Inhibition of H₂ Histamine Receptor-Mediated Cation Channel Opening by Protein Kinase C in Human Promyelocytic Cells

Byung-Chang Suh, Hyun Lee, Dong-Jae Jun, Jang-Soo Chun, Jong-Hee Lee and Kyong-Tai Kim

*J Immunol* 2001; 167:1663-1671; doi: 10.4049/jimmunol.167.3.1663

http://www.jimmunol.org/content/167/3/1663

References

This article cites 53 articles, 31 of which you can access for free at: http://www.jimmunol.org/content/167/3/1663.full#ref-list-1

Subscription

Information about subscribing to *The Journal of Immunology* is online at: http://jimmunol.org/subscription

Permissions

Submit copyright permission requests at: http://www.aai.org/About/Publications/JI/copyright.html

Email Alerts

Receive free email-alerts when new articles cite this article. Sign up at: http://jimmunol.org/alerts
Inhibition of H2 Histamine Receptor-Mediated Cation Channel Opening by Protein Kinase C in Human Promyelocytic Cells

Byong-Chang Suh,* Hyun Lee,* Dong-Jae Jun,* Jang-Soo Chun, † Jong-Hee Lee,* and Kyong-Tai Kim2‡

Histamine, through H2 receptors, triggers a prominent rise in intracellular free Ca2+ concentration ([Ca2+]i) in addition to an elevation of cAMP level in HL-60 promyelocytes. Here we show that the histamine-induced [Ca2+]i rise was due to influx of Ca2+ from the extracellular space, probably through nonselective cation channels, as incubation of the cells with SKF 96365 abolished the histamine-induced [Ca2+]i rise, Na+ influx, and membrane depolarization. The Ca2+ influx was specifically inhibited by pretreatment of the cells with PMA or extracellular ATP with 50% inhibitory concentrations of 0.12 ± 0.03 nM and 185 ± 17 μM, respectively. Western blot analysis of protein kinase C (PKC) isoforms revealed that PMA (≤1 nM) and ATP (300 μM) caused selective translocation of PKC-δ to the particulate/membrane fraction. Costimulation of the cells with histamine and SKF 96365 partially reduced histamine-induced granulocytogenic differentiation, which was evaluated by looking at the extent of fMet-Leu-Phe-induced [Ca2+]i rise and superoxide generation. In conclusion, nonselective cation channels are opened by stimulation of the H2 receptor, and the channels are at least in part involved in the induction of histamine-mediated differentiation processes. Both effects of histamine were selectively inhibited probably by the δ isoform of PKC in HL-60 cells. The Journal of Immunology, 2001, 167: 1663–1671.

© 2001 by The American Association of Immunologists 0022-1767/01/$02.00

Address correspondence and reprint requests to Dr. Kyong-Tai Kim, Department of Life Science, POSTECH, San 31, Hyoja Dong, Pohang 790-784, Korea. E-mail address: ktk@postech.ac.kr

1 This work was supported by the National Research Laboratory Program, the Brain Science and Engineering Research Program sponsored by the Ministry of Science and Engineering, the Korea Science and Engineering Foundation, and the Brain Korea 21 Program of the Ministry of Education.

2 Address correspondence and reprint requests to Dr. Kyong-Tai Kim, Department of Life Science, POSTECH, San 31, Hyoja Dong, Pohang 790-784, Korea. E-mail address: ktk@postech.ac.kr

3 Abbreviations used in this paper: [Ca2+]i, intracellular Ca2+; BzATP, 3’-O-(4-benzoyl)benzoyl ATP; DCFH-DA, 2’7’-dichlorodihydrofluorescein diacetate; TDA, 2-AFM, fura 2, pentamethoxybenzyl ester; IP3, inositol 1,4,5-trisphosphate; HTMT, 6-[2-(4-imidazolyl)ethylamino]-N-(4-trifluoromethylphenyl)heptane-carboxamidemaleate; PLC, phospholipase C; SBFI/AM, sodium-binding benzofuran isophthalate teta-acetoxyethyl ester; PKC, protein kinase C.

Materials and Methods

Materials

ATP, UTP, 3’-O-(4-benzoyl)benzoyl ATP (BzATP), dibutyryl cAMP, 8-bromo-cAMP, iMLP, thapsigargin, sulfinpyrazone, EGTA, EDTA, Trizma base, TCA, PMA, and inositol 1,4,5-trisphosphate (IP3) were purchased from Sigma (St. Louis, MO). [3H]IP3 and [3H]adenine were obtained from NEN Life Science Products (Boston, MA). R-(−)-a-methylhistamine, SKF 96365, and 6-[2-(4-imidazolyl)ethylamino]-N-(4-trifluoromethylphenyl)heptane-carboxamidemaleate (HTMT) were purchased from BIOMOL (Plymouth Meeting, PA). Histamine, dimaprit, ranitidine, triploridine, thioramidemaleate, GF 109203X, and Ro 20–1724 were obtained from
Research Biochemicals (Natick, MA). H89 was purchased from Seikagaku (Tokyo, Japan). Fura 2-penta-acetyloxymethylster (fura-2 AM), sodium-binding benzofuran isophthalate tetra-acetyoxymethyl ester (SBFI/AM), 2',7'-dichlorofluorescein diacetate (DCFH-DA), and bis-oxonol DSBAC (3) purchased from Molecular Probes (Eugene, OR).

Cell culture

Human promyelocytic leukemia HL-60 cells were maintained in RPMI 1640 medium (Life Technologies, Gaithersburg, MD) supplemented with 20% heat-inactivated bovine calf serum (HyClone, Logan, UT) plus 1% (v/v) penicillin-streptomycin (Life Technologies) under a humidified atmosphere of 5% CO2 at 37°C. Fresh medium was added to culture flasks every 2 days, and cells were subcultured once a week.

Measurement of [Ca2+]

The level of [Ca2+] was measured using fura 2-AM as previously described (14). 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. Thereafter, the cells were resuspended in Locke’s solution of the following composition: 154 mM NaCl, 5.6 mM KCl, 2.2 mM CaCl2, 1.2 mM MgCl2, 10 mM glucose, and 5 mM HEPES buffer adjusted to pH 7.4. In the Ca2+-free Locke’s solution, CaCl2 was omitted, and 100 μM EGTA was included. Sulfipyrazine (250 μM) was added to all solutions to prevent dye leakage (15). Changes in fluorescence ratios were measured at the dual excitation wavelengths of 340 and 380 nm, and the emission wavelength of 500 nm. [Ca2+] was calculated using the equation: 

$$[	ext{Ca}^{2+}] = K_a \frac{(R - R_{	ext{min}})(R_{	ext{max}} - R)}{(S_2 - S_0)}$$

where Rmin and Rmax are the ratios obtained when fura 2 is saturated with Ca2+ and when EGTA is used to remove Ca2+, respectively. To obtain Rmin and Rmax, 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 CaCl2. S2 and S0 are the proportionality coefficients of Ca2+-free fura 2 and saturated fura 2, respectively. Calibration of the fluorescence signal in term of [Ca2+] was performed according to the method described by Grynkiewicz et al. (16).

Measurement of intracellular Na+ level

The level of intracellular Na+ was determined by use of SBFi/AM, which is a fluorescence sodium indicator (17). Cells were harvested and incubated in serum-free RPMI 1640 medium with 15 μM SBFi/AM, 0.2% pluronac acid, and 250 μM sulfipyrazine at 37°C for 90 min under continuous stirring. Then the cells were washed with serum-free RPMI 1640 solution with 250 μM sulfipyrazine. Before measurement, a small aliquot of the cell suspension was removed, centrifuged, and, after the supernatant was removed, resuspended in Locke’s solution. For these experiments, the increase in cytosolic Na+ was measured as an increase in the fluorescence ratio determined at the dual excitation wavelengths of 340 and 380 nm and the emission wavelength of 520 nm at 37°C. Because the calibrations of the obtained fluorescence ratios for Na+ concentrations are not absolute (18), we expressed our results as fluorescence ratios.

Measurement of [H]cAMP

Intracellular cAMP generation was determined by [H]cAMP competition assay in binding to cAMP binding protein as described previously by Park et al. (19) with some modification. To determine the cAMP production induced by histamine or ATP analogs, the HL-60 cells were stimulated with 250 μM histamine or 250 μM ATP for 10 min, respectively. The samples were then centrifuged at 2500 g for 5 min. The supernatant was then added to 200 μl of assay buffer containing 4 mM CaCl2. The cAMP concentration was determined using an assay kit (Amersham, Piscataway, NJ). The samples were then washed twice with Locke’s solution. Then 20 μl of solution containing the binding protein was added, and the mixture was incubated for 15 min on ice and centrifuged at 2000 x g for 5 min. The pellet was resuspended in 100 μl water, and 1 ml scintillation cocktail was added to measure the radioactivity. The IP3 concentration in the sample was determined based on a standard curve and expressed as picoquimograms per milligram protein. The IP3 binding protein was prepared from bovine adrenal cortex according to the method of Challis et al. (22).

Measurement of IP3

The IP3 concentration in the cells was determined by [H]IP3 competition assay in binding to IP3 binding protein (21). To determine IP3 production, the HL-60 cells were stimulated with agonists for specific periods of time, and the reaction was terminated by aspirating the medium off the cells followed by addition of 0.3 ml ice-cold 15% (w/v) TCA containing 10 mM EDTA. The samples were then left on ice for 30 min to extract intracellular water-soluble inositol phosphates and then were centrifuged at 5000 x g for 10 min at 4°C. The extract was transferred to an Eppendorf tube, and TCA was removed by extractions with diethyl ether four times. Finally, the extract was neutralized with 200 mM Trizma base, and its pH was adjusted to ~7.4. Twenty microliters of the cell extract was added to 20 μl of the assay buffer (0.1 M tris(hydroxymethyl)-ammonium buffer containing 4 mM EDTA and 4 mg/ml BSA), and 20 μl [H]IP3 (0.1 μCi/ml) was added. Then 20 μl of solution containing the binding protein was added, and the mixture was incubated for 15 min on ice and centrifuged at 2000 x g for 5 min. The pellet was resuspended in 100 μl water, and 1 ml scintillation cocktail was added to measure the radioactivity. The IP3 concentration in the sample was determined based on a standard curve and expressed as picomoles per milligram protein. The IP3 binding protein was prepared from bovine adrenal cortex according to the method of Challis et al. (22).

Measurement of membrane potential with bisoxonol

Changes in membrane potential were monitored using a fluorescent potential-sensitive anionic dye, bisoxonol DSBAC (3), as reported by Barry and Cheek (23) with minor modifications. Briefly, HL-60 cells, after pre-incubation for 1 h at 37°C in incubation buffer (125 mM NaCl, 5 mM KCl, 1 mM MgSO4, 1 mM Na2HPO4, 5.5 mM glucose, 5 mM NaHCO3, 20 mM HEPES, and 200 μM EGTA, pH 7.4), were washed and resuspended with the above incubation buffer at a density of 1.5 × 106 cells/ml. The cells were then incubated with 300 nM bisoxonol for 7 min at 37°C before the addition of stimulants. Fluorescence was measured at the excitation wavelength of 450 nm and the emission wavelength of 580 nm.

Measurement of intracellular reactive oxygen species production

The production of intracellular reactive oxygen species such as superoxide and hydrogen peroxide was determined by a method based on the changes in fluorescence of DCFH-DA, an oxidation-sensitive fluorescence probe, following a previously published procedure (24, 25). Briefly, the cell suspension was incubated in fresh-serum-free RPMI 1640 medium with 2 μM DCFH-DA at 37°C for 40 min under continuous stirring. The loaded cells were then washed twice with Locke’s solution. Then 2 × 105 cells were placed into a cuvette in a thermostatically controlled cell holder at 37°C and continuously stirred. Fluorescence was measured when excited at 488 nm, and emission was recorded at 530 nm. The change in fluorescence intensity was monitored.

Cell fractionation and Western blot analysis of PKC isoforms

To separate the cell material into soluble/cytosolic and particulate/membrane fractions, the HL-60 cells were suspended in buffer A (20 mM Tris-HCl, pH 7.5, containing 250 mM sucrose, 2 mM EDTA, 2 mM EGTA, 10 μg/ml pepstatin A, 10 μg/ml leupeptin, 1 mM PMSF, and 10 μg/ml aprotinin). The cells were then sonicated twice for 5 s each time and centrifuged at 100,000 × g for 1 h. The supernatant was saved as the cytosolic fraction. The pellet was then extracted with buffer B (20 mM Tris-HCl, pH 7.5, containing 1% SDS, 150 mM NaCl, 1 mM EGTA, 1 mM EDTA, and protease inhibitors as described above for buffer A). Following centrifugation, the supernatant was saved as the particulate/membrane fraction (26).

Proteins (30 μg) from the cytosolic and particulate membrane fractions were separated by electrophoresis on an 8% polyacrylamide gel containing 0.1% SDS and transferred to a nitrocellulose membrane. The nitrocellulose sheet was blocked with 3% nonfat dry milk in Tris-buffered saline. PKC isoforms were detected with isofrom-specific-anti-PKC α, βI, βII, and δ isoforms (Transduction Laboratories, Lexington, KY). The blots were developed using a peroxidase-conjugated secondary Ab, either goat anti-rabbit or anti-mouse IgG, using the ECL system (Amersham, Arlington Heights, IL).

Analysis of data

Quantitative data are expressed as the mean ± SEM. Comparison between groups was analyzed using Student’s unpaired t test, and differences were considered significant when the degree of confidence in the significance was 95% or better (p < 0.05). Calculation of the 50% effective concentration was performed with the ALLFIT program (27).
Results
Nonselective cation channels activated by the H₂ receptor

Exposure of HL-60 cells to histamine (100 μM) results in an increase in \([Ca^{2+}]_i\), in the presence of 2.2 mM extracellular CaCl₂ (Fig. 1A). The specific H₂ agonist dimaprit (100 μM) also increased \([Ca^{2+}]_i\), with an effectiveness comparable to that of histamine. However, removal of extracellular Ca²⁺ completely abolished the histamine- and dimaprit-induced rises (Fig. 1A, right panel), indicating that the histamine-induced increase in \([Ca^{2+}]_i\), is mediated by Ca²⁺ influx from the extracellular medium. As shown in Fig. 1B, histamine and dimaprit increased \([Ca^{2+}]_i\), in a concentration-dependent manner with 50% effective concentrations of 10 ± 3 and 17 ± 5 μM, respectively. Moreover, stimulation of the cells with the maximal concentration of dimaprit (100 μM) resulted in complete inhibition of the subsequent histamine-induced Ca²⁺ mobilization (data not shown), suggesting that the \([Ca^{2+}]_i\) increase induced by histamine or dimaprit was mediated through a common receptor. The H₁ agonist HTMT and the H₃ agonist R(−)-α-methylhistamine had little effect on \([Ca^{2+}]_i\), suggesting that the histamine-mediated Ca²⁺ influx occurred exclusively through H₂ receptors in these cells. This was further confirmed in the experiment using selective antagonists. Fig. 1C shows that addition of the H₁ antagonist triploridine and the H₃ antagonist thioperamide-maleate did not affect the histamine-mediated \([Ca^{2+}]_i\), rise up to a 100-μM concentration, whereas the H₂ receptor antagonist ranitidine concentration-dependently inhibited the response with a 50% inhibitory concentration of 0.6 ± 0.2 μM. Because Ca²⁺ mobilization from intracellular stores is mediated by IP₃, we examined whether histamine treatment produced IP₃ in HL-60 cells. Table I shows that histamine and selective agonists had no effect on the generation of IP₃, whereas treatment with ATP and UTP significantly increased IP₃ contents. Therefore, the data clearly suggest that the histamine-induced \([Ca^{2+}]_i\), rise must be due to Ca²⁺ influx from the extracellular medium following the stimulation of H₂ receptors.

SKF 96365 blocks nonselective cation channels and inhibits receptor agonist-activated Ca²⁺ influx in neutrophils and HL-60 cells (28, 29). In the presence of extracellular Ca²⁺ we found that treatment with 10 μM SKF 96365 inhibited the subsequent histamine-induced \([Ca^{2+}]_i\), rise by ~85–90% (Fig. 2A). SKF 96365 also inhibited the ATP- and thapsigargin-induced \([Ca^{2+}]_i\), rises by 20–30% (Fig. 2A). However, SKF 96365 did not affect the ATP- and thapsigargin-mediated Ca²⁺ mobilization from intracellular stores (Fig. 2B), indicating that the decreased responses were due to the inhibition of store-operated Ca²⁺ entry from extracellular space. As shown in Fig. 1A, histamine had no effect on Ca²⁺ mobilization and the histamine-mediated Ca²⁺ influx was almost completely blocked by SKF 96365 (Fig. 2B). Fig. 2C shows the concentration-dependent inhibitory effect of SKF 96365 on the histamine- and ATP-induced \([Ca^{2+}]_i\), rise. SKF 96365 at concentrations greater than 30 μM produced complete inhibition of the histamine response, whereas it had a maximal inhibitory effect on the ATP response of about 25%. The results demonstrate that the histamine-induced \([Ca^{2+}]_i\), rise in HL-60 cells is exclusively due to Ca²⁺ influx through SKF 96365-sensitive nonselective cation channels, whereas the ATP-induced \([Ca^{2+}]_i\), rise is caused by Ca²⁺ mobilization from intracellular stores and Ca²⁺ influx from extracellular space through the store-operated Ca²⁺ channels; the latter is also partially sensitive to SKF 96365 (Fig. 2B).

We tested the permeation of Na⁺ ions through nonselective cation channels upon histamine stimulation. Histamine triggered Na⁺ influx in SBI-F2-loaded cells (Fig. 3A). However, the addition of ranitidine abolished the histamine-induced response, whereas it

---

**Table I. Effects of histamine on IP₃ generation in HL-60 promyelocytes**

<table>
<thead>
<tr>
<th>Treatment</th>
<th>IP₃ Generation (pmol/mg protein)</th>
</tr>
</thead>
<tbody>
<tr>
<td>Control</td>
<td>21.5 ± 1.6</td>
</tr>
<tr>
<td>Histamine</td>
<td>22.7 ± 1.2</td>
</tr>
<tr>
<td>HTMT</td>
<td>23.3 ± 2.6</td>
</tr>
<tr>
<td>Dimaprit</td>
<td>22.2 ± 2.8</td>
</tr>
<tr>
<td>R(−)-α-methylhistamine</td>
<td>20.3 ± 3.2</td>
</tr>
<tr>
<td>ATP</td>
<td>73.2 ± 6.5⁺</td>
</tr>
<tr>
<td>UTP</td>
<td>65.6 ± 3.7⁺</td>
</tr>
<tr>
<td>BzATP</td>
<td>25.3 ± 2.4</td>
</tr>
</tbody>
</table>

*HL-60 cells were stimulated with 100 μM histamine or each of the histamine receptor agonists and 300 μM ATP or other nucleotides for 15 s. IP₃ generation was measured as described in Materials and Methods. Data are means ± SEM of three independent experiments.

⁺ P < 0.01, compared with control level.
through the SKF 96365-sensitive cation channel. and maximal inhibitions at inhibited the histamine-mediated depolarization with half-maximal (data not shown). However, incubation of cells with SKF 96365 evoked membrane depolarization comparable to that of histamine.

A

B

C

D

FIGURE 2. Effect of SKF 96365 on histamine-induced Ca\(^{2+}\) mobilization. A, Fura 2-AM-loaded HL-60 cells treated with or without 10 \(\mu\)M SKF 96365 for 10 min were then stimulated with 100 \(\mu\)M histamine, 300 \(\mu\)M ATP, or 1 \(\mu\)M thapsigargin in the presence of extracellular 2.2 mM CaCl\(_2\). The experiments were conducted more than five times, and typical Ca\(^{2+}\) transients are presented. B, Fura 2-AM-loaded HL-60 cells were stimulated with 100 \(\mu\)M histamine, 300 \(\mu\)M ATP, or 1 \(\mu\)M thapsigargin initially in the absence of external Ca\(^{2+}\), and then 3 mM CaCl\(_2\) was added to the medium 1 min after histamine and ATP stimulation or 1.5 min after thapsigargin stimulation. C, Concentration-dependent inhibition of histamine- and ATP-mediated [Ca\(^{2+}\)], rise by SKF 96365. Cells pretreated with various concentrations of SKF 96365 were subsequently stimulated with histamine or ATP. The net increase in [Ca\(^{2+}\)], is expressed as a percentage of the control (histamine or ATP alone). Each point is the mean ± SEM of four independent experiments.

Negative regulation of histamine-activated nonselective cation channel upon PKC activation

Because it has been shown that histamine H\(_2\) receptor-mediated signaling is regulated by various protein kinases (30–32), we examined the involvement of PKC in the modulation of the histamine-mediated [Ca\(^{2+}\)], increase in HL-60 cells. We found that treatment of the cells with 100 pM PMA significantly inhibited the histamine-induced cytosolic Ca\(^{2+}\) increase (Fig. 5A). The inhibitory effect of PMA on the histamine-induced [Ca\(^{2+}\)], elevation was concentration dependent, with 50% inhibitory effect and maximum effect at 0.12 ± 0.03 and 1.0 ± 0.3 nM PMA, respectively (Fig. 5B). However, the cAMP generation induced by histamine was only barely influenced at the above concentrations and was maximally (60–65%) blocked at higher concentrations of PMA (~100 nM). An inactive PMA analog, 4\(\alpha\)-PMA, had no inhibitory effect at concentrations up to 100 nM (data not shown). The results thus indicate that the histamine-mediated cation channel activation was inhibited upon PKC activation without any link to the pathway of cAMP generation in HL-60 cells.

Because PKC is activated upon [Ca\(^{2+}\)], elevation and diacyl-glycerol generation elicited by phospholipase C (PLC)-coupled receptor activation, we tested whether activation of the P2 purinoceptor also had an inhibiting effect like that caused by PKC in...
Log[histamine], M

Dibutyryl cAMP neither increased nor inhibited the subsequent histamine-induced [Ca^{2+}]_{i} rise. Treatment of the cells with the adenylyl cyclase activator forskolin, PGE_2, or the cAMP analogs 8-bromo-cAMP and dibutyryl cAMP neither increased nor inhibited the subsequent histamine-mediated [Ca^{2+}]_{i} rise.

To find out whether PKC activation was responsible for the inhibiting effect of the P2 receptor, a PKC inhibitor was administered before stimulation with ATP, and the subsequent histamine response was measured. Fig. 7 shows that the addition of 3 μM GF109203X for 10 min before ATP stimulation blocked the inhibitory effect of ATP (300 μM) on the subsequent histamine-induced [Ca^{2+}]_{i} rise. In contrast, H89 (10 μM), which is a selective cAMP-dependent protein kinase inhibitor, had no impact on the ATP effect. These data clearly show that the signaling of the histamine receptor can be specifically inhibited by pretreatment with ATP via PKC activation.

**Differential regulation of PKC isoforms by PMA and extracellular ATP**

To investigate the PKC isoforms involved in the regulation of the histamine responses, we determined the isoforms of PKC translocated from cytosol to membranes upon treatment with PMA and ATP using Western blotting analysis with isoform-specific Abs. Each Ab recognized individual PKC isoforms in the lysate of rat brain, which was the positive control (33). The distribution of the expressed PKC isoforms between the soluble/cytosolic and the particulate/membrane fraction was determined by binding of Abs against the PKC-α, -β1, -δ, and -ε isoforms after stimulation of HL-60 cells (Fig. 8A). In untreated cells all PKC isoforms were detected predominantly in the cytosolic fraction. However, treatment of the cells with lower concentration (1 nM) of PMA selectively induced translocation of the cytosolic PKC-δ isoform to the particulate membrane fraction. Higher concentrations of PMA (>10 nM) selectively translocated PKC-ε in addition to PKC-δ, and at concentrations of >100 nM PMA all isoforms of PKC translocated to the membrane. The distribution of the atypical PKC-α was not affected by treatment with PMA (data not shown). Treatment with ATP (300 μM) also specifically induced translocation of PKC-δ, whereas it had little effect on translocation of the other isoforms of PKC. However, histamine did not affect the translocation of any PKC isozyme. Fig. 8B shows the time course of PKC translocation. The translocation of PKC-δ, but not other...

**FIGURE 5.** Inhibition of histamine-induced [Ca^{2+}]_{i} rise by PMA. A. Histamine (100 μM) was added to cells without (dotted line) or after (solid line) a 5-min pretreatment with 100 μM PMA in the presence of extracellular Ca^{2+}. B. Concentration dependence of the effect of PMA on the inhibition of subsequent histamine-induced [Ca^{2+}]_{i} rise (○) and cAMP generation (●). Cells preincubated with various concentrations of phorbol ester for 5 min were stimulated with 100 μM histamine. The net increase in [Ca^{2+}]_{i} and cAMP levels are expressed as a percentage of the level obtained after treatment with histamine alone.

**FIGURE 4.** Effect of histamine on plasma membrane depolarization in HL-60 cells. A, HL-60 cells were incubated at 37°C in Ca^{2+}-free standard saline solution at a concentration of 1 × 10^6 cells/ml in the presence of 300 nM bisoxonol. Histamine (100 μM) was added, followed by the addition of 30 mM KCl as the positive control. B, Concentration-dependent effect of histamine on membrane depolarization. Various concentrations of histamine were applied in the presence of bisoxonol. Depolarization is expressed as a percentage of the control (30 mM KCl). C and D, HL-60 cells pretreated with 3 or 10 μM SKF 96365 for 10 min were stimulated with histamine (100 μM) and 30 mM KCl.
types of PKC, was dramatically evoked within 5 min and sustained for up to 20 min after stimulation with ATP (300 μM) and PMA (1 nM). These data suggest that the specific translocation of novel PKC-δ may be involved in regulation of the nonselective channel-mediated Ca\(^{2+}\) influx induced by histamine.

Negative modulation of histamine-induced granulocytic differentiation of HL-60 cells

To assess the functional importance of the H\(_2\) receptor-mediated Ca\(^{2+}\) influx, we looked at its effect on cellular differentiation. Granulocytic differentiation of HL-60 promyelocytes results in increased expression of formyl peptide receptors that can be readily monitored by observing the increased effectiveness of fMLP in inducing a rise in [Ca\(^{2+}\)]\(_i\) (34). The responsiveness of HL-60 cells to fMLP was substantially increased when the cells were induced to differentiate by treatment with 1.25% DMSO, 100 μM histamine, or 100 μM dibutyryl cAMP (Fig. 9A). However, in cells simultaneously treated with histamine and SKF 96365, the fMLP

![FIGURE 7. Negation of ATP’s inhibitory effect on the histamine-induced [Ca\(^{2+}\)]\(_i\) rise by GF 109203X. Fura 2-loaded cells preincubated with vehicle (dotted line) or 3 μM GF 109203X (solid line) for 10 min were sequentially treated with 300 μM ATP and 100 μM histamine at a 5-min interval in the presence of extracellular Ca\(^{2+}\). Inset, The experiment was independently conducted more than three times, and the results are presented. Treatment of the cells with H89 (10 μM) had no effect on the ATP-mediated inhibitory effect on the histamine response.](image)

![FIGURE 8. Distribution of PKC isoforms in HL-60 cells. Total lysates (30 μg) extracted from HL-60 cells were separated by 8% SDS-PAGE, transferred to a nitrocellulose membrane, and reacted with isoform-specific Abs. A, HL-60 cells were treated with vehicle alone (Control) or with PMA, ATP (300 μM), or histamine (100 μM) for 10 min. B, HL-60 cells were treated with ATP (300 μM) or PMA (1 nM) for the indicated times (0, 5, 10, and 20 min). The cells were fractionated, and the lysates were used in immunoblot analyses with PKC isoform-specific Abs.](image)
with SKF 96365 also resulted in a decrease in fMLP-stimulated and Methods. The experiments were conducted more than three times and $p$-tiated with DMSO, histamine, and dibutyryl cAMP in the presence (dotted, PMA-induced production of reactive oxygen species in cells differen-

\[ \text{[Ca}^{2+}\text{]} \]

were conducted three times, and the values presented are the mean ± SEM. *, $p < 0.01$ compared with the histamine alone. C, Histamine-mediated cAMP generation. The cells incubated without or with SKF 96365 were stimulated with 100 $\mu$M histamine, and cAMP generation was measured. D, PMA-induced production of reactive oxygen species in cells differen-
tiated with DMSO, histamine, and dibutyryl cAMP in the presence (dotted trace) or the absence (solid trace) of SKF 96365. DCFH-loaded cells were stimulated with 1 $\mu$M PMA, and elevation of fluorescence was detected. none, Control fluorescence. The experiments were conducted three times, and the results were reproducible.

response in eliciting a $[\text{Ca}^{2+}]$, rise was significantly decreased, whereas inclusion of SKF 96365 had no effect on dibutyryl cAMP-mediated differentiation of HL-60 cells. Consistent with the $[\text{Ca}^{2+}]$, response, incubation of the cells with histamine together with SKF 96365 also resulted in a decrease in IMLP-stimulated IP$_3$ generation (Fig. 9B). However, incubation with SKF 96365 had no effect on histamine-mediated cAMP generation (Fig. 9C), suggesting that H$_2$ receptor-induced cAMP signaling to cellular differentiation was not affected by SKF 96365 treatment. The $[\text{Ca}^{2+}]$ effect on histamine-mediated differentiation of HL-60 cells was also detected by monitoring the production of reactive oxygen species. Fig. 9D shows that PMA evoked the generation of superoxide in DMSO-, histamine-, and dibutyryl cAMP-treated cells. However, simultaneous treatment of the cells with histamine and SKF 96365 resulted in a lesser elevation of fluorescence in cells loaded with DCFH-DA, whereas in cells treated with dibutyryl cAMP plus SKF 96365 PMA-mediated superoxide generation was not affected by SKF 96365 inclusion. Therefore, the results indicate that the H$_2$ histamine receptor-mediated granulocytic differentiation could be functionally regulated by the $\text{Ca}^{2+}$ influx through H$_2$ receptor-activated cation channels.

**Discussion**

The present study demonstrates that stimulation of HL-60 promyelocytes with histamine causes an increase in $[\text{Ca}^{2+}]$, through PLC-independent pathways and that the $[\text{Ca}^{2+}]$, rise is negatively regulated by PKC activation in a subtype-selective manner. Furthermore, we found that the H$_2$ receptor-mediated $[\text{Ca}^{2+}]$, rise is involved in the histamine-mediated granulocytic differentiation of these cells. Thus, the aim of this work was to investigate the possible mechanism of PKC activation and the specific regulation of the H$_2$ receptor-mediated signal transduction pathways and its contribution to differentiation.

The data presented in this study show that the effect of histamine on the $[\text{Ca}^{2+}]$, increase in HL-60 cells is mediated via H$_1$ receptors, as had been suggested by several previous studies (11, 12, 35). The H$_2$ receptor antagonist ranitidine completely inhibited the stimulatory effects of histamine on the $[\text{Ca}^{2+}]$, rise, whereas H$_1$ and H$_3$-selective antagonists did not have this effect (see Fig. 1). In addition, the H$_2$ receptor agonist dimaprit increased $[\text{Ca}^{2+}]$, to an extent comparable to that of histamine, whereas the H$_1$ agonist HTMT and the H$_3$ agonist $R$-(-)-$\alpha$-methylhistamine had little effect. The H$_2$ receptor-mediated $[\text{Ca}^{2+}]$, increase resulted from $\text{Ca}^{2+}$ influx from the extracellular medium and not from $\text{Ca}^{2+}$ released from internal stores, because histamine did not induce a $[\text{Ca}^{2+}]$, rise in the absence of extracellular $\text{Ca}^{2+}$ and did not stimulate significant IP$_3$ production (Fig. 1 and Table I). In addition, the experiment to test PKC activation subsequently occurred after activation of the PLC pathway shows that any translocation of PKC isoforms was not detected by the histamine treatment, suggesting that histamine does not apparently activate PLC. Our study also shows that the histamine-mediated increase in $[\text{Ca}^{2+}]$, resulted from the opening of nonselective cation channels, because SKF 96365 treatment could almost completely inhibit the histamine-stimulated $\text{Ca}^{2+}$ entry, $\text{Na}^+$ influx, and membrane depolarization. Nevertheless, we cannot exclude the possibility that PLC activation may also be required to activate the nonselective cation channel. Previously, many studies have shown that nonselective cation channels are simultaneously activated upon stimulation of PLC-coupled receptors such as P2 purinoceptors or fMLP receptors in HL-60 cells, probably via a G-protein-dependent pathway (11, 36), but this mechanism has not yet been studied systemati-
cally. It seems likely that the activation of nonselective cation channels results from H$_2$ receptor-coupled PLC activation, although in amounts below our detection limit. There still remains the possibility that a localized increase in IP$_3$ close to a $\text{Ca}^{2+}$ storage organelle might occur during the H$_2$ histamine receptor activation and lead to the concomitant opening of nonselective cation channels.

It has been known that activation of the H$_2$ histamine receptor elicits both cAMP production and a rise in $[\text{Ca}^{2+}]$, in various cells. This was confirmed in experiments with a cloned H$_2$ receptor in

**FIGURE 9.** Effect of SKF 96365 inclusion on the histamine-mediated granulocytic differentiation of HL-60 cells. The cells were incubated with 100 $\mu$M histamine or 100 $\mu$M dibutyryl cAMP in the presence or the absence of 10 $\mu$M SKF 96365 for 48 h, and then the cells were transferred to fresh medium and further incubated for 24 h, because treatment of the cells with 10 $\mu$M SKF 96365 longer than 48 h caused cell death. Cells were also treated with 1.25% (v/v) DMSO for 72 h to provide controls for the differentiation of HL-60 cells. A, fMLP-mediated $[\text{Ca}^{2+}]$, rise in cells treated with DMSO, histamine, or dibutyryl cAMP in the presence or the absence of SKF 96365. Fura 2-AM-loaded cells were stimulated with 1 $\mu$M fMLP, and the $[\text{Ca}^{2+}]$, level was measured as described in Materials and Methods. The experiments were conducted more than three times and typical $\text{Ca}^{2+}$ transients are presented. B, hemocyanin-loaded IP$_3$ generation in HL-60 cells treated with DMSO, histamine, or dibutyryl cAMP in the presence or the absence of SKF 96365. The cells were stimulated with fMLP (1 $\mu$M) for 20 s, and IP$_3$ generation was measured. The experiments were conducted three times, and the values presented are the mean ± SEM. * , $p < 0.01$ compared with the histamine alone. C, Histamine-mediated cAMP generation. The cells incubated without or with SKF 96365 were stimulated with 100 $\mu$M histamine, and cAMP generation was measured. D, PMA-induced production of reactive oxygen species in cells differen-
tiated with DMSO, histamine, and dibutyryl cAMP in the presence (dotted trace) or the absence (solid trace) of SKF 96365. DCFH-loaded cells were stimulated with 1 $\mu$M PMA, and elevation of fluorescence was detected. none, Control fluorescence. The experiments were conducted three times, and the results were reproducible.
which the direct linkage of a single receptor to both adenyllyl cyclase and PLC via separate GTP-dependent mechanisms was demonstrated (37, 38). Several other reports also showed that a single type of receptor may be associated with more than one G protein and thus lead to multiple intracellular signaling systems, although the mechanism by which different factors may be involved in regulation of the plurality of receptor-mediated signaling remains unknown.

The mechanism involved in the PKC-mediated regulation of the histamine receptor-histamine-mediated channel opening is also involved in the histamine-induced differentiation, PKC plays a role in the cellular differentiation of HL-60 cells. At present, the action mechanism by which the histamine receptor-mediated cation channel activation and membrane depolarization might result in the inhibition of differentiation of HL-60 promyelocytes. Although a physiological role for ATP in the immune system has not yet been firmly established, the high concentration of ATP stored in bone marrow-derived megakaryocytes and its release upon extracellular stimulation suggest a functional relevance for extracellular nucleotides in the physiology of hemopoietic cells (51). Moreover, it has been shown that P2 purinergic receptors are present on various immature bone marrow-derived cells and are involved in the regulation of the proliferation of hemopoietic stem cells by the release of histamine from mast cells (52, 53). Recently, Seifert et al. (11) also reported that the histamine-mediated Ca2+ rise plays a role in the cellular differentiation of HL-60 cells. Therefore, we may yet discover an important physiological relevance in the cross-communication between PLC-coupled receptor and receptor-activated nonselective cation channel opening, displaying distinct biological characteristics. In conclusion, our results show that PMA and a physiological agonist, ATP, can inhibit Ca2+ influx induced by the natural stimulant histamine by selective activation of PKC-δ in HL-60 cells.

Acknowledgments
We gratefully acknowledge Chun-Do Oh and Young-Mee Yoon (Kwangju Institute of Science and Technology) for help with the Western blot analysis of PKC isoforms. We thank G. Hoschek for editing the manuscript. References


