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Histamine H1 receptor antagonist blocks histamine-induced proinflammatory cytokine production through inhibition of Ca^{2+}-dependent protein kinase C, Raf/MEK/ERK and IKK/IκB/NF-κB signal cascades

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Abstract

Histamine H1 receptor (H1R), a therapeutic target for alleviation of acute allergic reaction, may be also involved in mediating inflammatory responses via effects on cytokine production. However, the mechanisms whereby histamine induces cytokine production are poorly defined. In this study, we comprehensively investigated the signaling pathway involved in cytokine expression caused by histamine, using native human epidermal keratinocytes. We confirmed the expression of functional H1R by reverse transcription-polymerase chain reaction (RT-PCR), Western blotting and histamine-induced Ca^{2+} elevation. Histamine induced concentration- and time-dependent production of granulocyte-macrophage-colony stimulating factor (GM-CSF), interleukin (IL)-8 and IL-6, which was completely blocked by olopatadine, an H1 antagonist. Histamine activated the phosphorylation of protein kinase C (PKC), c-Raf, mitogen-activated protein/extracellular signal-regulated kinase kinase (MEK), extracellular signal-regulated kinase (ERK), IκB kinase (IKK), inhibitory κB (IκB)-α and nuclear factor-KB (NF-κB) p65, which was inhibited by Ro-31-8220, a PKC inhibitor. Also, Ro-31-8220 significantly suppressed the expression of these cytokines. BAPTA-AM, an intracellular Ca^{2+} chelator, also reduced PKC phosphorylation and cytokine expression. PD98059, a MEK inhibitor, and BAY 11-8702, an IκB-α inhibitor, reduced ERK and NF-κB cascade activation, respectively, with little effect on PKC phosphorylation. PD98059 preferentially inhibited GM-CSF production whereas BAY 11-8702 prevented IL-8 and IL-6 production. Furthermore, in addition to the above cytokines, histamine stimulated the biosynthesis and/or release of numerous keratinocyte-derived mediators, which are probably regulated by the ERK or NF-κB cascades. Our study suggests that histamine activates Ca^{2+}-dependent PKC isoforms that play crucial roles in the activation of Raf/MEK/ERK and IKK/IκB/NF-κB cascades, leading to up-regulation of cytokine expression. Thus, the anti-inflammatory benefit of H1 antagonists may be in part due to prevention of cytokine production.

Keywords: Histamine H1 receptor; Ca^{2+}; PKC; ERK; NF-κB; Inflammation

Abbreviations: H1R, histamine H1 receptor; RT-PCR, reverse transcription-polymerase chain reaction; GM-CSF, granulocyte-macrophage-colony stimulating factor; IL, interleukin; olopatadine, olopatadine hydrochloride; PKC, protein kinase C; MEK, mitogen-activated protein/extracellular signal-regulated kinase kinase; ERK, extracellular signal-regulated kinase; IKK, IκB kinase; IκB, inhibitory κB; NF-κB, nuclear factor-κB; PTX, pertussis toxin; DAG, diacylglycerol; MAPK, mitogen-activated protein kinase; cetirizine, cetirizine hydrochloride; fexofenadine, fexofenadine hydrochloride; bepotastine, bepotastine besilate; chlorpheniramine, chlorpheniramine maleate; IBMX, 3-isobutyl-1-methylxanthine; PMA, phorbol 12-myristate 13-acetate; BAPTA-AM, 1,2-bis(*o*-aminophenoxy)ethane-*N*,*N*,*N*′,*N*′-tetraacetic acid-acetoxymethyl ester; TNF, tumor necrosis factor; ICAM-1, intercellular adhesion molecule-1; MMP, matrix metalloprotease; GAPDH, glyceraldehyde-3-phosphate dehydrogenase; PAGE, polyacrylamide gel electrophoresis; ELISA, enzyme-linked immunosorbent assay

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1. Introduction

Histamine is a biogenic amine that plays important pathophysiological roles in central and peripheral tissues [1]. Histamine, which is mainly released from stimulated mast cells, contributes to several biological processes that characterize allergic responses [1,2]. The pathophysiological functions of histamine are mediated through four distinct G-protein coupled receptors that are classified as H1, H2, H3 and H4 [1,3]. H1R antagonists are one of the most often used class of drugs to relive the symptoms of allergic diseases, such as allergic rhinitis, atopic dermatitis, psoriasis, allergic conjunctivitis [1,4–8]. The binding of histamine to H1R results in an increase in intracellular calcium, which induces smooth muscle contraction and vascular permeability associated with acute allergic responses [1]. However, the involvement of histamine signaling in late allergic responses is not clear [1]. The late response is characterized by an increase in cytokine synthesis and infiltration of inflammatory cells, followed by acute allergic responses.

The proposal pattern of signal transduction through H1R is as follows. H1R induces activation of phospholipase C via pertussis toxin (PTX) resistant $G_{q/11}$-proteins [1]. Stimulation of phospholipase C generates inositol-1,4,5-trisphosphate and diacylglycerol (DAG) from phosphatidylinositol-4,5-bisphosphate derived from plasma membranes. Inositol-1,4,5-trisphosphate releases Ca^{2+} from intracellular calcium stores, and subsequently activates store-operated calcium channels, whereas DAG activates specific isoforms of PKC [1,9].

It has been reported that PKC might be involved in activation of ERK and NF-κB pathways in response to several stimuli [10–18]. ERK is a well-characterized mitogen-activated protein kinase (MAPK) and is primarily associated with the regulation of proliferation, anti-apoptosis, differentiation and gene expression (e.g. cytokine gene expression) [19,20]. NF-κB, which is located in cytoplasm in an inactivated form due to association with its inhibitors, IκB proteins, plays pivotal roles in inflammatory processes [21]. In response to several stimuli, IκBs are phosphorylated by IKKs, ubiquitinated, and proteolytically degraded thereby allowing NF-κB to translocate to the nucleus, inducing transcription of proinflammatory genes [21]. NF-κB exists as a dimer composed of various combinations of structurally related proteins; p65 is the transcriptional component of the most common form of the NF-κB heterodimer [21,22].

However, the precise signal transduction and understanding of the pathophysiological functions downstream of PKC via H1R remain unresolved. In order to analyze the signaling of H1R, we chose epidermal keratinocytes to perform experiments because these cells not only constitute the major epidermal cell population but also synthesize numerous cytokines and chemokines, which are responsible for the skin immune response [20,23]. Mast cells, which release abundant histamine, infiltrate into the dermis in chronic skin disorders, such as atopic dermatitis and psoriasis [24,25]. Indeed, histamine is reported to promote production of IL-6 and IL-8 from keratinocytes [26]. Moreover, we have recently demonstrated that histamine also simulates GM-CSF production [20]. These observations suggest that histamine participates in inflammation of chronic skin disorders. Moreover, in this study, we demonstrated that H1R, but not H2R, H3R nor H4R, is expressed in human epidermal keratinocytes, suggesting that histamine-induced effects on intracellular signaling pathways are achieved by actions on H1R in these cells.

Knowledge on the signal pathways for cytokine production via H1R is important for an understanding of the precise pharmacological action of present clinically available H1 antagonists, such as olopatadine hydrochloride (olopatadine) [4], cetirizine hydrochloride (cetirizine) [5], fexofenadine hydrochloride (fexofenadine) [6] and loratadine [7]. Also, the insight in pharmacological action may help the development of H1 antagonists that treat allergic disorders more effectively than the existing drugs. In this study, using human epidermal keratinocytes, we examined the effects of H1R on acute (e.g. calcium mobilization) and late intracellular responses (e.g. proinflammatory cytokine synthesis) and focused on exploration of the mechanism of signal transduction pathways leading to the induction of cytokine expression.

2. Materials and methods

2.1. Reagents

Olopatadine, cetirizine, fexofenadine and bepotastine besilate (bepotastine) were synthesized in our laboratories. The following materials were purchased from the indicated commercial source: loratadine (Pharm Chemical Shanghai Lansheng); chlorpheniramine maleate (chlorpheniramine) (Sigma–Aldrich); PD98059 (Cell Signaling Technology); Ro-31-8220 (Calbiochem); Gö 6976 (Calbiochem); histamine (Sigma); cimetidine (Sigma); 3-isobutyl-1-methylxanthine (IBMX), (Sigma); phorbol 12-myristate 13-acetate (PMA) (Sigma); 1,2-bis(*o*-aminophenoxy)ethane-*N*,*N*,*N*′,*N*′-tetraacetic acid-acetoxymethyl ester (BAPTA-AM) (Biomol Research Laboratories); BAY 11-7082 (Biomol Research Laboratories); clobenpropit (Tocris); PTX (List Biological Laboratories); recombinant human tumor necrosis factor (TNF)-α (Strathmann Biotec Ag). All chemical reagents except PTX and TNF-α were dissolved in DMSO at a concentration of 0.1 mol/L and stored at −20 °C. The final concentration of DMSO in the assay system described below was 0.1% (v/v). The concentrations of DMSO did not affect the physiological responses of cultured keratinocytes, such as calcium mobilization,

protein phosphorylation and proinflammatory cytokine expression. At the concentrations used in this study, none of these reagents exhibited significantly cytotoxicity to cultured keratinocytes. PTX and TNF-α were dissolved in the supplied medium just before assay.

2.1.1. Cell culture

Primary human epidermal keratinocytes were purchased from Bio Whittaker and grown in KGM-2 with the addition of supplements containing bovine pituitary extract, human epidermal growth factor, insulin, epinephrine, transferrin, hydrocortisone, gentamycin and amphotericin B according to the manufacturer's instruction (Clonetics). Cultures were maintained under 5% CO_2 at 37 °C in tissue culture flasks. When cells were cultured at confluent state, the cells were rapidly washed with DMEM (Nissui) and resuspended in KGM-2 medium without hydrocortisone on the day before assay. Cells between passages 3 and 6 were used.

2.1.2. Semi-quantification of RT-PCR

Confluent cells in 6-well plates were treated with the indicated concentrations of compounds for 1 h in KGM-2 without hydrocortisone, and then stimulated with 30 μmol/L histamine for 3 h. After the incubation, the cells were treated with ice-cold PBS. Total RNA was isolated according to the manufacturer's instruction (RNeasy). First strand-cDNA synthesis was performed from 1.5 μg of total RNA in 20-μL volumes with oligo (dT) priming using the Superscript First-strand Synthesis System (Invitrogen). PCR was performed from the generated cDNA using ExTaq polymerase (Takara). Based on previous reports [3,21,27], the following primer pairs (including PCR product sizes in parentheses) were synthesized by Invitrogen: H1R, 5′-GAC TGT GTA GCC GTC AAC CGG A-3′ and 5′-TGA AGG CTG CCA TGA TAA AAC C-3′ (318 bp); H2R, 5′-TCG TGT CCT TGG CTA TCA C-3′ and 5′-CTT TGC TGG TCT CGT TCC T-3′ (332 bp); H3R, 5′-TCA GCT ACG ACC GCT TCC TGT CGG TCA C-3′ and 5′-TTG AGT GAG CGC GGC CTC TCA GTG CCC C-3′ (171 bp); H4R, 5′-GAA TTG TCT GGC TGG ATT AAT TTG CTA ATT TG-3′ and 5′-AAG AAT GAT GTG ATG GCA AGG ATG TAC C-3′ (596 bp); GM-CSF, 5′-GGC GTC TCC TGA ACC TGA GTA G-3′ and 5′-GTC GGC TCC TGG AGG TCA AA-3′ (91 bp); IL-8, 5′-CTA GCC AGG ATC CAC AAG TCC-3′ and 5′-AGC ACT ACC AAC ACA GCT GGC-3′ (503 bp); IL-6, 5′-TGA CAA CTC ATC TCA TTC TGC-3′ and 5′-AAG TTA GCC ATT TAT TTG AGG TAA GC-3′ (411 bp); TNF-α, 5′-AAG ACC TCA CCT AGA AAT TGA CAC-3′ and 5′-TAG GCC GAT TAC AGA CAC AAC-3′ (419 bp); intercellular adhesion molecule-1 (ICAM-1), 5′-TAT GGC AAC GAC TCC TTC T-3′ and 5′-CAT TCA GCG TCA CCT TGG-3′ (238 bp); matrix metalloprotease (MMP)-1, 5′-ATT TCT CCG CTT TTC AAC TT-3′ and 5′-ATG CAC AGC TTT CCT CCA CT-3′ (167 bp); MMP-3, 5′-ATG AAG AGT CTT CCA ATC CT-3′ and 5′-GTC CTT TCT CCT AAC AAA CT-3′ (167 bp); MMP-9, 5′-CAT CTT CCA AGG CCA ATC CTA CTC-3′ and 5′-GAT GCC ATT CAC GTC GTC CTT ATG C-3′ (441 bp); MMP-13, 5′-TGC TGC ATT CTC CTT CAG GA-3′ and 5′-ATG CAT CCA GGG GTC CTG GC-3′ (184 bp); glyceraldehyde-3-phosphate dehydrogenase (GAPDH), 5′-CCC ATC ACC ATC TTC CAG GAG-3′ and 5′-TTC ACC ACC TTC TTG ATG TCA-3′ (572 bp). The denaturation, annealing, and extension conditions for H1R, H2R, H3R, H4R, GM-CSF, IL-8, ICAM-1, MMP-1, MMP-3, MMP-9, MMP-13 and GAPDH were 94 °C for 60 s, 60 °C for 45 s and 72 °C for 45 s, respectively, and that for TNF-α and IL-6 and were 94 °C for 30 s, 65 °C for 30 s and 72 °C for 30 s, respectively. The PCR cycle conditions were 26, 36, 36, 36, 28, 34, 23, 32, 28, 23, 26, 26, 32 and 20 cycles for H1R, H2R, H3R, H4R, GM-CSF, IL-6, IL-8, TNF-α, ICAM-1, MMP-1, MMP-3, MMP-9, MMP-13 and GAPDH, respectively. These conditions are the optimal settings for each gene. PCR reaction was performed with a GeneAmp PCR system 9700 (Applied Biosystems). The PCR products were electrophoresed in 2% (w/v) agarose gels and stained with SYBR Green I (Molecular Probes). The detection and densitometric analysis of each band were performed with a Lumino Image Analyzer LAS-1000 plus (Fuji Photo Film).

2.1.3. Western blot analysis

Confluent cells in 6-well plates were pre-incubated with KGM-2 without all supplements for 12 h. The cells were treated with the indicated concentrations of compounds for 1 h, and then stimulated with 30 μmol/L histamine for 7.5 min. After incubation, the cells were washed with ice-cold PBS. The cells were lysed with ice-cold lysis buffer (150 mmol/L NaCl, 1 mmol/L Na_2EDTA, 1 mmol/L EGTA, 1% (v/v) Triton X-100, 2.5 mmol/L sodium pyrophosphate, 1 mmol/L β-glycerophosphate, 1 mmol/L Na_3VO_4, 1 μg/mL leupeptin, 10 mmol/L NaF, 1 mmol/L phenylmethylsulfonyl fluoride, 20 mmol/L Tris–HCl, pH 7.5) for 30 min on ice. Insoluble materials were removed by centrifugation and the supernatant was boiled with Laemmli's sample buffer containing dithiothreitol for 3 min. Equivalent amounts of protein in each sample (5–10 μg/lane) were separated by 7.5 or 10% SDS–polyacrylamide gel electrophoresis (PAGE) and blotted onto a PVDF membrane (Immobilon, Millipore). Proteins were detected using an immunoblotting technique with the following antibodies: anti-H1R antibody (Chemicon); anti-actin antibody (Santa Cruz Biotechnology); anti-phospho-PKC (pan) antibody (which only detects endogenous phosphorylation of PKCα, βI, βII, δ, ε and η isoforms at a carboxy-terminal residue homologous to serine 660 of PKCβII, Cell Signaling Technology); anti-p44/42 MAP kinase (ERK1/2) antibody (Cell Signaling Technology); anti-phospho-p44/42 MAP kinase (Thr202/Tyr204) antibody (Cell Signaling Technology); anti-phospho-IKKα/β

(Ser176/180) antibody (Cell Signaling Technology); anti-phospho-IκB-α (Ser32) antibody (Cell Signaling Technology); anti-phospho-c-Raf (Ser338) antibody (Cell Signaling Technology); anti-phospho-MEK1/2 (Ser217/221) antibody (Cell Signaling Technology); anti-phospho-NF-κB p65 (Ser536) antibody (Cell Signaling Technology). Horseradish peroxidase-linked whole antibody (Amersham Bioscience) was used as a secondary antibody. The antibodies were detected with Super Signal West Pico Chemiluminescent Substrate (Pierce) and analyzed using a Lumino Image Analyzer LAS-1000 Plus (Fuji Photo Film).

2.1.4. Measurement of intracellular Ca^{2+}

Sub-confluent cells in a 35-mm glass-based dish were washed with Hanks' solution containing 0.1% (w/v) BSA. The fura-2 loading was achieved by exposing the cells to 5 μmol/L fura-2-AM (Dojindo) in the dark for 30 min. The fura-2 loaded cells were immediately placed on the Ca^{2+} imaging equipment Arugus-50/Ca system (Hamamatsu Photonics). The cells were treated with olopatadine for 5 min, and then stimulated with 30 μmol/L histamine by surface perfusion. The fluorescence ratio of intracellular fura-2 was alternately monitored at the excitation wavelengths of 340 and 380 nm with an emission wavelength of 510 nm.

2.1.5. Measurement of cAMP

Confluent cells in 24-well plates were stimulated with 30 μmol/L histamine in the presence of 1 mmol/L IBMX for 45 min. The termination of reactions and cAMP measurements were carried out as described in the manual of cAMP kit (CIS bio international).

2.1.6. Immunocytochemistry

Confluent cells in 6-well plates were pre-incubated with KGM-2 without all supplements for 8 h. The cells were treated with or without 1 μmol/L olopatadine for 1 h, and then stimulated with 30 μmol/L histamine for 7.5 min. After incubation, the cells were washed with ice-cold PBS and fixed with 3.7% (w/v) formaldehyde on ice for 15 min. The fixed cells were permeabilized by treatment

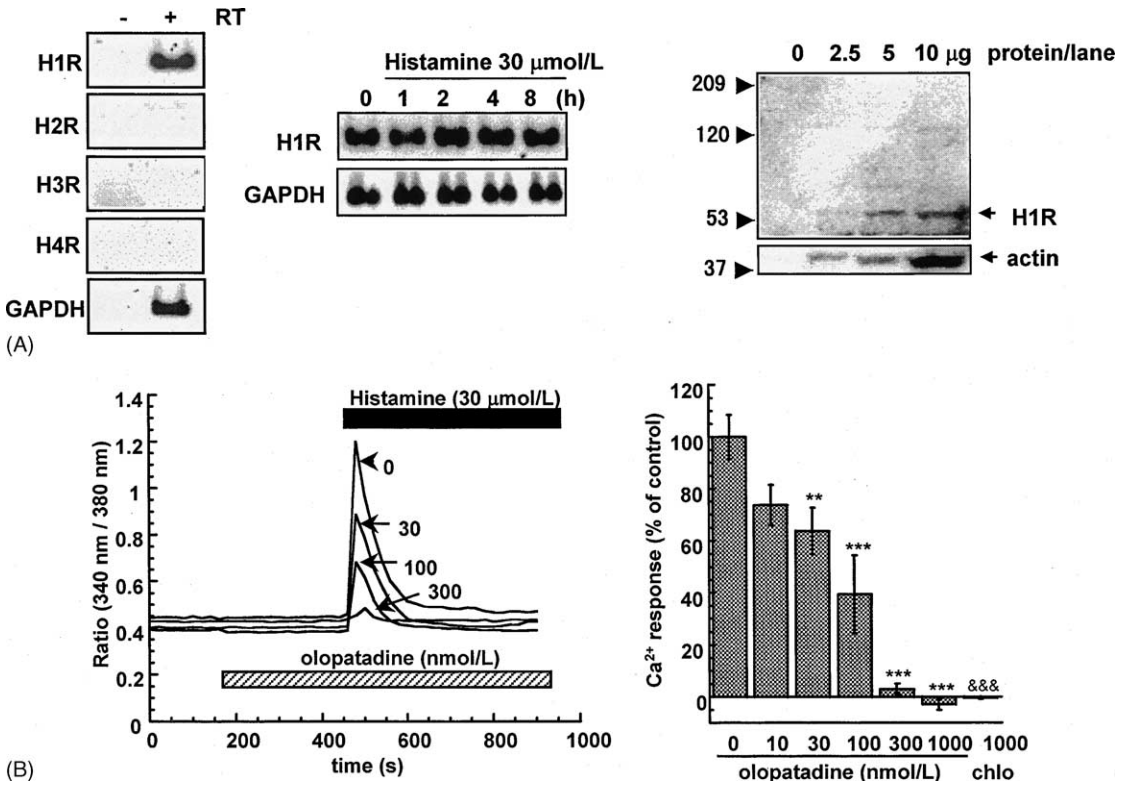

Fig. 1. Functional expression of histamine H1 receptor (H1R) in human epidermal keratinocytes. (A) Expression of H1R, H2R, H3R and H4R mRNA was analyzed by RT-PCR with specific primer sets (left panels). Reverse transcriptase (RT) negative samples were included as a control for the genomic contamination. Keratinocytes were stimulated with 30 μmol/L histamine for indicated time. Time course of H1R mRNA levels was analyzed by RT-PCR (center panels). Total cell lysate was analyzed by Western blotting with anti-H1R and anti-actin antibodies (right panels). The upper arrow shows apparent molecular mass 57 kDa for H1R. (B) Histamine-stimulated intracellular Ca^{2+} mobilization in keratinocytes was studied using the fluorescent Ca^{2+} probe fura-2-AM. Fura-2-loaded keratinocytes were pretreated with indicated concentrations of olopatadine, a H1R antagonist, for 5 min prior to stimulation with 30 μmol/L histamine. The fluorescence ratio (340 nm/380 nm) of fura-2 was measured (left panel). The results of Ca^{2+} response are shown as % of the histamine-induced response without drugs (right panel). Chlorpheniramine (Chlo) is another H1R antagonist. Values represent the mean of 27–30 cell fluorescent images ±S.E. in the same experiment. $^{*}P < 0.05$, $^{**}P < 0.01$, $^{***}P < 0.001$ compared with the control group (Dunnett). $^{\&\&\&}P < 0.001$ compared with the control group (Aspin–Welch).

with PBS containing 0.1% (w/v) Triton X-100 and blocked with 5.5% (w/v) goat serum at room temperature for 1 h. A primary antibody to detect phospho-p44/42 MAP kinase (Thr202/Tyr204) antibody (Cell Signaling Technology) or phospho-NF-κB p65 (Ser536) (Cell Signaling Technology) was incubated at 4 °C overnight. Subsequently, addition of secondary antibody (Vectastatin ABC kit, Vector Laboratories) and staining (DAB substrate kit, Vector Laboratories) were carried out according to the manufacturer's instructions.

2.1.7. Measurement of mediator production

Confluent cells in 24-well plates were treated with the indicated concentrations of agents for 1 h in KGM-2 without hydrocortisone, and then stimulated with 30 μmol/L histamine for 24 h. GM-CSF, IL-8, IL-6 or MMP-1 levels in the supernatant were determined using commercially available enzyme-linked immunosorbent assay (ELISA) according to the manufacturer's instructions. The ELISA kits used were human GM-CSF AN'ALYZA (Genzyme/Teche), human IL-8 ELISA Kit (BioSource International), Endogen Human IL-6 (PIERCE) and human MMP-1 Biotrak ELISA system (Amersham Bioscience).

2.1.8. Immune complex in vitro kinase assay

Confluent cells in 100-mm dishes were pre-incubated with KGM-2 without all supplements for 12 h. The cells were treated with the indicated concentrations of compounds for 1 h, and then stimulated with 30 μmol/L histamine for 7.5 min. After incubation, the cells were washed with ice-cold PBS. The cells were lysed with ice-cold lysis buffer for 30 min on ice. Insoluble materials were removed by centrifugation. The supernatant was incubated with anti-phospho-p44/42 MAP kinase (Thr202/Tyr204) antibody (Cell Signaling Technology) overnight at 4 °C on a rocking platform. Twenty microliters of protein G sepharose (Amersham Bioscience) slurry was added and rocking continued for another 5 h. The immunoprecipitates were washed twice in lysis buffer and twice in kinase assay buffer (10 mmol/L $MgCl_2$, 2 mmol/L dithiothreitol, 5 mmol/L β-glycerophosphate, 0.1 mmol/L Na_3VO_4, 25 mmol/L Tris–HCl, pH 7.5). The immune complexes were incubated with 1 μg Elk-1 fusion protein (Cell Signaling Technology) in the kinase assay buffer containing 200 μmol/L ATP in a final volume of 40 μL for 30 min at 30 °C with gentle agitation. Reactions were stopped by the addition of 20 μL of 3 [X] Laemmli's sample buffer, followed by boiling of the samples and SDS–PAGE. Phosphorylation of Elk-1 was analyzed by Western blotting technique using anti-phospho-Elk-1 (Ser383) antibody (Cell Signaling Technology).

2.1.9. Luciferase reporter assay

Transient transfections of keratinocytes were performed using Lipofectamine 2000 (Invitrogen), according to the

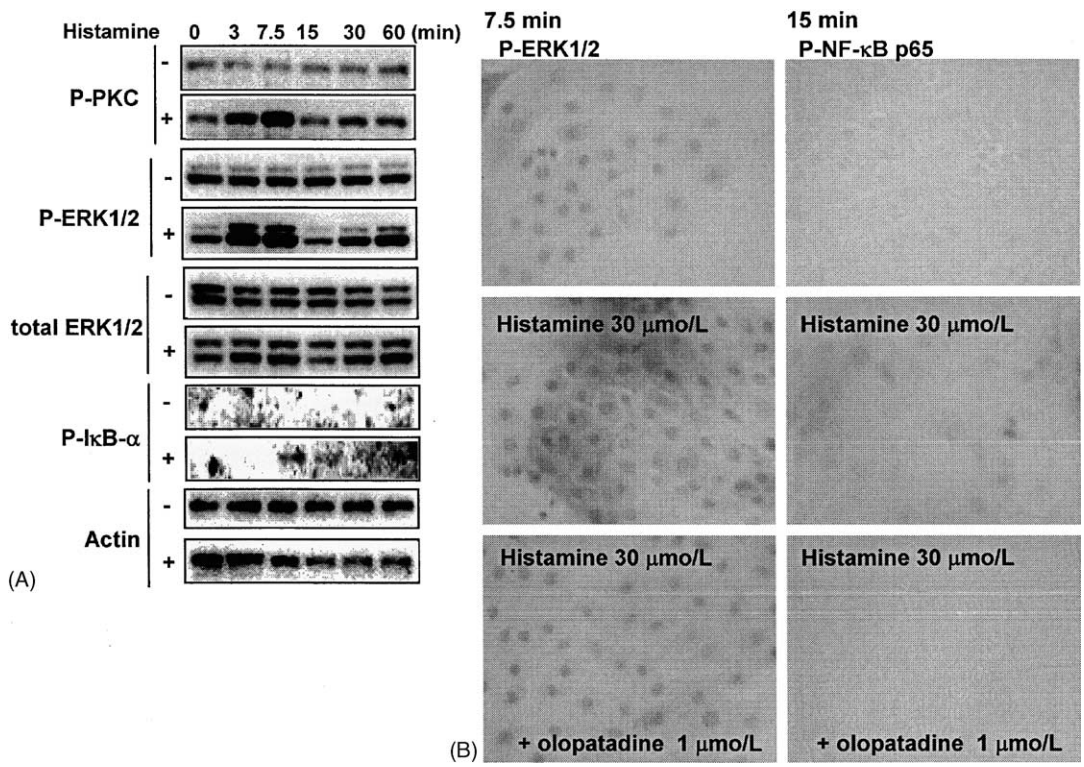

Fig. 2. Histamine-induced PKC, ERK and NF-κB activation. (A) Time course of histamine-induced phosphorylation levels of PKC, ERK and IκB-α was analyzed by Western blotting using phospho-protein specific antibodies. (B) Histamine-stimulated keratinocytes were subjected to immunocytochemistry with anti-phospho ERK or phospho NF-κB p65 antibody. Keratinocytes were pretreated with 1 μmol/L olopatadine for 1 h prior to activation with 30 μmol/L histamine for 7.5 or 15 min to detect phospho-ERK or phospho-NF-κB p65, respectively. One representative result of several images is shown.

manufacturer's instructions. Eighty to ninety percent of Confluent keratinocytes in 96-well plates were transfected with the pNF-κB reporter plasmid encoding the triple consensus NF-κB binding sites (gggaaattcc) upstream of the luciferase gene for 5 h. After incubation, cultures were maintained in the normal growth medium KGM-2 for 24 h. The transfected cells were treated with the indicated concentrations of compounds for 1 h, and then stimulated with 100 μmol/L histamine for 6 h. Luciferase activity was detected with the Steady-Glo luciferase assay system (Promega) using Microplate Scintillation & Luminescence Counter TopCount NXT (Packard, Groningen, The Netherlands).

2.2. Cell surface ELISA for determination of ICAM-1

Confluent cells were cultured in 96-well plates. The cells were treated with 0.1 μmol/L olopatadine or 3 μmol/L chlorpheniramine for 1 h, and then stimulated with 30 μmol/L histamine for 24 h. After incubation, the medium was removed and the cells were fixed with 3.7% formaldehyde on ice for 30 min. The plates were then washed with ice-cold PBS and blocked with 1% (w/v) BSA on ice for 1 h. A primary monoclonal antibody to ICAM-1 (clone 6.5B5, Cymbus Biotechnology Ltd.) was added at 1:100 dilution, and the plates were incubated on ice for 1 h. The plates were then washed and incubated with

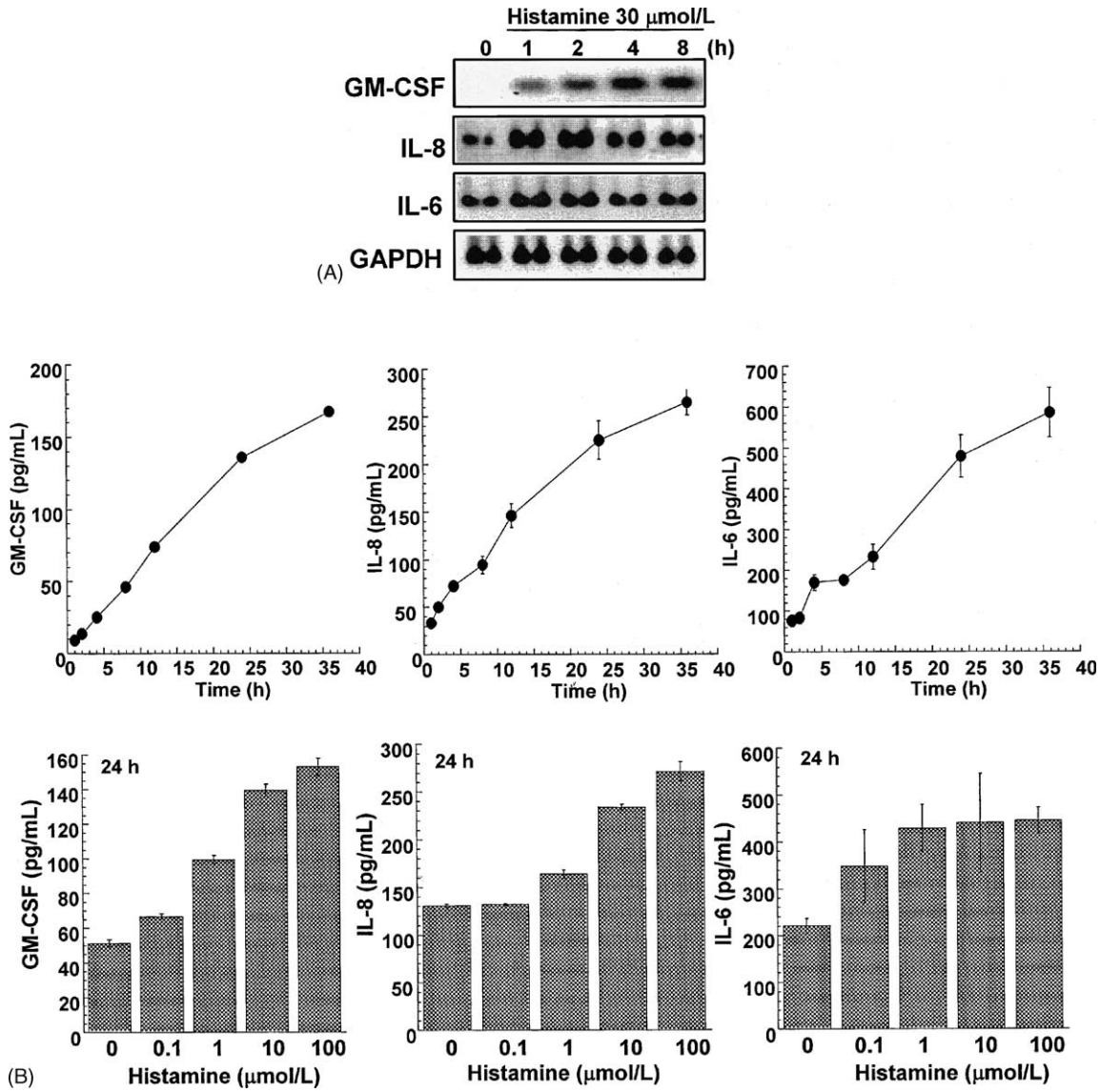

Fig. 3. Histamine-induced expression and production of GM-CSF, IL-8 and IL-6 in human epidermal keratinocytes. (A) Keratinocytes were stimulated with 30 μmol/L histamine for indicated times. Time course of mRNA levels was analyzed by RT-PCR. One representative result of three independent experiments is shown. (B) Keratinocytes were stimulated with indicated concentrations of histamine. Protein released into the supernatant was measured by ELISA. Time course and histamine-concentration response of production were shown in upper panels and lower panels, respectively. Values represent means ± S.E. of three determinations.

horseradish peroxidase-conjugated anti-mouse secondary antibody (Amersham Bioscience) at 1:1000 dilution on ice for 1 h. After the incubation, the plates were washed again. The antibodies were detected with Super Signal West Pico Chemiluminescent Substrate (Pierce) using Microplate Scintillation & Luminescence Counter TopCount NXT (Packard).

2.2.1. Statistical analysis

Statistical analysis was performed using the Dunnett, Aspin–Welch or Student's test. P-values <0.05 were considered significant.

3. Results

3.1. Functional expression of H1R on human epidermal keratinocytes

Since the existence of H1R molecules on keratinocytes is supposed [20,26], the expression of mRNA and protein of H1R was confirmed in cultured human epidermal keratinocytes whereas mRNAs of H2R, H3R and H4R were not (Fig. 1A). Stimulation of cells with histamine did not affect H1R expression (Fig. 1A). To evaluate the functionality of H1R in keratinocytes, whether or not

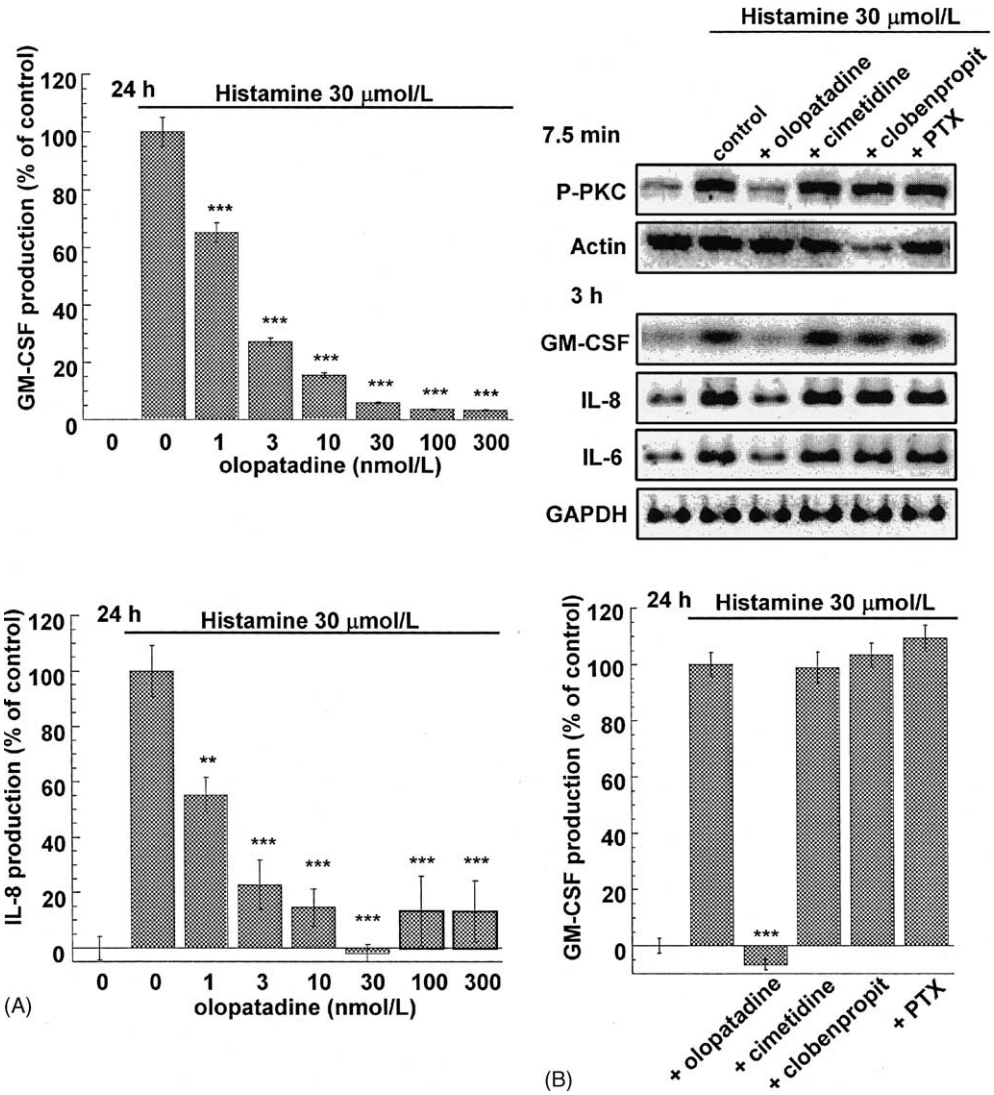

Fig. 4. Effects of histamine receptor antagonists or PTX on cytokine production and PKC phosphorylation in human epidermal keratinocytes. (A) Effects of olopatadine, an H1R antagonist, on GM-CSF and IL-8 production. Keratinocytes were pretreated with indicated concentrations of olopatadine for 1 h prior to stimulation with 30 μmol/L histamine for 24 h. Protein released into the supernatant was measured by ELISA. Values represent means ± S.E. of six determinations of two separate experiments. $^{**}P < 0.01$, $^{***}P < 0.001$ compared with the control group (Dunnett). (B) Keratinocyte were pre-incubated with 1 μmol/L olopatadine (H1R antagonist), 10 μmol/L cimietidine (H2R antagonist) and 10 μmol/L clobenpropit (H3R antagonist) for 1 h, or 100 ng/mL PTX for 18 h, and then stimulated with 30 μmol/L histamine for 7.5 min, 3 h or 24 h to determine phosphorylation of PKC, cytokine mRNA expression or GM-CSF production, respectively. Phosphorylation levels of PKC were analyzed by Western blotting using phospho-PKC specific antibody (upper panels). GM-CSF, IL-8 and IL-6 mRNA levels were analyzed by RT-PCR (middle panels). One representative result of two independent experiments is shown. GM-CSF production was measured by ELISA (lower panels). The results are shown as % of control. Values represent means ± S.E. of three determinations. $^{***}P < 0.001$ compared with the control group (Student's t).

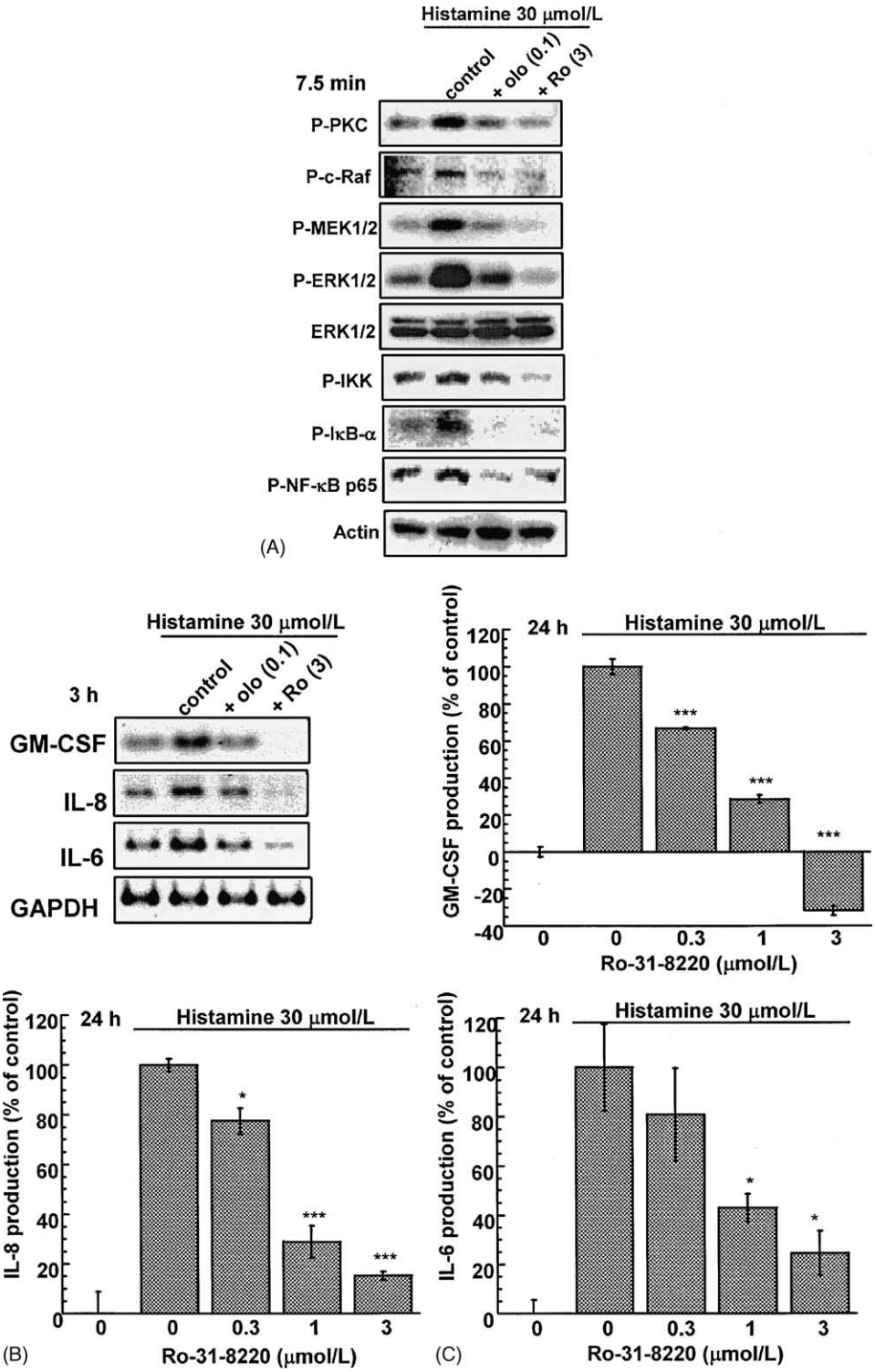

Fig. 5. Effects of Ro-31-8220, a specific PKC inhibitor, on protein phosphorylation and cytokine production in human epidermal keratinocytes. Keratinocytes were pretreated with indicated concentrations of Ro-31-8220 (Ro) and 100 nmol/L olopatadine (olo) for 1 h prior to stimulation with 30 μmol/L histamine for 7.5 min, 3 h or 24 h to determine phosphorylation of proteins, mRNA expression or production of cytokines, respectively. (A) Phosphorylation of PKC, c-Raf, MEK1/2, ERK1/2, IKK, IκB-α and NF-κB p65 was detected by Western blotting using phospho-protein specific antibodies. (B) GM-CSF, IL-8 and IL-6 mRNA levels were analyzed by RT-PCR. One representative result of two independent experiments is shown. (C) Production of GM-CSF, IL-8 and IL-6 were measured by ELISA. The results are shown as % of control. Values represent means ± S.E. of three determinations. $^{*}P<0.05$, $^{**}P<0.01$, $^{***}P<0.001$ compared with the control group (Dunnett).

histamine increases in intracellular calcium was examined. Histamine caused a temporal elevation of intracellular calcium, and this calcium mobilization was inhibited by treatment with olopatadine [4,28] in a concentration-dependent manner (Fig. 1B). Chlorpheniramine, a classical H1 antagonist, also prevented the calcium mobilization (Fig. 1B). Histamine did not increase intracellular cAMP (data not shown), suggesting that the histamine receptor is not coupled to Gs protein for activation of adenylate cyclase like H2R.

Moreover, we confirmed histamine-induced signal transduction by detection of protein phosphorylation in keratinocytes. Histamine transiently stimulated phosphorylation of PKC, ERK and IκB-α (Fig. 2A). Also, olopatadine, which was used as a positive control for H1 antagonist, significantly inhibited histamine-evoked phosphorylation of these proteins (see Figs. 4B, 5A, 6A, 7A and 8A). Chlorpheniramine also prevented histamine-induced protein phosphorylation (data not shown). Phospho-ERK and phospho-NF-κB p65 in cytoplasm and nucleus, respectively, were detected by immunocytochemical analysis of keratinocytes stimulated with histamine (Fig. 2B). Furthermore, the H1 antagonist olopatadine also completely inhibited the phosphorylation (Fig. 2B).

3.2. Effects of histamine on mRNA expression and protein production of GM-CSF, IL-8 and IL-6

Keratinocytes spontaneously released cytokines (Fig. 3B), which may be dependent of some components included in the control medium, such as growth factors. Effects of histamine on mRNA and protein production of GM-CSF, IL-8 and IL-6 [29–31] were verified. Treatment with histamine concentration-dependently enhanced mRNA expression and production of these cytokines (Fig. 3A and B).

3.3. Effects of antihistamines or PTX-treatment on histamine-induced cytokine production and PKC activation

Olopatadine concentration-dependently suppressed histamine-induced production of GM-CSF and IL-8 (Fig. 4A). Other H1 antagonists (cetirizine [5], fexofenadine [6], loratadine [7], bepotastine [8] and chlorpheniramine) also inhibited histamine-enhanced GM-CSF and IL-8; IC_{50} values for each drug are shown in Table 1. In contrary to the H1 antagonists, an H2 antagonist (cimetidine [1]) and an H3 antagonist (clobenpropit [1]) did not suppress histamine-induced GM-CSF expression and production (Fig. 4A).

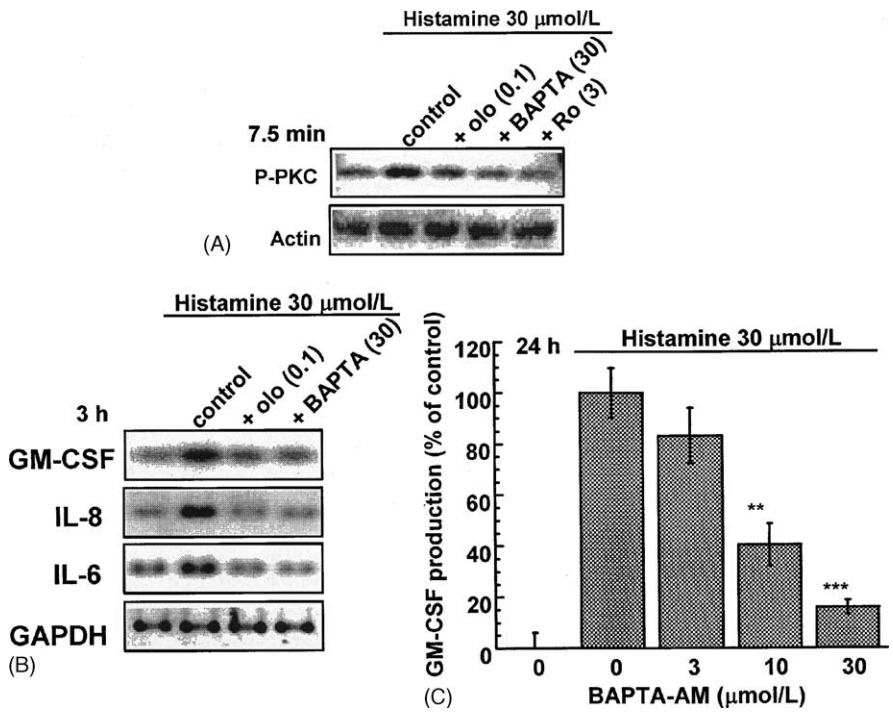

Fig. 6. Effects of BAPTA-AM, an intracellular calcium chelator, on PKC phosphorylation and cytokine production in human epidermal keratinocytes. Keratinocytes were pretreated with indicated concentrations of BAPTA-AM (BAPTA), Ro-31-8220 (Ro) and 100 nmol/L olopatadine (olo) for 1 h prior to stimulation with 30 μmol/L histamine for 7.5 min, 3 h or 24 h to determine phosphorylation of PKC, mRNA expression or production of cytokines, respectively. (A) Phosphorylation of PKC was detected by Western blotting using phospho-protein specific antibodies. (B) GM-CSF, IL-8 and IL-6 mRNA levels were analyzed by RT-PCR. One representative result of two independent experiments is shown. (C) GM-CSF production was measured by ELISA. The results are shown as % of control. Values represent means ± S.E. of three determinations. $^{**}P < 0.01$, $^{***}P < 0.001$ compared with the control group (Dunnett).

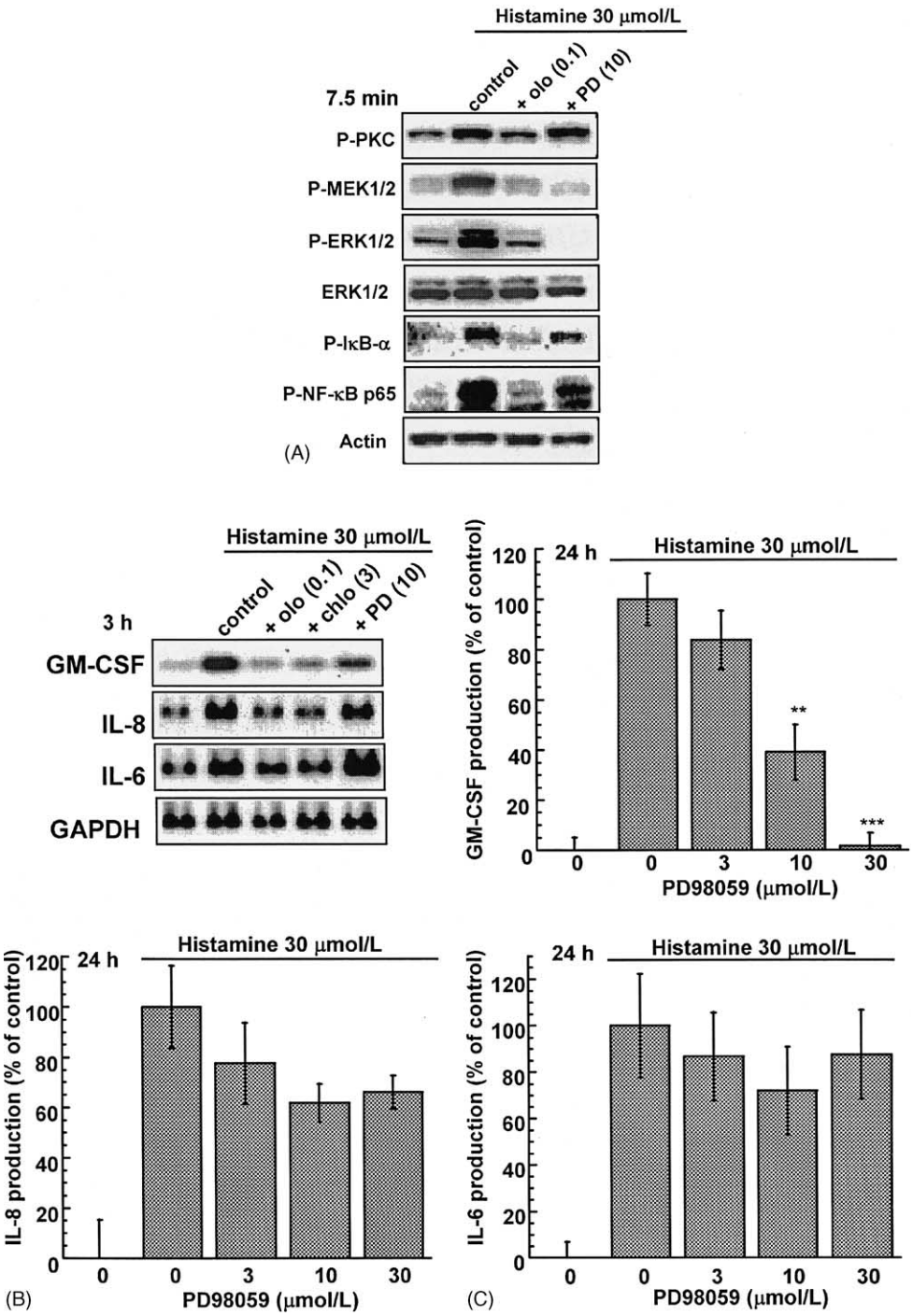

Fig. 7. Effects of PD98059, a specific MEK-1 inhibitor, on protein phosphorylation and cytokine production in human epidermal keratinocytes. Keratinocytes were pretreated with indicated concentrations of PD98059 (PD), 100 nmol/L olopatadine (olo) and 3 μmol/L chlorpheniramine (chlo) for 1 h prior to stimulation with 30 μmol/L histamine for 7.5 min, 3 h or 24 h to determine phosphorylation of proteins, mRNA expression or production of cytokines, respectively. (A) Phosphorylation of PKC, MEK1/2, ERK1/2 IκB-α and NF-κB p65 was detected by Western blotting using phospho-protein specific antibodies. (B) GM-CSF, IL-8 and IL-6 mRNA levels were analyzed by RT-PCR. One representative result of two independent experiments is shown. (C) Production of GM-CSF, IL-8 and IL-6 were measured by ELISA. The results are shown as % of control. Values represent means ± S.E. of three determinations. $^{**}P < 0.01$, $^{***}P < 0.001$ compared with the control group (Dunnett).

Phosphorylation of PKC was inhibited by the H1 antagonist, but not by the H2 antagonist or the H3 antagonist (Fig. 4B), in accordance with the biological responses. Pretreatment with PTX had no effect on histamine-induced PKC phosphorylation and cytokine production (Fig. 4B), suggesting that H1R is coupled to PTX-insensitive G proteins.

3.4. Roles of PKC signaling in histamine-induced cytokine production

In order to characterize the function of PKC activated by histamine in keratinocytes, effects of Ro-31-8220 [32], a specific inhibitor of PKC, on histamine-induced cytokine

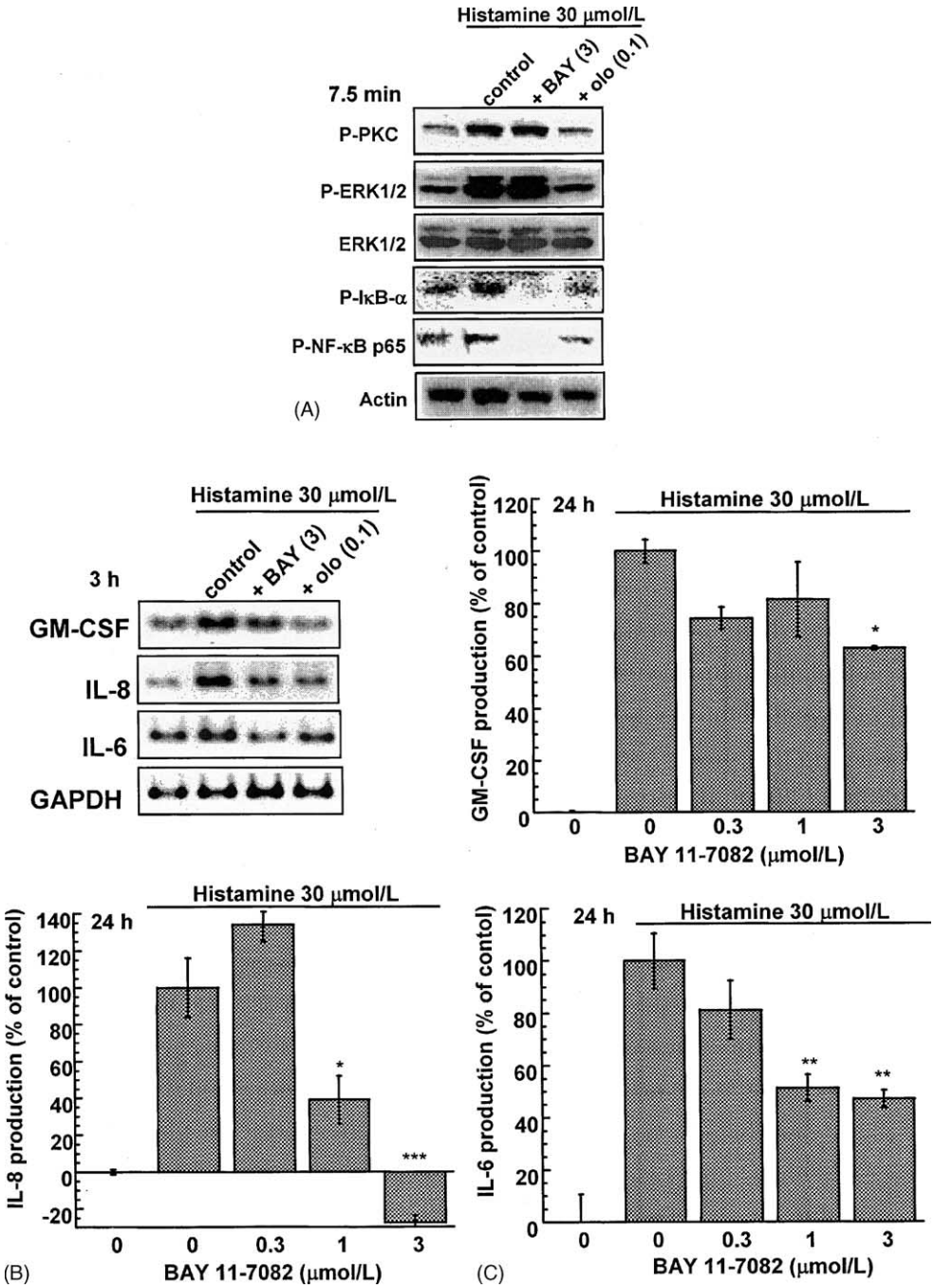

Fig. 8. Effects of BAY 11-7082, an inhibitor for IκB-α phosphorylation, on protein phosphorylation and cytokine production in human epidermal keratinocytes. Keratinocytes were pretreated with indicated concentrations of BAY 11-7082 (BAY), 100 nmol/L olopatadine (olo) for 1 h prior to stimulation with 30 μmol/L histamine for 7.5 min, 3 h or 24 h to determine phosphorylation of proteins, mRNA expression or production of cytokines, respectively. (A) Phosphorylation of PKC, ERK1/2, IκB-α and NF-κB p65 was detected by Western blotting using phospho-protein specific antibodies. The indicated phospho-protein levels in each sample were analyzed. (B) GM-CSF, IL-8 and IL-6 mRNA levels were analyzed by RT-PCR. One representative result of two independent experiments is shown. (C) Production of GM-CSF, IL-8 and IL-6 were measured by ELISA. The results are shown as % of control. Values represent means ± S.E. of three determinations. $^{*}P < 0.05$, $^{**}P < 0.01$, $^{***}P < 0.001$ compared with the control group (Dunnett).

expression and production were examined. Ro-31-8220 markedly suppressed histamine-induced phosphorylation of PKC, c-Raf, MEK1/2, ERK1/2, IKK, IκB-α and NF-κB p65 (Fig. 5A). Also, Ro-31-8220 inhibited histamine-induced mRNA expression and production of GM-CSF, IL-8 and IL-6 (Fig. 5B and C), indicating that PKC activation is essential for histamine-enhanced cytokine production, and that ERK and NF-κB cascades may play parts in the PKC signaling.

The PKC family consists of calcium-dependent conventional and calcium-independent isoforms [9]. BAPTA-AM, an intracellular calcium chelator, reduced histamine-

Table 1
Effects of clinically available H1 antagonists on cytokine production from epidermal keratinocytes

Drug	IC_{50} (95% confidence limits, nmol/L)	
	GM-CSF	IL-8
Olopatadine	1.4 (0.97–2.0)	0.40 (0.09–0.96)
Cetirizine	27 (20–35)	33 (25–43)
Loratadine	25 (18–37)	160 (78–470)
Fexofenadine	110 (79–140)	75 (57–100)
Bepostaine	36 (26–50)	55 (41–76)
Chlorpheniramine	360 (290–460)	510 (420–660)

Keratinocytes were pretreated with indicated drugs for 1 h prior to stimulation with 30 μmol/L histamine for 24 h. Production of GM-CSF and IL-8 were measured by ELISA. IC_{50} value was calculated with six determinations of two separate experiments.

induced PKC phosphorylation and cytokine expression (Fig. 6), suggesting that H1R connects with calcium-dependent PKC isoforms in keratinocytes.

3.5. Involvement of ERK cascade in histamine-induced cytokine production

To explore the involvement of ERK cascade, effects of PD98059 [33], a specific inhibitor of MEK1, on histamine-induced cytokine expression and production were investigated. PD98059 inhibited histamine-induced ERK phosphorylation without having influence on PKC phosphorylation (Fig. 7A). Moreover, PD98059 partially blocked histamine-induced phosphorylation of IκB-α and NF-κB p65. PD98059 considerably suppressed histamine-induced GM-CSF mRNA expression but it did not inhibit IL-8 and IL-6 mRNA expression (Fig. 7B). The suppression of histamine-enhanced production of these cytokines by PD98059 was similar to that of mRNA induction (Fig. 7C).

3.6. Involvement of NF-κB cascade in histamine-induced cytokine production

To investigate the contribution of NF-κB cascade, effects of BAY 11-7082 [34,35], a specific inhibitor for IκB-α, phosphorylation, on histamine-induced cytokine expression and production were examined. BAY 11-7082 inhibited histamine-induced NF-κB p65 phosphorylation without affecting PKC and ERK phosphorylation (Fig. 8A). BAY 11-7082 suppressed significantly histamine-induced IL-8 and IL-6 mRNA expression and partially inhibited the GM-CSF mRNA induction (Fig. 8B). Also, BAY 11-7082 inhibited histamine-enhanced production of these cytokine (Fig. 8C).

3.7. Effects of inhibitors on histamine-induced ERK and NF-κB activation

In order to re-examine the effects of used inhibitors on histamine-induced ERK and NF-κB activation, in vitro kinase assay using immunoprecipitated ERK and NF-κB inducible reporter assay, respectively, were carried out. Ro-31-8220, BAPTA-AM and PD98059 markedly inhibited histamine-induced ERK kinase activities whereas BAY 11-7082 did not (Fig. 9A). Ro-31-8220, BAPTA-AM and BAY 11-7082 significantly suppressed histamine-induced NF-κB reporter activities, and PD98059 partially prevented it (Fig. 9B). Thus, inhibition of activation of ERK and NF-κB by these reagents was consistent with that of phosphorylation of ERK and NF-κB p65. PMA, a PKC activator, which is substituted for DAG, also activated ERK and NF-κB, and produced GM-CSF and IL-8 (Fig. 9C).

3.8. Coordinative effects of histamine and TNF-α on cytokine production

Histamine augmented TNF-α-induced GM-CSF, IL-8 and L-6 expression (Fig. 10A). Olopatadine only prevented the increase in GM-CSF production caused by histamine; it did not block TNF-α-elevated GM-CSF production (Fig. 10B).

3.9. Histamine up-regulates several mediators

Since histamine-evoked PKC activation plays critical roles in both ERK and NF-κB signaling pathways, it is tempting to investigate effects of histamine on the expression of other keratinocyte-derived mediators, which are suggested to be regulated by both pathways [36,37]. Histamine induced TNF-α, ICAM-1, MMP-1, MMP-3, MMP-9 and MMP-13 expression, and this induction was blocked by olopatadine and chlorpheniramine (Fig. 11A and B). Histamine-induced MMP-1 production was significantly blocked by Ro-31-8220 and PD98059, but not by BAY 11-7082 (Fig. 11C).

4. Discussion

In the present study, the expression profile of histamine receptor classes in human epidermal keratinocytes was studied for the first time. H1R but not H2R, H3R and H4R was expressed in cultured human keratinocytes so that the signal transduction through H1R can be simply evaluated with the exception of involvement of other histamine receptors.

Histamine concentration-dependently induced the expression and production of cytokines, chemokines and matrix metalloproteases from human epidermal keratinocytes. The histamine-induced expression of mediators, such as GM-CSF, IL-8 and IL-6, were blocked by all tested H1 antagonists, but neither by an H2 antagonist nor an H3 antagonist. The IC_{50} values for inhibition of for GM-CSF or IL-8 production by olopatadine were 1.4 and 0.4 nmol/L, respectively, which may relate to its receptor binding affinity for H1R (Kd; 2.5 nmol/L and Ki; 1.8 nmol/L)

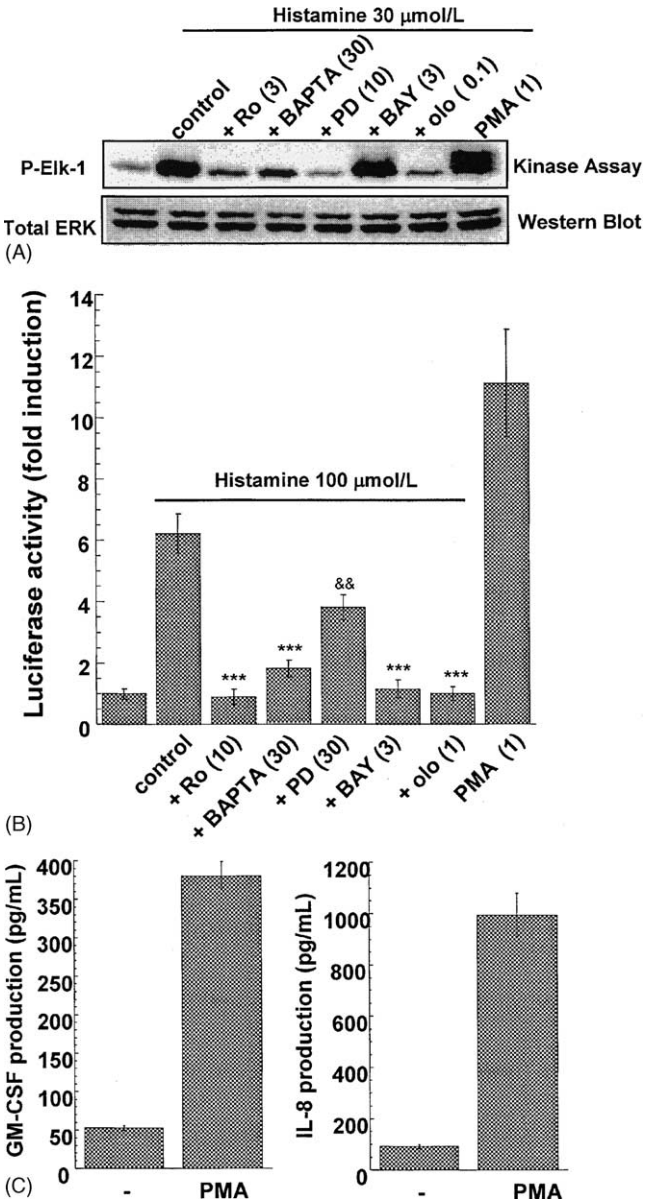

Fig. 9. Effects of several inhibitors on ERK and NF-κB activation in human epidermal keratinocytes. (A) Keratinocytes were pretreated with indicated concentrations of reagents; Ro-31-8220 (Ro), BAPTA-AM (BAPTA), PD98059 (PD), BAY 11-7082 (BAY), olopatadine (olo), for 1 h prior to stimulation with 30 μmol/L histamine or 1 μmol/L PMA for 7.5 min. As a control, the cell lysate was subject to Western blotting using the total ERK specific antibody (lower panel). The same lysate was immunoprecipitated with the phospho-ERK specific antibody. The immune complexes were incubated with ATP and Elk-1 protein, as a substrate. The phosphorylation of Elk-1 by immunoprecipitates was determined by Western blotting using the phospho-Elk-1 specific antibody (upper panel). One representative result of two independent experiments is shown. (B) Keratinocytes were transiently transfected with the NF-κB inducible reporter plasmid. After transfection, cells were pretreated with the indicated concentrations of reagents for 1 h prior to stimulation with 100 μmol/L histamine or 1 μmol/L PMA for 6 h. Then, luciferase activity was determined. Values represent means ± S.E. of 16 determinations of two separate experiments. The results are shown as fold induction from non-stimulated cells. $^{***}P < 0.001$ compared with the control group (Student's t). $^{\&\&}P < 0.01$ compared with the control group (Aspin–Welch). (C) Keratinocytes were stimulated with 1 μmol/L PMA for 24 h. Then, production of GM-CSF and IL-8 were measured by ELISA. Values represent means ± S.E. of three determinations.

previously obtained in Chinese hamster ovary cells expressing H1R [38]. Moreover, olopatadine exhibited very low affinities for H2R and H3R in comparison with H1R [28]. Olopatadine did not affect histamine-induced Ca^{2+} elevation using H4R-transfected cells (data not shown). Thus, it is likely that histamine mediates these functional responses via H1R on human keratinocytes.

The phosphorylation of PKC, c-Raf, MEK, IKK and NF-κB p65 caused by histamine was detected for the first time. Stimulation of PKC, c-Raf/MEK/ERK and IKK/IκB/NF-κB by histamine suggests that activation of these molecules could be involved in histamine-induced physiological responses. Pharmacological approaches using specific inhibitors for signal transduction were carried out to elucidate the function of each signaling pathway in cytokine production, as discussed below.

Ro-31-8220 [32], a specific PKC inhibitor significantly reduced the expression and production of histamine-stimulated GM-CSF, IL-8 and IL-6, suggesting that PKC signaling plays crucial roles in cytokine expression. Gö 6976 [39], a conventional PKC isoform inhibitor, also inhibited the cytokine production (data not shown). Ro-31-8220 decreased the phosphorylation of Raf, MEK and ERK, and inhibited the kinase activities of ERK. PKC is reported to phosphorylate c-Raf, which further phosphorylates MEK, a MAPKK upstream of ERK [40]. Accordingly, H1R-induced PKC activation may trigger Raf/MEK/ERK cascade in keratinocytes. Ro-31-8220 also reduced the phosphorylation of IKK, IκB-α and NF-κB p65, resulting in the inhibition of NF-κB inducible luciferase activities. These results indicated that PKC signaling is also required for the activation of IKK/IκB/NF-κB cascade. It is consistent with above views that stimulation of PKC by PMA induced activation of both ERK and NF-κB pathways, resulting in production of GM-CSF and IL-8.

Reducing intracellular calcium by BAPTA-AM, a Ca^{2+} chelator, decreased the phosphorylation of PKC and the expression of GM-CSF, IL-8 and IL-6, suggesting that histamine-mediated PKC signaling is calcium-dependent. Hence, histamine-elevated intracellular calcium may contribute to not only acute allergic responses but also production of proinflammatory cytokines, resulting in the late allergic responses.

The PKC family consists of at least 12 different isoforms [9]. These isoforms are divided into four groups, which are the conventional PKC, novel PKC, atypical PKC and PKCμ, based on amino acid sequence similarity and sensitivity to Ca^{2+} and DAG [9]. Of the four groups, only the conventional PKC types α, βI, βII and γ require Ca^{2+} [9]. Keratinocytes express PKCα, δ, ε, η and ζ, isoforms [41]. Anti-phospho-PKC (pan) antibody, which was used in this study, detects endogenous phosphorylation of PKCα, βI, βII, δ, ε and η isoforms. Taken together, the data suggest that conventional PKCα is the most likely functional PKC isoforms involved in H1R simulated intracellular signaling. Previously, it was reported that the

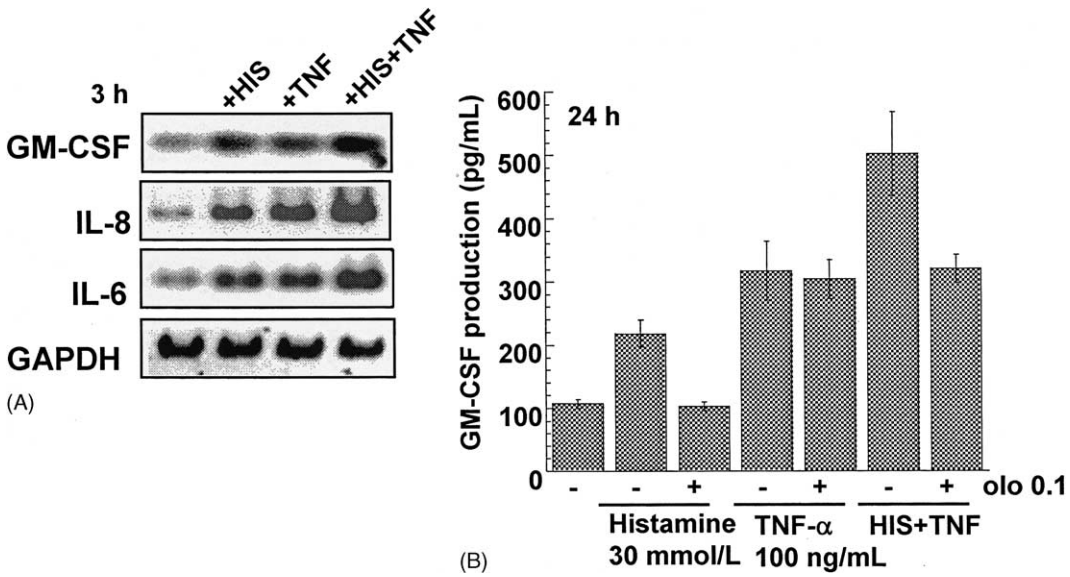

Fig. 10. Additive effects of histamine and TNF-α on expression and production of cytokines in human epidermal keratinocytes. (A) Keratinocytes were stimulated with both 30 μmol/L histamine and 100 ng/mL TNF-α for 3 h. GM-CSF, IL-8 and IL-6 mRNA levels were analyzed with the RT-PCR method. (B) Keratinocytes were pretreated with 100 nmol/L olopatadine for 1 h prior to stimulation with both histamine and TNF-α for 24 h. GM-CSF production was measured by ELISA. Values represent means ± S.E. of three determinations.

PKCα isoform plays critical roles in H1R-induced c-fos expression in Chinese hamster ovary cells expressing recombinant H1R [42]. More recently, another group has reported that histamine affects the PKCα translocation leading to especially GM-CSF expression [43], in consistent with our studies. Thus, it is likely that H1R may be linked to PKCα isoform in native epidermal keratinocytes.

PD98059 [33], a specific MEK-1 inhibitor, and BAY 11-7082 [34,35], a specific IκB inhibitor, reduced histamine-induced ERK activation and NF-κB activation, respectively, whereas both inhibitors did not affect PKC phosphorylation, suggesting that ERK and NF-κB cascades are not located upstream of PKC. Moreover, the inhibition pattern of cytokine production was different between PD98059 and BAY 11-7082. For example, PD98059 inhibited GM-CSF production more effectively than BAY 11-7082 while BAY 11-7082 prevented IL-8 and IL-6 production more potently than PD98059. These findings suggest that the ERK and NF-κB cascades mainly regulate GM-CSF production and IL-8/IL-6 production, respectively. However, regulation of the production of each cytokine is not fully dependent on the ERK or NF-κB cascades because the analysis using BAY 11-7082 indicated that NF-κB, in part, mediates GM-CSF expression. In view of the results so far achieved, both ERK and NF-κB cascades are separate processes regulated from PKC activation (Fig. 12). In the present study, PD98059 partially inhibited IκB-α/NF-κB p65 phosphorylation and NF-κB inducible reporter activities. Recently, ERK has been also implicated in other stimulus-induced NF-κB activation [44,45]. Our findings suggest that the ERK pathway may show some interaction with the NF-κB cascade (Fig. 12). ERK and NF-κB might contribute in an interdependent or a synergistic fashion to an efficient histamine-induced cytokine expression. Moreover, histamine induces expression of numerous genes other than GM-CSF, IL-8 and IL-6 in keratinocytes. Probably, both ERK and NF-κB cascades participate in the expression of other genes. At least, we demonstrated that the MMP-1 production is mainly regulated by the ERK cascade via PKC activation.

GM-CSF, which is overproduced in keratinocytes from patients with atopic dermatitis, is critical to the development and function of antigen-presenting cells [24,29]. IL-8, which is highly expressed at the lesion site of atopic dermatitis and psoriasis, stimulates the migration of neutrophils [30]. ICAM-1, which potently causes adherence of T lymphocytes and keratinocytes, acts as a co-stimulant of the activation of T lymphocytes [36]. TNF-α and IL-6 are involved in skin inflammation [31]. MMPs are believed to degrade collagen and prompt cell migration [37]. Histamine augmented TNF-α induced cytokine production from keratinocytes. We speculate that synergistic stimulations with histamine may result in a more serious form of inflammatory disease.

In this study, all clinically available H1 antagonists prevented histamine-induced cytokine production. Epidermal keratinocytes in vivo would be influenced by the recruited mast cells to propagate inflammation [24,25]. We have recently demonstrated that olopatadine prevents the increase in GM-CSF in skin inflammation using atopic dermatitis-pone animal models [46]. Our pharmacological results suggest that keratinocytes are responsive to mast cell-derived histamine with potential ability to elaborate proinflammatory cytokines, which contribute to amplification of the inflammation under the pathological conditions, such as atopic dermatitis and psoriasis.

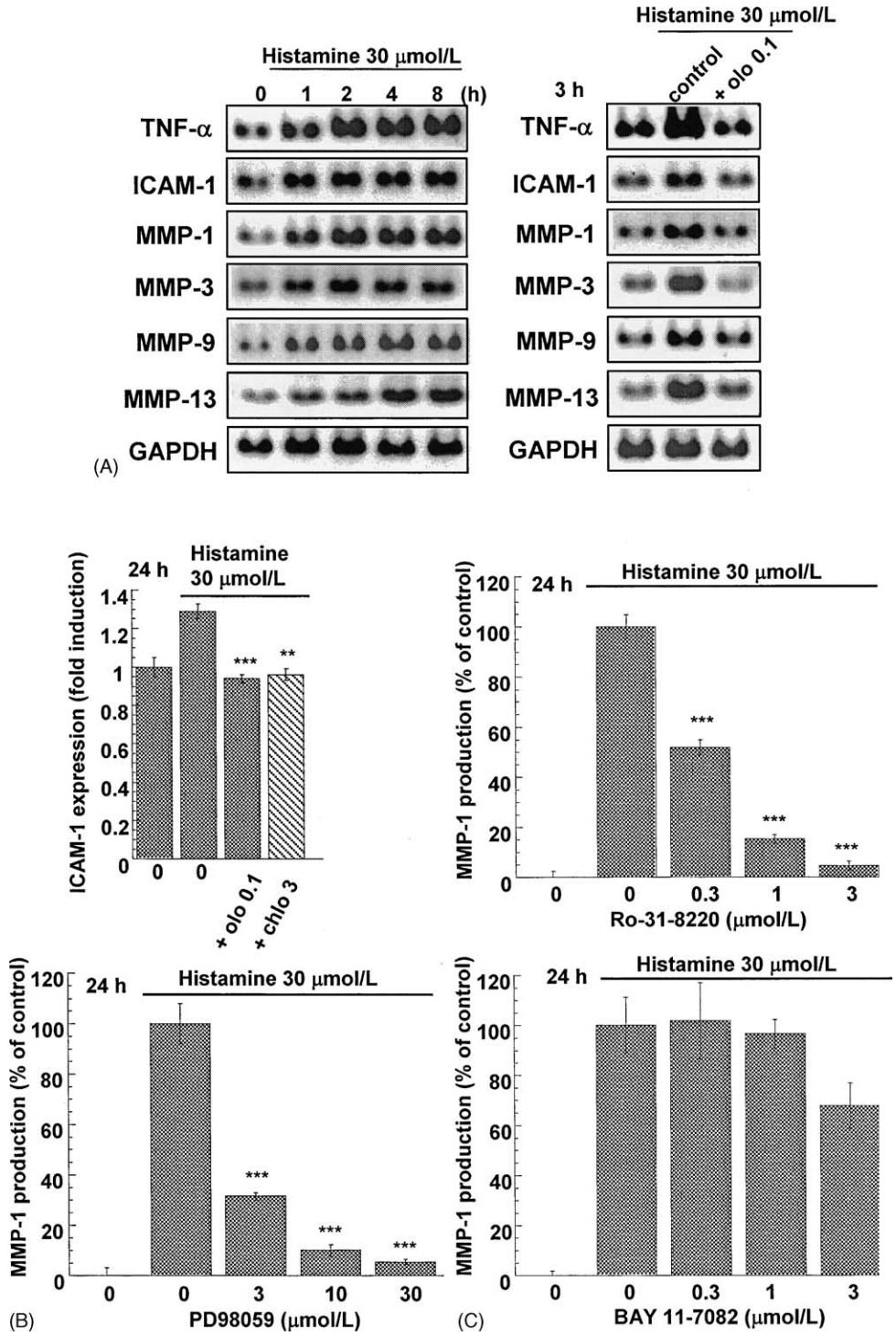

Fig. 11. Effects of histamine on production of inducible mediators in human epidermal keratinocytes. (A) Keratinocytes were stimulated with 30 μmol/L histamine for indicated times. Time course of mRNA levels of indicated genes was analyzed by RT-PCR. One representative result of three independent experiments is shown (left panel). Keratinocytes were pretreated with 100 nmol/L olopatadine (olo) for 1 h prior to stimulation with histamine for 3 h. mRNA levels of indicated genes were analyzed by RT-PCR (right panel). (B) Keratinocytes were pretreated 100 nmol/L olopatadine (olo) and 3 μmol/L chlorpheniramine (chlo) for 1 h prior to stimulation with 30 μmol/L histamine for 24 h. The expression of ICAM-1 protein on the surface of cells was determined by the cell surface ELISA method. The results are shown as fold induction from non-stimulated cells. Values represent means ± S.E. of four determinations. $^{**}P<0.01$, $^{***}P<0.001$ compared with the control group (Student's *t*). (C) Keratinocytes were pretreated with indicated concentrations of Ro-31-8220, PD98059 and BAY 11-7082 for 1 h prior to stimulation with 30 μmol/L histamine for 24 h. MMP-1 production was measured by ELISA. The results are shown as % of control. Values represent means ± S.E. of three determinations. $^{***}P<0.001$ compared with the control group (Dunnett).

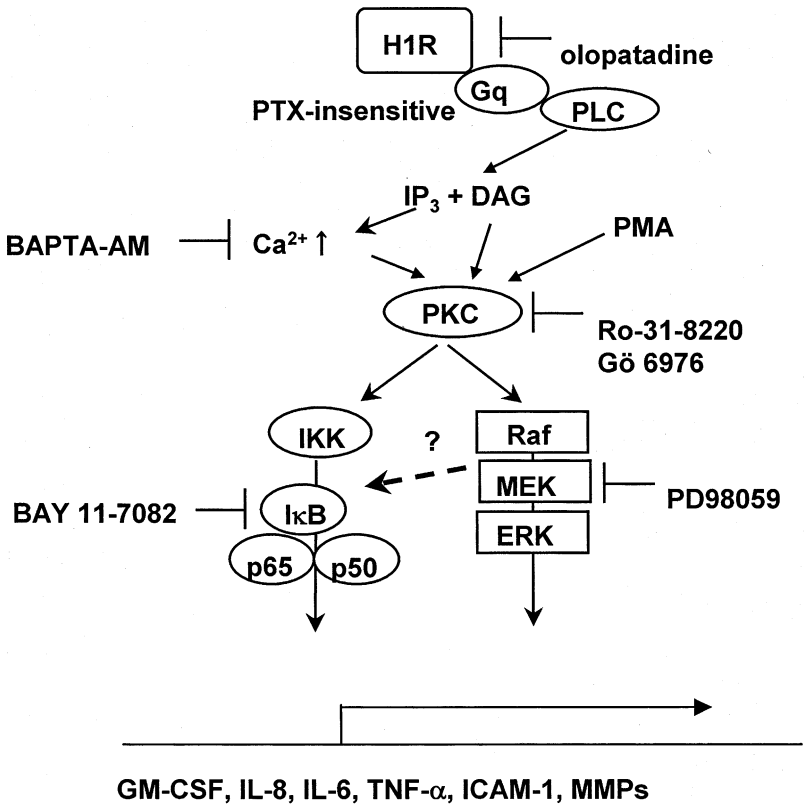

Fig. 12. Schematic summary of signaling pathways via H1R for cytokine production. Histamine induced activation of ERK and NF-κB cascades may be triggered through the Ca^{2+}-dependent PKC stimulation. Two cascades cooperatively lead to expression of several mediators.

In conclusion, H1R-stimulated PKC is required for induction of ERK and NF-κB cascades in response to histamine, inducing production of proinflammatory mediators from epidermal keratinocytes. These findings suggest that treatment with H1 antagonists offers additional therapeutic benefits by preventing production of proinflammatory cytokines, resulting in alleviating histamine-induced allergic late response. In addition, this signal pathways leading to cytokine production from H1R may serve a model for estimation of potential action of the other ligand of G-protein coupled receptors.

Acknowledgements

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