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Histamine inhibits ATP-induced $[Ca^{2+}]_i$ rise through the activation of protein kinase A in HL-60 cells

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Abstract

We investigated the cross-talk between the histamine and ATP receptors in HL-60 human promyelocytes. While both histamine and extracellular ATP increase intracellular Ca^{2+} concentration ($[Ca^{2+}]_i$), we found that histamine treatment causes a decrease in the subsequent ATP-induced Ca^{2+} release from intracellular stores and Ca^{2+} influx from extracellular space. In addition, histamine also inhibited the subsequent ATP-induced inositol 1,4,5-trisphosphate (IP_3) generation in a manner comparable to the Ca^{2+} release. However, histamine did not inhibit thapsigargin-induced Ca^{2+} release and influx, thus indicating that histamine does not directly inhibit the Ca^{2+} release-activated channel (CRAC). Ca^{2+} elevation induced by 2′- and 3′-*O*-(4-benzoylbenzoyl) ATP (BzATP), which does not produce IP_3, was also inhibited by treatment with histamine, suggesting the presence of ATP-gated channels that are regulated by histamine. Treatment with dibutyryl cAMP or 8-bromo-cAMP inhibited the subsequent ATP-induced response similar to histamine. Moreover, the incubation of cells with *N*-[2-(*p*-bromocinnamylamino)ethyl]-5-isoquinolinesulfonamide (H89), a protein kinase A inhibitor, abolished histamine's inhibitory effect on the ATP-induced $[Ca^{2+}]_i$ rise and IP_3 formation. These results suggest that histamine inhibits both ATP-induced IP_3 production and ATP-activated channel opening, through protein kinase A activation.

Keywords: Histamine; ATP, extracellular; $[Ca^{2+}]_i$; HL-60 cell

1. Introduction

Histamine is found in every human tissue and can play various roles such as the role of a local hormone, a mediator in processes related to allergy and inflammation, or a neurotransmitter. Histamine may, in particular, have an important function in immune response and in immediate hypersensitivity reactions. It is involved in the inhibition of the chemotactic responsiveness of basophils, in the release of histamine from mast cells, and in various lymphocyte functions, including the proliferative response to mitogens, antibody synthesis, cell-mediated cytolysis and the production of lymphokines (reviewed by Hill, 1990).

In addition to histamine, extracellular ATP is also known as a modulator of immune responses through the activation of P_2 purinoceptors. Extracellular ATP may trigger or modulate various lymphocyte functions, including DNA synthesis, blastogenesis, cell-mediated killing, and apoptosis. In the inflammatory system, ATP can stimulate the upregulation of the expression of surface adhesion molecules, the priming of formyl peptide receptor-induced superoxide release (Cockcroft and Stutchfield, 1989a,b; Seifert et al., 1989), and the degranulation of mast cells (reviewed by Gordon, 1986; Dubyak and El-Moatassim, 1993).

In the immune system, neutrophils play an important role in the defense against acute bacterial infection and in other inflammatory events. Neutrophils and HL-60 promyelocytes carry both histamine receptors and P_2 purinoceptors. The histamine receptor on neutrophils and HL-60 cells is of the H_2 subtype that is coupled to adenylyl cyclase and nonselective cation channels. Therefore, the stimulation of these cells with histamine results in an elevation of cAMP level and intracellular Ca^{2+} concentration ($[Ca^{2+}]_i$) (Gespach et al., 1982; Seifert et al., 1992b). The several mechanisms by which activation of the P_2 purinoceptors on HL-60 cells leads to an increase in

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$[Ca^{2+}]_i$ are not yet completely understood. Extracellular ATP stimulates phospholipase C-coupled P_2 purinoceptors. Since UTP can increase cytosolic Ca^{2+} with a potency similar to ATP, the receptors are thought to be of the P_{2U} subtype (Dubyak and El-Moatassim, 1993). ATP also triggers influx of extracellular Ca^{2+} through nonselective cation channels in differentiated HL-60 cells (Krautwurst et al., 1992; Bean, 1992).

Both histamine and extracellular ATP are mediators of the immune response and can induce differentiation of promyelocytes (Chaplinski and Niedel, 1982; Nonaka et al., 1992; Seifert et al., 1992b; Cowen et al., 1989). Since histamine and extracellular ATP can activate the cells simultaneously or sequentially within a short interval, we investigated the cross-talk between the two receptor signalings. We used undifferentiated HL-60 cells, because histamine receptors and P_2 purinoceptors exist during the undifferentiated as well as the differentiated state, while the formyl peptide receptors, which induce the release of superoxide, arachidonic acid, and proteinases, are expressed only on differentiated cells. Here we report that histamine has an inhibitory effect on the ATP-induced $[Ca^{2+}]_i$ increase through a cAMP-dependent pathway.

2. Materials and methods

2.1. Materials

RPMI 1640 and penicillin-streptomycin were purchased from Gibco (Grand Island, NY, USA). Bovine calf serum was obtained from HyClone Laboratories (Logan, UT, USA). Histamine 2HCl, ATP, UTP, BzATP, cAMP, dibutyryl cAMP, 8-Br-cAMP, ranitidine HCl, thapsigargin, sulfinpyrazone, EGTA, EDTA, Trizma base, trichloroacetic acid (TCA) and IP_3 were purchased from Sigma (St. Louis, MO, USA). H89 was purchased from Seikagaku (Chuo-ku, Tokyo, Japan). [^{3}H]IP_3 and [^{3}H]adenine were obtained from NEN (Boston, MA, USA). Ionomycin and isobutylmethylxanthine (IBMX) were obtained from Research Biochemicals International (Natick, MA, USA). Fura-2 pentaacetoxymethylester (fura-2/AM) was purchased from Molecular Probes (Eugene, OR, USA).

2.2. Cell culture

HL-60 cells were maintained at 37°C in RPMI 1640 supplemented with 10% (v/v) heat-inactivated bovine calf serum and 1% (v/v) penicillin-streptomycin in a humidified atmosphere of 95% air and 5% CO_2. The culture medium was changed every 2 days.

2.3. Measurement of $[Ca^{2+}]_i$

The level of intracellular Ca^{2+} was measured using fura-2/AM as previously described (Suh and Kim, 1994). Briefly, cell suspensions were incubated in fresh serum-free RPMI 1640 medium with 3 μM fura-2/AM at 37°C for 40 min under continuous stirring. After this the cells were resuspended in Locke's solution of the following composition: 154 mM NaCl, 5.6 mM KCl, 2.2 mM $CaCl_2$, 1.2 mM $MgCl_2$, 10 mM glucose and 5 mM HEPES buffer adjusted to pH 7.4. In the Ca^{2+}-free Locke's solution, $CaCl_2$ was omitted and 100 μM EGTA included. Sulfinpyrazone (250 μM) was added to all solutions to prevent dye leakage. Changes in fluorescence ratios were measured at the dual excitation wavelengths of 340 nm and 380 nm and the emission wavelength of 500 nm. $[Ca^{2+}]_i$ was calculated using the equation

$$[Ca^{2+}]_i = K_d[(R - R_{min})/(R_{max} - R)](S_{f2}/S_{b2})$$

where R_{max} and R_{min} are the ratio obtained when fura-2 is saturated with Ca^{2+} and when EGTA is used to remove Ca^{2+}, respectively. To obtain R_{min} and R_{max}, the fluorescence ratios of the cell suspension were measured successively at final concentrations of 4 mM EGTA, 30 mM Trizma base and 0.1% Triton X-100, and then at a final concentration of 4 mM $CaCl_2$. S_{f2} and S_{b2} are the proportionality coefficients of Ca^{2+}-saturated fura-2 and free fura-2, respectively. Calibration of the fluorescence signal in terms of $[Ca^{2+}]_i$ was performed according to Grynkiewicz et al. (1985).

2.4. Measurement of cyclic AMP

Intracellular cyclic AMP was determined by measuring the formation of [^{3}H]cAMP from [^{3}H]adenine nucleotide pools as described previously by Salomon (1991). Briefly, cells were harvested and aliquoted into a million cells per tube. The cells were then loaded with [^{3}H]adenine (2 μCi/ml) in complete medium for 24 h. After loading, the cells were washed two times with Locke's solution. The stimulation reaction was stopped by adding 1 ml of ice-cold 5% (v/v) TCA containing 1 μM unlabeled cAMP. The tubes were left on ice for 30 min to extract the water-soluble components including cAMP and ATP. After the extraction on ice, the tubes were centrifuged at $15\,000 \times g$ for 10 min to precipitate cell debris. [^{3}H]cAMP and [^{3}H]ATP were separated using sequential chromatography on Dowex AG50W-X4 (200–400 mesh) cation exchanger and a neutral alumina column. The [^{3}H]ATP fraction was recovered from the Dowex column by elution with 2 ml of distilled water. Then a sequential elution with 3.5 ml distilled water was loaded onto the alumina column. The alumina column was eluted with 4 ml imidazole buffer (0.1 M, pH 7.2) into scintillation vials containing 15 ml scintillation fluid and the radioactivity of the [^{3}H]cAMP was measured. The increase in the intracellular cAMP concentration was calculated as [^{3}H]cAMP/([^{3}H]ATP + [^{3}H]cAMP) $\times 10^3$. The data were expressed as mean ± S.E.M of triplicate measurements.

2.5. Measurement of IP_3

IP_3 mobilization in the cells was determined by competition assay with $[^3H]IP_3$ binding to IP_3 binding protein. As previously described in detail (Suh et al., 1995), IP_3 production was measured based on a standard curve. The amount of IP_3 is expressed as pmol/mg of total cellular protein. The IP_3 binding protein was prepared from bovine adrenal cortex according to the method of Challis et al. (1990).

2.6. Analysis of data

All quantitative data are expressed as mean ± S.E.M. Comparison between two groups was performed using Student's unpaired *t*-test, and comparison among groups more than two was carried out using one-way analysis of variance (ANOVA). Differences were considered to be significant when the degree of confidence in the significance was 95% or better ($P < 0.05$). Calculation of EC_{50} was performed with the Allfit program (De Lean et al., 1978).

3. Results

Application of various concentrations of histamine and ATP in the presence of 2.2 mM extracellular $CaCl_2$ resulted in a concentration-dependent $[Ca^{2+}]_i$ increase in HL-60 cells. ATP increased $[Ca^{2+}]_i$ with an EC_{50} of 0.9 μM and maximum at 100 μM. Histamine increased $[Ca^{2+}]_i$ with an EC_{50} of 2 μM and maximum at 100 μM (Fig. 1A). ATP-induced Ca^{2+} elevation was approximately 4-fold higher than the histamine-induced one when compared with maximum responses. Histamine also increased intracellular cAMP level with an EC_{50} of 0.5 μM. Maximal cAMP level was reached at 100 μM after 3 min treatment (Fig. 1B). We used, however, excessively 300 μM of each agonist in the subsequent experiments to fully activate both receptors in studying cross-talk between histamine and P_2 purinoceptors.

In the presence of extracellular $CaCl_2$, histamine triggered a relatively small increase in Ca^{2+}, but it inhibited a subsequent ATP-induced $[Ca^{2+}]_i$ rise (Fig. 2A). In the absence of extracellular Ca^{2+}, the Ca^{2+} mobilization induced by ATP was also inhibited by pretreatment with histamine, even though the histamine-induced Ca^{2+} release was hardly detected (Fig. 2B). The data suggest that histamine triggers only Ca^{2+} influx from the extracellular medium and that the elevated cytosolic Ca^{2+} is not responsible for the inhibition of the subsequent ATP-induced response. To confirm this result, we treated the cells with ionomycin. Treatment with 0.5 nM ionomycin induced a cytosolic Ca^{2+} increase similar to the one obtained by histamine treatment, but it did not affect the subsequent ATP-induced $[Ca^{2+}]_i$ rise (data not shown). Inhibition of

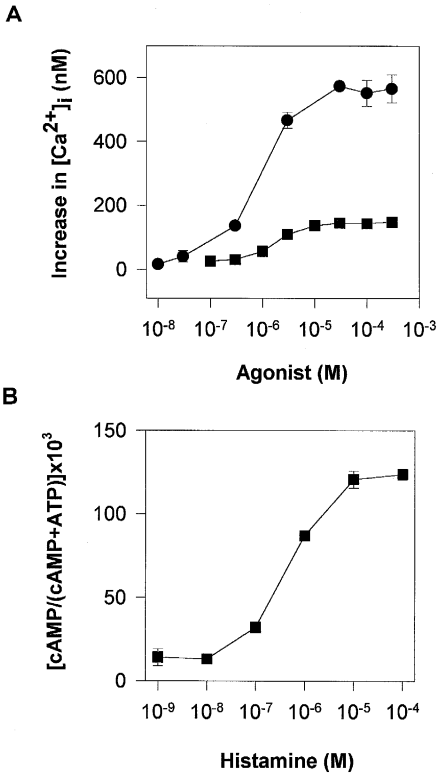

Fig. 1. Concentration-dependent increase in intracellular Ca^{2+} (A) and cAMP production (B) by extracellular ATP and histamine in undifferentiated HL-60 cells. (A) Fura-2/AM-loaded cells were stimulated with various concentrations of ATP (●) and histamine (■) in the presence of extracellular 2.2 mM $CaCl_2$ and the peak level of $[Ca^{2+}]_i$ was measured. The net increase in $[Ca^{2+}]_i$ was obtained by subtracting the basal level from the level after agonist treatment. Each point is the mean ± S.E.M of three experiments. (B) $[^3H]$Adenine-loaded cells were preincubated with 1 mM IBMX in Locke's solution for 20 min and then stimulated with various concentration of histamine for 3 min. The cAMP measurement as described in Section 2. In this report, all experiments were independently carried out more than three times and results were reproducible.

ATP-induced $[Ca^{2+}]_i$ elevation in the presence of extracellular Ca^{2+} and ATP-induced Ca^{2+} release in the absence of extracellular Ca^{2+} by histamine was 24 ± 2.9% and 48 ± 5.8%, respectively, quantified by measuring the peak levels. In view of the area under the Ca^{2+} traces, inhibition of ATP-induced $[Ca^{2+}]_i$ elevation by histamine was about 43% in the presence of extracellular Ca^{2+}, suggesting that the downhill part of the cytosolic Ca^{2+} level after peak was more significantly inhibited by histamine.

To further investigate the inhibitory effect of histamine on ATP-induced $[Ca^{2+}]_i$ rise, inositol 1,4,5-trisphosphate (IP_3) was measured. Fig. 3 shows that ATP induced IP_3 production, with the peak level reached 15 s after stimulation, whereas histamine treatment hardly produced IP_3 at all. However, histamine treatment also decreased the ATP-induced IP_3 production. The data clearly show that histamine inhibits ATP-induced Ca^{2+} mobilization by decreasing the IP_3 production thus reducing the capacitative Ca^{2+} entry through Ca^{2+} release-activated channels

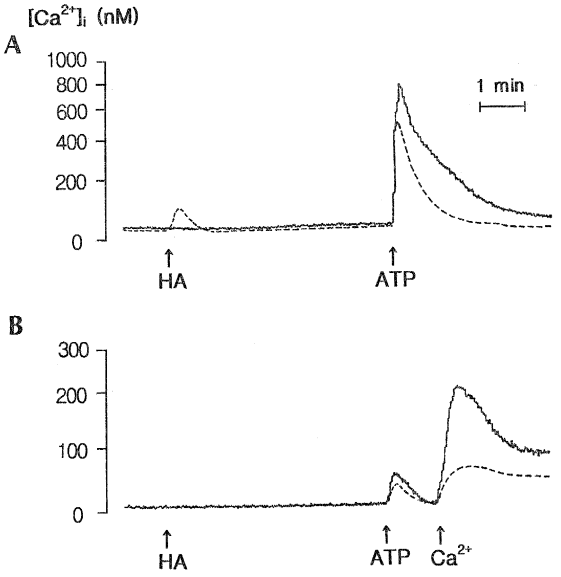

Fig. 2. Inhibition of extracellular ATP-induced $[Ca^{2+}]_i$ increase by histamine. 300 μM ATP was added to cells without (solid line) and with (dotted line) a 5 min pretreatment with 300 μM histamine (HA) in the presence (A) and the absence (B) of extracellular Ca^{2+}. In B, 3 mM Ca^{2+} was added extracellularly after the completion of the ATP-induced Ca^{2+} release to test histamine's effect on Ca^{2+} influx. These experiments were performed with the same batch of cells. Similar results were obtained in more than 10 experiments performed with different preparations of HL-60 cells.

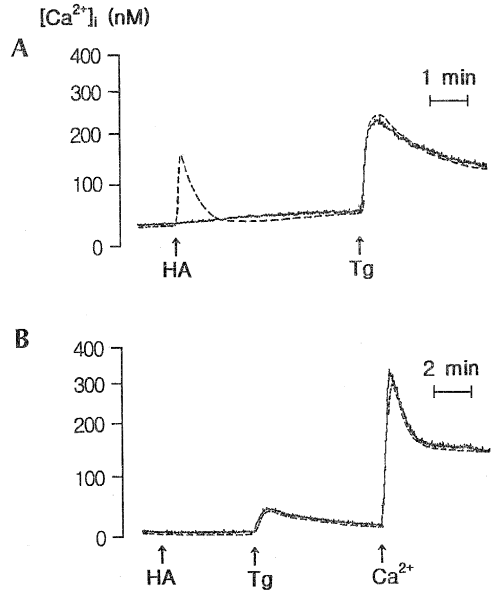

Fig. 4. Effect of histamine on thapsigargin-induced $[Ca^{2+}]_i$ increase. 1 μM thapsigargin (Tg) was added to cells without (solid line) and with (dotted line) preincubation with 300 μM histamine (HA) for 5 min in the presence (A) and absence (B) of extracellular Ca^{2+}. In B, extracellular Ca^{2+} (3 mM) was added after 7 min of thapsigargin stimulation to test histamine's effect on capacitative Ca^{2+} entry.

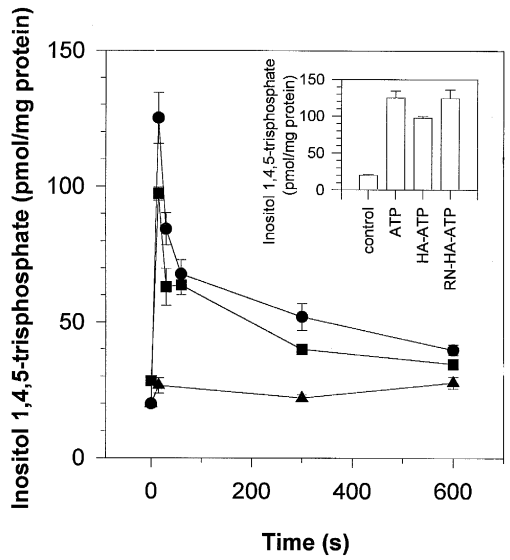

Fig. 3. Time dependence of IP_3 production after ATP stimulation. HL-60 cells were treated with 300 μM histamine (■) or with control medium (●) for 5 min and then stimulated with 300 μM ATP for various lengths of time (0, 15, 30, 60, 180, 300, 600 s). IP_3 production after histamine treatment (▲) was also measured at 0, 300 and 600 s after stimulation. In the inset, cells were stimulated with 300 μM ATP for 15 s with and without the 300 μM histamine pretreatment. In the case of the ranitidine experiment (RN), 10 μM ranitidine was applied for 5 min before the 300 μM histamine treatment after which the cells were stimulated with 300 μM ATP. Data are the means ± S.E.M from triplicate measurements. Decrease in the ATP-induced IP_3 production by histamine is evident with 95% confidence by analyzing with one-way ANOVA.

(CRACs) which are sensitive to the depletion of the intracellular Ca^{2+} stores. To show that the histamine effect was mediated through the activation of histamine H_2 receptors on the HL-60 cells, we used a specific H_2 antagonist, ranitidine. The IP_3 level by sequential treatment of histamine and ATP in the presence of 10 μM ranitidine was not significantly different than the IP_3 level of ATP treatment (inset). The inhibitory effect of histamine on the ATP-induced IP_3 production was completely blocked by incubation with ranitidine. We also found that 10 μM ranitidine completely blocked the histamine-induced cAMP production (data not shown).

In testing the possibility that histamine might directly inhibit CRAC, the cells were treated with thapsigargin, a microsomal Ca^{2+}-ATPase inhibitor, to deplete the intracellular calcium stores without IP_3 production and trigger the capacitative calcium entry from the external space through CRAC. Histamine had no influence on the thapsigargin-induced Ca^{2+} release and influx (Fig. 4), suggesting that it does not directly inhibit CRAC.

Extracellular ATP increases $[Ca^{2+}]_i$ through IP_3-independent nonselective cation channels in addition to IP_3-mediated Ca^{2+} release and subsequent Ca^{2+} influx through CRAC in HL-60 cells. To analyze both the IP_3-dependent and the IP_3-independent pathway, we used UTP and BzATP in distinguishing between the phospholipase C-dependent process and the phospholipase C-independent process. UTP increased IP_3 level 4.2-fold compared to the level of unstimulated control, while histamine inhibited UTP-induced IP_3 production, as was seen in the ATP-induced

response. BzATP, however, increased IP_3 level only 1.2-fold, which was not different compared to control with 95% confidence, and it did not trigger Ca^{2+} release from intracellular Ca^{2+} stores in the absence of extracellular Ca^{2+}, as shown in Fig. 5D. The data, therefore, suggest that UTP preferentially triggers IP_3-dependent $[Ca^{2+}]_i$ rise, while BzATP preferentially triggers Ca^{2+} influx in an IP_3-independent manner. Histamine inhibited the UTP-induced $[Ca^{2+}]_i$ increase in the presence and in the absence of extracellular Ca^{2+} (Fig. 5A and B) as expected because of the inhibition of UTP-induced IP_3 production. BzATP elicited Ca^{2+} influx without internal Ca^{2+} mobilization, since BzATP-induced $[Ca^{2+}]_i$ rise was only seen in the presence of extracellular Ca^{2+}. The BzATP-induced $[Ca^{2+}]_i$ rise was also inhibited by histamine (Fig. 5C and D). The data, therefore, suggest that histamine inhibits not only Ca^{2+} release from IP_3-sensitive calcium stores and Ca^{2+} influx through CRAC but also ATP-gated cation channels.

Histamine elevates intracellular cAMP, as well as Ca^{2+} in HL-60 cells (Gespach et al., 1982). To test whether histamine-induced cAMP signaling plays a critical role in the inhibition of the ATP effect, we tested the cell-permeable cAMP analogs, dibutyryl cAMP and 8-bromo-cAMP (Table 1). Treatment with the cAMP analogs produced a similar inhibitory effect as was obtained with histamine. Simultaneous treatment with a cAMP analog and histamine achieved almost the same inhibition as treatment with the cAMP analog alone. Nonpermeable cAMP, on the other hand, did not inhibit ATP-induced $[Ca^{2+}]_i$ elevation. The cAMP-producing agonist prostaglandin E_2 also inhibited ATP-induced $[Ca^{2+}]_i$ elevation. The data suggest that a cAMP-dependent pathway is involved in the inhibitory action of histamine on the ATP responses.

Since accumulated cAMP can activate protein kinase A, we tested the involvement of protein kinase A by using H89, a relatively selective inhibitor of protein kinase A. Treatment with H89 completely reversed the inhibitory effect of histamine on the ATP-induced Ca^{2+} elevation (Fig. 6A). We can exclude the possibility that H89 is an antagonist of histamine H_2 receptors, because treatment with H89 did not have any influence on the production of cAMP by histamine (data not shown). On the other hand, GF109203X, which is a selective protein kinase C (PKC) inhibitor, had no impact on the histamine action (data not shown). These results suggest that the histamine effect is mediated by activation of protein kinase A upon elevation of intracellular cAMP. The inhibition of ATP- and UTP-induced IP_3 production by histamine was also blocked by

Table 1
Inhibition of extracellular ATP-induced $[Ca^{2+}]_i$ increase by cAMP analogs

Treatment	ATP-induced $[Ca^{2+}]_i$ rise (%)
Control	100
Histamine (300 μM)	78.49 ± 1.57 [a]
8-Bromo-cAMP (1 mM)	70.91 ± 0.48 [a]
Histamine + 8-bromo-cAMP	76.87 ± 0.01 [a]
Dibutyryl cAMP (1 mM)	80.60 ± 1.5 [a]
Histamine + dibutyryl cAMP	79.10 ± 1.99 [a]
cAMP (1 mM)	97.54 ± 2.95 [b]
Prostaglandin E_2 (10 μM)	85.88 ± 0.47 [a]

Cells were stimulated with 300 μM ATP after a 5 min pretreatment with cAMP analogs (1 mM dibutyryl cyclic AMP, 1 mM 8-bromo-cyclic AMP and 1 mM nonpermeable cAMP) with or without 300 μM histamine or 10 μM prostaglandin E_2 in the presence of extracellular Ca^{2+}. The heights of the peak elicited by each stimulation were compared. Data are the mean ± S.E.M and expressed as percentage of control stimulations, responses to ATP without any pretreatment. Comparisons were analyzed using one-way ANOVA followed by Student's unpaired *t*-test. [a] $P < 0.01$; [b] $P > 0.05$.

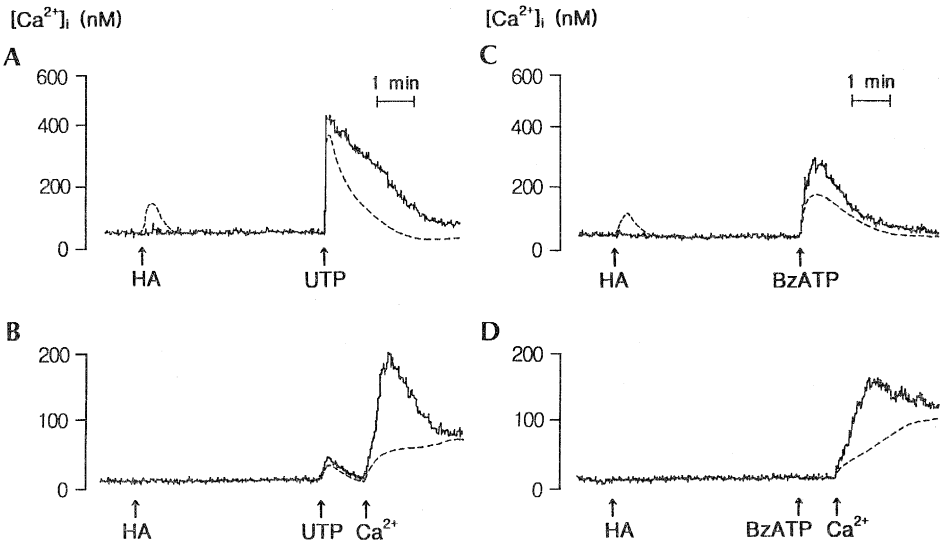

Fig. 5. Inhibition of UTP- and BzATP-induced $[Ca^{2+}]_i$ rise by histamine. UTP or BzATP, 300 μM each, was added to cells without (solid line) and with (dotted line) a 5 min pretreatment with 300 μM histamine (HA) in the presence (A, C) and the absence (B, D) of extracellular Ca^{2+}. In B and D, extracellular 3 mM Ca^{2+} was added to the cells after each nucleotide-induced Ca^{2+} release was completed to test histamine's effect on Ca^{2+} influx.

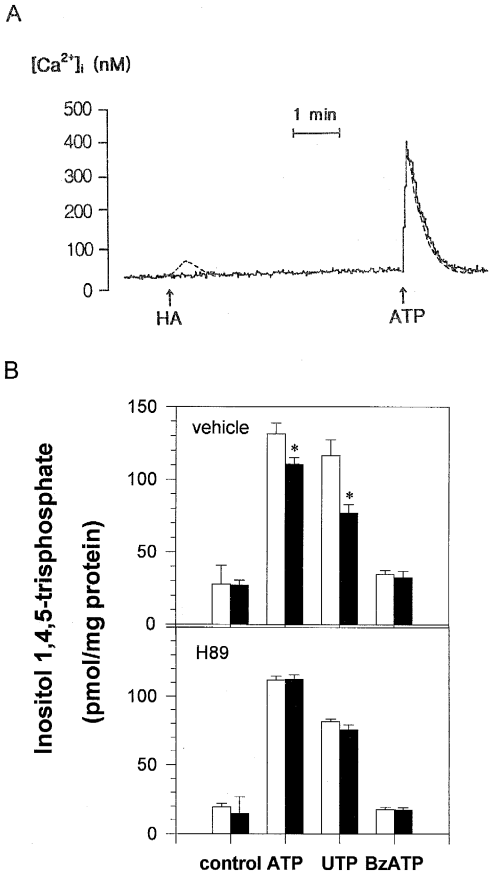

Fig. 6. Negation of histamine's effect on ATP-induced $[Ca^{2+}]_i$ rise and IP_3 production by H89. A: Cells were preincubated with 100 μM H89 for 1 h, treated with 300 μM histamine (dotted line) or with control medium (solid line) for 5 min, and then stimulated with 300 μM ATP in the presence of extracellular Ca^{2+}. Four independent experiments yielded reproducible Ca^{2+} traces. B: Control cells treated with vehicle (upper panel) and cells treated with H89 for 1 h (lower panel) were stimulated for 15 s with control medium, 300 μM ATP, 300 μM UTP, or 300 μM BzATP. The histograms represent the responses obtained to the nucleotide stimulations with (black bar) or without (white bar) pretreatment with histamine. The results are the mean ± S.E.M from triplicate measurements. $^{*}P < 0.01$, compared with the IP_3 production without histamine treatment in one-way ANOVA.

pretreatment with H89 (Fig. 6B), even though the inhibition of ATP- and UTP-induced IP_3 production was 21% and 33%, respectively, in the absence of H89. The different extent of inhibition by histamine of the ATP- and UTP-induced IP_3 production might be due to differential inhibitory effects on the response caused by a strong agonist and a mild agonist. The data also indicate that protein kinase A mediates the inhibitory effect of histamine on IP_3 production.

4. Discussion

Histamine receptors have been divided into three major subtypes, H_1, H_2, H_3. It is generally accepted that histamine H_1 receptors are coupled to phospholipase C and H_2 receptors to adenylyl cyclase. Almost nothing is known about the intracellular signaling system activated via H_3 receptors (Leurs et al., 1995). Although histamine H_1 and/or H_2 receptors were characterized in HL-60 cells, the cells were dibutyrylcAMP- or dimethylsulfoxide (DMSO)-differentiated HL-60 cells (Seifert et al., 1992c; Mitsuhashi et al., 1989). In the undifferentiated HL-60 promyelocytes we used, however, histamine increased cAMP level, but scarcely induced IP_3 generation nor Ca^{2+} release from intracellular Ca^{2+} stores. Therefore, undifferentiated HL-60 cells seem not to possess H_1 subtypes. Although $[Ca^{2+}]_i$ was increased by histamine, it is not likely to be caused by H_1 receptors activating the phospholipase C system. There is a report that H_2 receptors activate nonselective cation channels and increase $[Ca^{2+}]_i$ (Seifert et al., 1992a,b).

In HL-60 cells, the ATP-induced $[Ca^{2+}]_i$ rise is inhibited by protein kinase A activated by an increase in cAMP after histamine treatment, which stimulated histamine H_2 receptors. $[Ca^{2+}]_i$ induced by histamine might not affect ATP-induced $[Ca^{2+}]_i$. The extent of inhibition by histamine is bigger in Ca^{2+}-free rather than in Ca^{2+}-containing medium, because subsequent Ca^{2+} influx and refilling of Ca^{2+} stores which then release Ca^{2+} again after ATP stimulation occurs in Ca^{2+}-containing medium but not in Ca^{2+}-free medium. The source of Ca^{2+} for the ATP-induced $[Ca^{2+}]_i$ rise can be presumed to consist of the following components: (i) Ca^{2+} release from intracellular Ca^{2+} stores through activation of IP_3 receptors, (ii) capacitative calcium entry activated after depletion of the Ca^{2+} stores, (iii) Ca^{2+} influx through ATP-gated cation channels.

Our results show that histamine decreases IP_3 production that is stimulated by ATP or UTP, and that the histamine effect is attenuated by H89 in undifferentiated HL-60 cells. In our view the more acceptable possibility is that protein kinase A activated by histamine decreases phospholipase C activation. It has been reported that the hydrolysis of phosphatidylinositol 4,5-bisphosphate (PIP_2) is inhibited by protein kinase A in platelets, neutrophils, insulin-secreting islets, kidneys, smooth muscle, glomerulosa cells, lymphocytes, and neurotumor NCB-20 cells (Lazarowski and Lapetina, 1989; Takai et al., 1982; Takenawa et al., 1986; Kim et al., 1989; Linden and Delahunty, 1989). Misaki et al. (1989) also demonstrated that protein kinase A inhibited GTPγS-stimulated phosphatidylinositol (PI) hydrolysis in the membrane of differentiated HL-60 cells. In contrast, elevated cAMP enhanced PI hydrolysis (Kaibuchi et al., 1982) or Ca^{2+} influx (Kass et al., 1994) in hepatocytes. In Schwann cells, cAMP up-regulates P_{2Y} purinoceptors that are linked to phospholipase C resulting in increased Ca^{2+} release from internal Ca^{2+} stores (Lyons et al., 1994). These apparently contradictory results suggest that protein kinase A might act at various sites in the PI signaling pathway, including phosphatidylinositol kinase, receptors responsive to Ca^{2+} mobilizing agonists, G

proteins involved in coupling the receptor to phospholipase C (Misaki et al., 1989), and phospholipase C itself (Kim et al., 1989), but the actual site has not yet been elucidated in HL-60 cells. Our results, for the first time, demonstrate that a physiological agonist, histamine, inhibits the PI turnover induced by a natural stimulant, ATP, by protein kinase A activation. However, we cannot exclude the possibility that protein kinase A activated by histamine stimulation may reduce the ATP-induced $[Ca^{2+}]_i$ elevation which in turn inhibits the facilitatory effect of Ca^{2+} on receptor-mediated phospholipase C activation because cytosolic Ca^{2+} is involved in the activation of phospholipase C (Wojcikiewicz et al., 1994).

The capacitative calcium entry exists in many cells and occurs through CRAC stimulated by a messenger that is generated by depleted Ca^{2+} stores (reviewed in Berridge, 1995). It has been reported that phosphorylation down-regulates capacitative calcium entry (Montero et al., 1993, 1994; Randriamampita and Tsien, 1995). However, we found that histamine-induced protein kinase A activation could not directly inhibit thapsigargin-induced capacitative calcium influx following the depletion of intracellular Ca^{2+} stores in an IP_3 receptor-independent manner. This result also suggests that a change in Ca^{2+}-ATPase is not involved in the histamine response. We also measured and found inhibition of Mn^{2+} entry by histamine with fura-2 fluorescence quenching (data not shown), suggesting that the histamine-induced inhibition did not occur through activation of the plasma membrane Ca^{2+}-ATPase because Mn^{2+} flux is unidirectional and not extruded by the action of Ca^{2+}-ATPase.

ATP-induced Ca^{2+} influx is composed of capacitative Ca^{2+} influx and Ca^{2+} influx through cation channels. To possibly distinguish between these two components, UTP and BzATP were used. We found that UTP increases $[Ca^{2+}]_i$ by Ca^{2+} mobilization triggered by IP_3 following the activation of P_{2U} purinoceptors in HL-60 cells (Stutchfield and Cockcroft, 1990). In contrast, BzATP increases $[Ca^{2+}]_i$ without significant IP_3 production. We, therefore, thought that UTP and BzATP could demonstrate ATP-activated Ca^{2+} mobilization and Ca^{2+} influx through ATP-gated cation channels, respectively, and that they offered a useful approach to the separation of the Ca^{2+} influx pathways.

Histamine inhibited both UTP- and BzATP-induced $[Ca^{2+}]_i$ increases. Inhibition of the BzATP-induced $[Ca^{2+}]_i$ rise by histamine suggests that purinoceptors are coupled to cation channels and are regulated by protein kinase A. It has been reported that neutrophils, DMSO- or dibutyryl cAMP-differentiated HL-60 cells possess ATP- and fMLP-activated nonselective cation channels that lack a voltage-dependent gating mechanism. Ca^{2+} and Na^+ influxes through nonselective cation channels are involved in the activation of β-glucuronidase release and superoxide production (Krautwurst et al., 1992). Elevation of cAMP and activation of PKC play inhibitory roles in the regulation of nonselective cation channels in neutrophils (Schumann et al., 1992; McCarthy et al., 1989; Seifert et al., 1992a). Purinoceptors that mediate Ca^{2+} influx might be ATP-gated nonselective cation channels, P_{2X} purinoceptors, or pore-forming ATP receptors, P_{2Z} purinoceptors. The P_{2X} purinoceptor is a nonselective cation channel that is permeable to Ca^{2+} and Na^+ and is found in smooth muscle, brain, heart, and spleen. The P_{2Z} purinoceptor, potentially activated by ATP^{4-}, forms non-selective pores that pass molecules up to 1 kDa (Gordon, 1986) and is present in mast cells, macrophages, and the vas deferens. Although major purinoceptors in HL-60 cells are identified as belonging to the P_{2U} subtype (Xing et al., 1992; Dubyak and El-Moatassim, 1993; Montero et al., 1995), our results suggest that ATP-gated cation channels also exist in undifferentiated HL-60 cells. It is well known that BzATP is a potent agonist to the P_{2Z} purinoceptor (Nuttle and Dubyak, 1994; El-Moatassim and Dubyak, 1992), with a potency order of BzATP > ATP = ATPγS. However, HL-60 cells may not have classical P_{2Z} purinoceptors, since BzATP was not as potent as ATP and ethidium bromide (about 300 Da) could not pass into the cells (data not shown). These BzATP-activated cation channels need to be characterized in more detail. There is also a possibility that a small amount of Ca^{2+} entry caused by BzATP triggered intracellular Ca^{2+} release by Ca^{2+}-induced Ca^{2+} release (CICR). However, there was no change in $[Ca^{2+}]_i$ by ryanodine treatment in HL-60 cells (data not shown). The results suggest that CICR may not be involved in the cell because ryanodine receptor is known to be principally involved in the CICR process in nonmuscle cells (Furuichi et al., 1994).

In HL-60 cells, histamine leads to the differentiation to neutrophil-like cells via cAMP accumulation (Chaplinski and Niedel, 1982; Nonaka et al., 1992; Seifert et al., 1992b). In addition to histamine, extracellular ATP also induces differentiation of leukocytes via $[Ca^{2+}]_i$ rise (Cowen et al., 1989). It has been speculated that cross-talk between histamine and ATP would be required in regulating the start and the rate of differentiation in these cells. After differentiation, ATP plays an important role as an activator or modulator in exocytotic secretion and superoxide generation (Cockcroft and Stutchfield, 1989a,b; Karoki and Minakami, 1989; Seifert et al., 1989). Therefore, it seems likely that, when histamine stimulates cells prior to ATP, it can negatively regulate the subsequent ATP-activated functions through the mechanisms suggested in the present study.

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