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ATP-Induced Histamine Release Is in Part Related to Phospholipase A_2-Mediated Arachidonic Acid Metabolism in Rat Peritoneal Mast Cells

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Histamine and arachidonic acid (AA) release was measured using the P2-purinoceptor antagonists, phospholipase A_2 (PLA_2) and cyclooxygenase (COX)/lipoxygenase (LOX) inhibitors to determine whether or not ATP-induced histamine release is associated with arachidonic acid (AA) release in rat peritoneal mast cells. ATP increased histamine release in a dose dependent manner, whereas adenosine did not. PPADS (a selective P2X-purinoceptor antagonist) and suramin (a nonselective P2X,2Y-purinoceptor antagonist) inhibited ATP-induced histamine release in a dose dependent manner. However, RB-2 (a P2Y-purinoceptor antagonist) did not block ATP-induced histamine release. Manoalide and oleyloxyethyl phosphorylcholine (OPC), secretory PLA_2 inhibitors, also inhibited ATP-induced histamine release dose-dependently. Both COX inhibitors (ibuprofen and indomethacin) and LOX inhibitors (baicalein and caffeic acid) inhibited ATP-induced histamine in a dose dependent manner. ATP significantly increased [^{3}H]AA release by 54%. PPADS and suramin significantly inhibited ATP-induced [^{3}H]AA release by 81% and 39%, respectively. ATP-induced histamine release was significantly inhibited by a variety of protein kinase inhibitors, such as bisindolmaleimide, genistein, methyl 2,5-dihydroxycinnamate, W-7 and trifluoperazine. Overall, the results suggest that ATP-induced histamine release is in part related to the PLA_2-mediated AA metabolism and P2X-purinoceptors.

Key words: ATP, Arachidonic acid, Purinoceptor, Histamine, Mast cells

INTRODUCTION

ATP has long been known to induce the exocytotic release of histamine from mast cells (Diamant and Kruger, 1967; Dahlquist and Diamant, 1974; Jaffar and Pearce, 1990). Recently, ATP has been reported to increase the release of histamine and prostaglandin D_2 from rat peritoneal mast cells significantly (Jaffar and Pearce, 1990). Varsani and Pearce (1997) reported that anti-IgE-induced histamine was inhibited by phospholipase A_2 (PLA_2) inhibitor release in rat peritoneal mast cells. This suggests that histamine release in rat peritoneal mast cells is associated with arachidonic acid (AA) release by PLA_2.

In addition, non-steroidal anti-inflammatory drugs have been reported to inhibit histamine release induced by the antigen, ionophore A23187 and compound 48/80 in rats (Champion *et al.*, 1977; Lewis and Whittle, 1977; Conroy and DeWeck, 1981; Gomes and Pearce, 1988). It has also been suggested that the lipoxygenase (LOX) activity is involved in the release of histamine stimulated by an ionophore and antigen in MC9 cells (Musch *et al.*, 1985; Musch and Siegel, 1986). However, there is little evidence suggesting that LOX inhibitors influence histamine release in mast cells. However, these findings suggest that the AA metabolites produced by COX and LOX may be involved in histamine release. Therefore, the effect of the PLA_2, COX or LOX inhibitors on ATP-induced histamine release was examined to determine whether or not ATP-induced histamine release from rat peritoneal mast cells is related to AA release and/or its metabolites.

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MATERIALS AND METHODS

Materials

ATP, adenosine, pyridoxal phosphate-6-azophenyl-2',4'-disulphonic acid (PPADS), suramin, reactive blue 2 (RB-2), CGS15943, 8-cyclopentyl-1,3-dipropylxanthine (DPCPX), manoalide, oleyloxyethyl phosphorylcholine (OPC), ibuprofen, indomethacin, baicalein, caffeic acid, bisindolylmaleimide, genistein, methyl 2,5-dihydroxycinnamate (DHC), W-7 and trifluoperazine were purchased from the Sigma Chemical Co. (St. Louis, Mo, USA). [^{3}H]AA was purchased from Amersham Pharmacia.

Isolation of rat peritoneal mast cell

Male Sprague-Dawley rats weighing 250 to 350 g were housed over one week in a normal environmentally controlled animal room (temperature 24 ± 2°C, humidity: 50 ± 5% and a 12 h/12 h day/night regime) with food and water provided *ad libitum*. The rats were sacrificed under ether anesthesia and the peritoneal cavity was opened and injected with 20 ml of Krebs buffer (mM: NaCl 137, KCl 2.7, Na_2HPO_4 0.4, $MgCl_2$ 0.5, HEPES [pH 7.4] 10, $CaCl_2$ 1.8, glucose 5) with the abdomen being gently massaged for 1-2 min. The peritoneal fluid was centrifuged at 1000 rpm for 10 min at 4°C, and the peritoneal mast cells were isolated by Percoll density centrifugation (Knudsen and Johasen, 1989). The mast cells that constituted 90-100% of the cell suspension were suspended into 10^5 cells/ml with Krebs buffer and used to investigate histamine and [^{3}H]AA release. The cells were incubated with a variety of inhibitors for 5 min and then stimulated with ATP.

Histamine assay

The released histamine was assayed using a fluorometric method (Shore *et al.*, 1959). Various antagonists or inhibitors were added to the cell suspension and incubated for 5 min at 36°C. The cells were then stimulated with 100 μM ATP for 10 min at 36°C. After centrifugation, the histamine concentration in both the supernatant and the pellet were measured with 0.1 ml of 1% *o*-phthaldialdehyde in methanol. After 4 min, the reaction was quenched by adding 0.2 ml of 3 N HCl. The fluorescence intensity was measured using excitation and emission wavelengths of 355 and 455 nm, respectively with a microplate fluorescence reader (FL600, Bio-Tek).

[^{3}H]AA release

The isolated mast cells were suspended in 10 ml of Krebs buffer and incubated with [^{3}H]AA (0.2 μCi/ml) for 2 h at 37°C. The cells were washed twice with Krebs buffer containing 0.5 mg/ml bovine serum albumin to trap the liberated [^{3}H]AA. The cells were incubated with ATP in the presence or absence of the receptor antagonists for 10 min at 37°C. The [^{3}H]AA radioactivity released by ATP in the medium was measured by a scintillation counter (Jesus *et al.*, 1994).

Data analysis

The results are represented as a mean ± S.D. and were analyzed statistically by an analysis of the variance (ANOVA), and the differences between groups were determined using a Newman-Keuls test. The significance level was set at less than 5%.

RESULTS

Effects of purinoceptor antagonists on histamine release induced by ATP

ATP increased the histamine release in mast cells in a dose dependent manner and its maximum effect was observed at 100 μM, whereas adenosine did not have any influence on histamine release (Fig. 1). This suggests that the ATP-induced histamine release is mediated via P2-purinoceptors rather than the adenosine receptors. Consequently, the effects of the purinoceptor antagonists on ATP-induced histamine release were examined to investigate which P2-purinoceptor is involved. PPADS (a P2X-purinoceptor antagonist) and suramin (a nonselective P2X,2Y-purinoceptor antagonist) inhibited ATP-induced histamine release in a dose dependent manner. However, the RB-2 (a P2Y-purinoceptor antagonist) did not inhibit the release of ATP-induced histamine (Fig. 2). CGS15943 (a nonselective adenosine receptor antagonist) and DPCPX

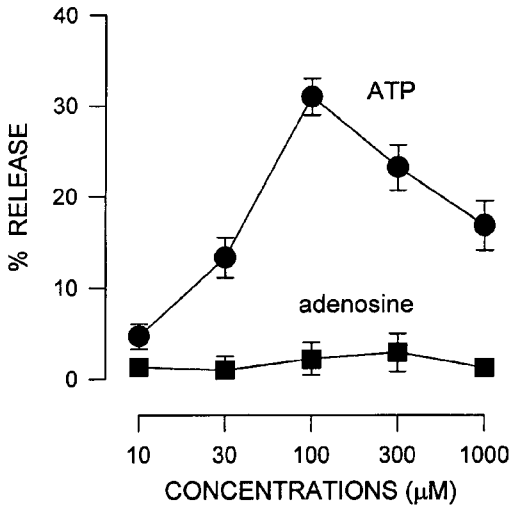

Fig. 1. Dose-response of histamine release from mast cells to ATP and adenosine. The results are shown as a mean ± SD of 5 separate experiments and are expressed as the % release (histamine concentration in the supernatant/histamine contents in supernatant and pellet × 100).

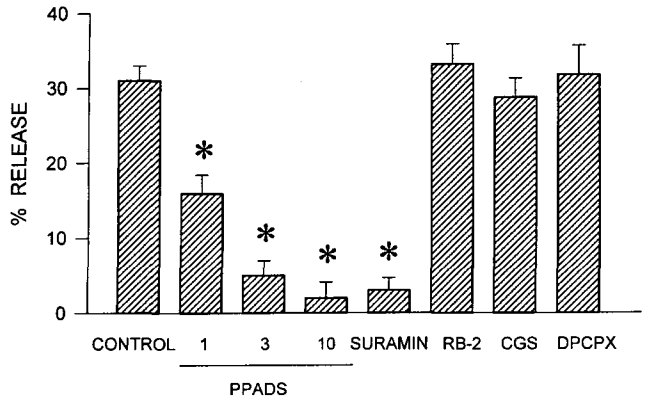

Fig. 2. Effects of the receptor antagonists on ATP-induced histamine release. The mast cells were preincubated with PPADS (a P2X-purinoceptor antagonist; 1, 3, 10 μM), suramin (a non-selective P2X,2Y-purinoceptor antagonist, 300 mM), RB-2 (P2Y-purinoceptor antagonist, 100 μM), CGS (CGS 15943, a non-selective adenosine receptor antagonist, 10 nM) and DPCPX (an A1 adenosine receptor antagonist, 50 nM) for 5 min then stimulated with 100 mM ATP for 10 min. The control value induced by 100 mM ATP is 31 ± 2%. The results are reported as a mean ± SD of 5 separate experiments. * $P<0.05$ vs. control

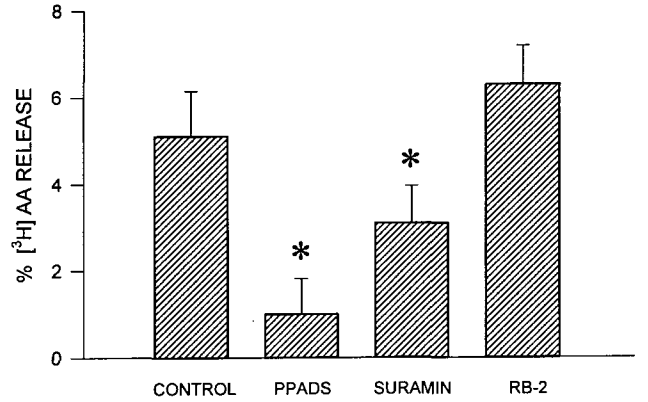

Fig. 3. Effects of phospholipase A_2 inhibitors on ATP-induced histamine release. The mast cells were preincubated with manoalide and oleyloxyethyl phosphorylcholine (OPC) for 5 min then stimulated with 100 μM ATP for 10 min. The results are reported as a mean ± SD of 5 separate experiments. *$P<0.05$ vs. control

(an A1 adenosine receptor antagonist) also did not affect ATP-induced histamine release (Fig. 2).

Effects of PLA_2, COX and LOX inhibitors

Manoalide and OPC, secretory PLA_2 inhibitors, inhibited the ATP-induced histamine release in a dose dependent manner (Fig. 3). Both COX (ibuprofen and indomethacin) and LOX inhibitors (baicalein and caffeic acid) also inhibited ATP-induced histamine release in a dose dependent manner (Figs. 4 & 5).

Effect of ATP on [^{3}H]AA release

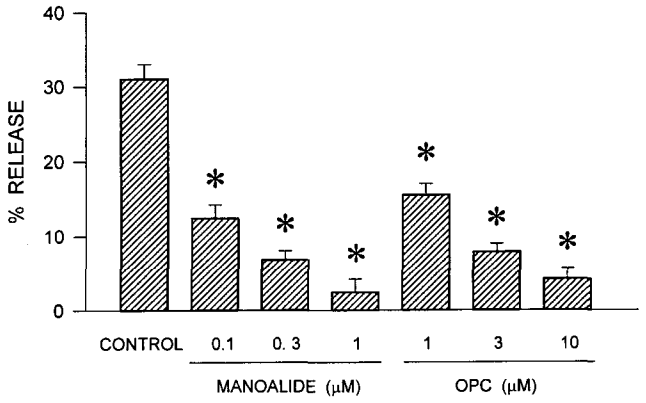

Fig. 4. Effects of cyclooxygenase inhibitors on histamine release induced by ATP. The mast cells were preincubated with ibuprofen and indomethacin for 5 min then stimulated with 100 μM ATP for 10 min. The results are reported as a mean ± SD of 5 separate experiments. * $P<0.05$ vs. control

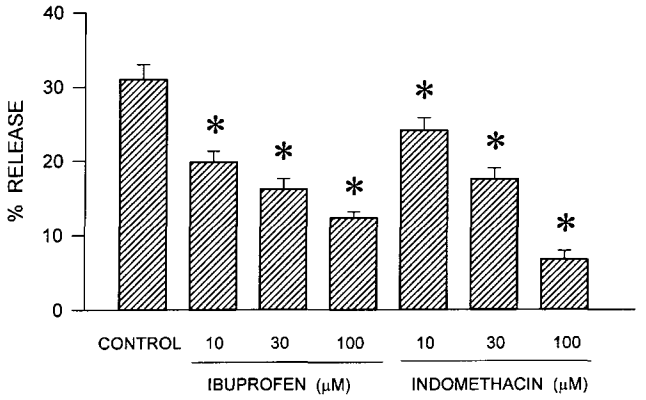

Fig. 5. Effects of lipoxygenase inhibitors on histamine release induced by ATP. The mast cells were preincubated with baicalein and caffeic acid for 5 min then stimulated with 100 μM ATP for 10 min. The results are reported as a mean ± SD of 5 separate experiments. *$P<0.05$ vs. control

ATP-induced histamine release was significantly inhibited by PLA_2, COX and LOX inhibitors, which suggests that ATP-induced histamine release is associated with AA release and its metabolites. In this experiment, we investigated whether or not ATP increases AA release and if so, which receptor is involved. ATP significantly increased the release of AA by 54%. Both PPADS and suramin significantly inhibited ATP-induced AA release by 80% and 39%, respectively. However, RB-2 did not (Fig. 6), suggesting that AA release by ATP is mediated via the P2X-purinoceptors.

Effects of protein kinase inhibitors

0.1 μM Bisindolylmaleimide, a protein kinase C inhibitor, inhibited ATP-induced histamine release by 62%. Genistein and DHC, both tyrosine kinase inhibitors, significantly

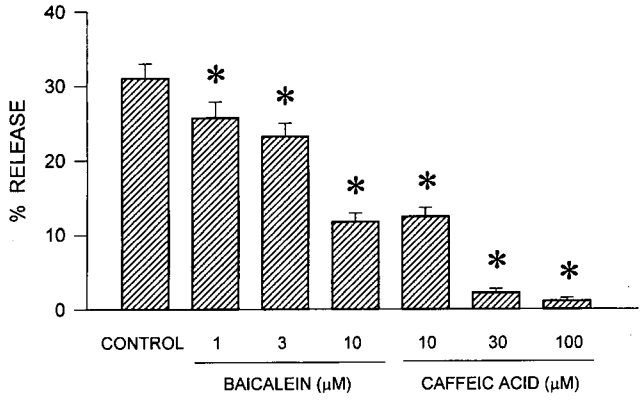

Fig. 6. Effects of receptor antagonists on arachidonic acid release induced by ATP. The mast cells labeled with [^{3}H] arachidonic acid (AA) were preincubated with PPADS (10 μM), suramin (300 μM) and RB-2 (100 μM) for 5 min then stimulated with 100 μM ATP for 10 min. The control value induced by 100 μM ATP is 5.1 ± 1.1%. The results are reported as a mean ± SD of 5 separate experiments and are expressed as the % release ([^{3}H]AA radioactivity in supernatant /[^{3}H]AA radioactivity in supernatant and pellet × 100). *P<0.05 vs. control

inhibited histamine release by 30% at 10 μM and 50% at 1 μM, respectively. W-7 and trifluoperazine, both calmodulin antagonists, significantly inhibited ATP-induced histamine release by 60% and 74% at 3 μM, respectively (Fig. 7).

DISCUSSION

The results showed that ATP increased histamine release in rat peritoneal mast cells in a dose dependent manner, whereas adenosine did not. At a concentration > 300 μM, ATP-induced histamine release was lower. The decreasing pattern of histamine release by ATP has been reported (Jaffar and Pearce, 1993). In a subsequent

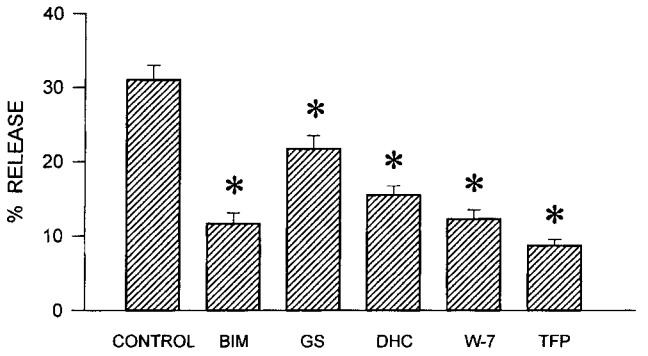

Fig. 7. Effects of protein kinase inhibitors on histamine release induced by ATP. The mast cells were preincubated with bisindolmaleimide (BIM, 0.1 μM), genistein (GS, 10 μM), methyl 2,5-dihydroxycinnamate (DHC, 1 μM), W-7 (3 μM) and trifluoperazine (TFP, 3 μM) for 5 min then stimulated with 100 μM ATP for 10 min. The results are reported as a mean ± SD of 5 separate experiments. *P< 0.05 vs. control

study, the effects of the various purinoceptor antagonists on ATP-induced histamine release were measured to determine which purinoceptors were involved in histamine release. PPADS and suramin dose-dependently inhibited ATP-induced histamine release. However, RB-2, CGS 15943 and DPCPX did not block ATP-induced histamine release. This suggests that ATP causes histamine release from the rat peritoneal mast cells via the P2X-purinoceptor rather than the P2Y-purinoceptor and adenosine receptor (P1-purinoceptor).

The effects of PLA_2, COX and LOX inhibitors were examined to determine whether or not the PLA_2 and AA metabolites were involved in ATP-induced histamine release. PLA_2 inhibitors decreased the ATP-induced histamine release in a dose dependent manner, which suggests that ATP causes PLA_2 activation in rat peritoneal mast cells. In this experiment, secretory PLA_2 inhibitors, manoalide and OPC, were used. Many types of eicosanoid-producing cells, such as macrophages, mast cells and platelets, contained secretory PLA_2. In those cells that contained secretory PLA_2, the bulk of AA release appears to be mediated by the secretory PLA_2, not by the calcium-dependent cytosolic PLA_2 (Balsinde and Dennis, 1996; Balsinde *et al.*, 1998). Whether or not cytosolic PLA_2 has any function in histamine release in peritoneal mast cells will be the subject of a future study.

Both ibuprofen and indomethacin, cyclooxygenase inhibitors, inhibited the ATP-induced histamine in a dose dependent manner. This finding is consistent with earlier reports suggesting that non-steroidal anti-inflammatory drugs inhibit histamine release induced by the antigens, ionophore A23187 and compound 48/80 in rats (Champion *et al.*, 1977; Lewis and Whittle, 1977; Conroy and DeWeck, 1981; Gomes and Pearce, 1988). Both baicalein and caffeic acid, LOX inhibitors, also dose-dependently inhibited ATP-induced histamine release. It has been previously reported that LOX activity was involved in the histamine release stimulated by the ionophore and antigen in MC9 cells (Musch *et al.*, 1985; Musch and Siegel, 1986). There are few reports showing the effect of the LOX inhibitor on histamine release in mast cells. The results in this study suggest that the AA metabolites produced by both COX and LOX may be involved in the ATP-induced histamine release induced by ATP.

Considering previous reports showing that the protein kinase C that is activated by the PLC pathway results in PLA_2 and PLD stimulation (Balsinde *et al.*, 1997), it is difficult to determine whether ATP activates PLA_2 directly. However, in this experiment, ATP significantly increased [^{3}H]AA release by 55% in rat peritoneal mast cells. PPADS (a P2X-purinoceptor antagonist) and suramin (a nonselective P2X, 2Y-purinoceptor antagonist) significantly inhibited ATP-induced AA release, but RB-2 (a P2Y-purinoceptor antagonist) did not. This indicates that ATP causes AA release via the P2X-purinoceptors, which concurred with the

results of histamine release.

In a subsequent experiment, the involvement of protein kinase in histamine release by ATP was investigated. Various protein kinase Inhibitors, such as the protein kinase C, tyrosine kinase inhibitors and calmodulin antagonists significantly inhibited the ATP-induced histamine release. It has been suggested that these protein kinase inhibitors significantly blocked the histamine release by concanavalin A, ionophore A23187 and compound 48/80 in mast cells (Suzuki et al., 1983; Gulbenkian *et al.*, 1987; Izushi *et al.*, 1989; Ozawa *et al.*, 1993). This suggests that protein kinase C, tyrosine kinase and calmodulin-dependent pathway were involved in the ATP-induced histamine release from the mast cells. In conclusion, ATP-induced histamine release is in part related to the PLA_2-mediated AA metabolism P2X-purinoceptors.

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