H^+ exchange. An enhancement of an Na^+/H^+ exchange by A23187 has been suggested for various cells [5, 6,12–14].

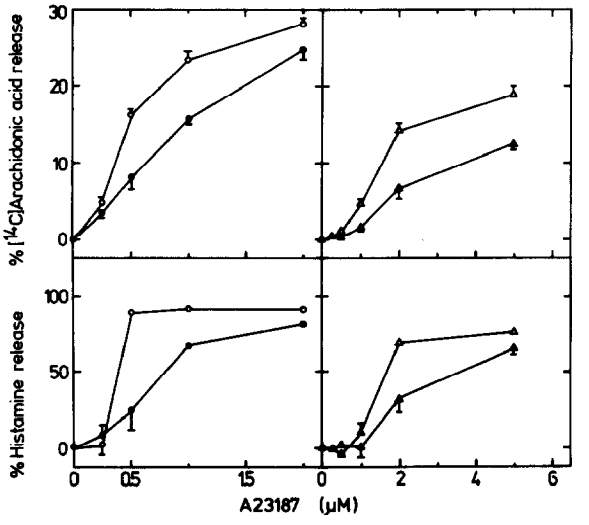

Fig.1. Effect of amiloride and of removal of extracellular Na^+ on A23187-induced [^{14}C]AA and histamine release. [^{14}C]AA prelabelled mast cells were stimulated by A23187 in the presence of amiloride (left) or in an Na^+-free buffer (right). The values for [^{14}C]AA release have been corrected for the control values (left, 2700 ± 100 cpm; right, 820 ± 50 cpm); the total cell uptake was 12000 ± 400 cpm (left) of 9200 ± 600 cpm (right). Results are the mean ± SE of three separate experiments each in duplicate (○, △, controls; ●, amiloride; ▲, Na^+-free buffer).

In mast cells, Na^+/H^+ exchange stimulation could be secondary to activation of protein kinase C [8] by DG or by calcium [15]. To characterize the role of protein kinase C in A23187-induced mast cell activation, two different protein kinase C inhibitors were used. Both [^{14}C]AA and histamine releases were slightly inhibited in a dose-dependent manner with H-7 [16] and with sphingosine [17], respectively (not shown). Fig.2 shows the maximal inhibition obtained with H-7, which was achieved at 100 μM, and with sphingosine, which was achieved at 1.0 μM. At 0.5 μM A23187 both sphingosine and H-7 reduced the [^{14}C]AA release and the histamine secretion by approx. 25%. The weak effect of inhibitors of protein kinase C suggests that this enzyme was only to a minor degree involved in mediating the effect of A23187. Furthermore, it indicates that an increase in intracellular calcium concentration may account for a major part of the stimulation of an Na^+/H^+ exchange in A23187-stimulated mast cells.

In agreement with previous results [9] and with results reported by Imai et al. [18], the release of [^{14}C]AA was accompanied by a decrease of radioactivity in PC and to a lesser extent in TG (fig.3), suggesting activation of phospholipase A_2 and TG-lipase. The hydrolysis of PC was inhibited by amiloride, while there was no effect on hydrolysis of TG. The small increase in ^{14}C-labelling of MG, DG and PA may also indicate activation of other lipases. However, suggestions about the role of these other pathways in mast cell activation must await further studies.

The apparent inhibition of phospholipase A_2 activation by inhibitors of Na^+/H^+ exchange in the present work is in agreement with results of Sweatt et al. [19] using human platelets, i.e. an enhanced Na^+/H^+ exchange facilitates the activation of phospholipase A_2 by calcium ions. Furthermore, our results suggest that the effect of an increased intracellular calcium concentration on histamine secretion is partly mediated by a stimulated Na^+/H^+ exchange. At high A23187 concentrations, where a high and unphysiological intracellular calcium concentration probably was achieved, the dependence of an Na^+/H^+ exchange

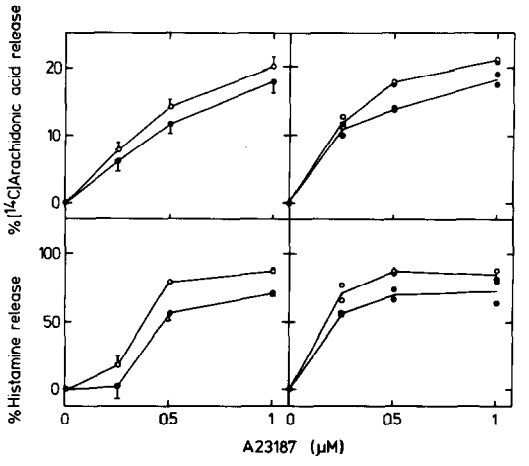

Fig.2. Effect of protein kinase C inhibitors H-7 (left) and sphingosine (right) on A23187-induced [^{14}C]AA and histamine release. [^{14}C]AA prelabelled mast cells were preincubated for 10 min without (○) or with (●) 100 μM H-7 or 1.0 μM sphingosine and stimulated by A23187 for 10 min. The values for [^{14}C]AA release have been corrected for the control values (left, 3100 ± 100 cpm; right, 3700 ± 500 cpm); the total cell uptake was 13 000 ± 600 cpm (left) or 13 400 cpm (right). In the lefthand panels results are the mean ± SE of three separate experiments each in duplicate. In the righthand panels each point represents one of two separate experiments, each in duplicate.

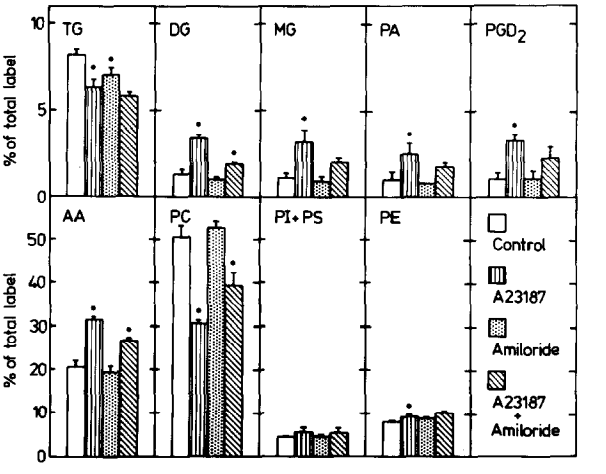

Fig.3. Effect of A23187 stimulation on the distribution of ^{14}C-labelling in [^{14}C]AA prelabelled mast cells in the presence and absence of amiloride. Prelabelled mast cells were preincubated 5 min with 1.0 mM amiloride and stimulated for 10 min by the addition of 0.5 μM A23187. The values are shown as % of total amount of radioactivity recovered by TLC (100% = 20 500 ± 600 cpm). Results are the mean ± SE of three experiments, one in triplicate and two in duplicate. Asterisks above columns 2, 3 and 4 indicate significant differences ($p < 0.05$, Student's t-test) between the values: control vs A23187 (column 2); control vs amiloride (column 3); A23187 vs A23187 + amiloride (column 4). 4% of the radioactivity was found in non-identified compounds.

was overcome both with respect to histamine secretion and to arachidonic acid release.

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