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Prostaglandin E_2 Inhibits Proteinase-Activated Receptor 2-Signal Transduction through Regulation of Receptor Internalization

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ABSTRACT. Proteinase-activated receptor (PAR) is expressed on various cells, and the PAR family consists of PAR1, PAR2, PAR3, and PAR4. Individual PARs are activated during inflammatory conditions in which they regulate inflammatory responses in various diseases. For example, PAR activation is known to induce prostaglandin E_2 (PGE_2) production, and then upregulated PGE_2 suppresses PAR1 expression in a negative feedback loop. Surprisingly, PGE_2 effects on PAR2, which is a well-researched and attractive target for drug development, remain unknown. Therefore, we investigated PAR2 regulation by PGE_2. Using HEK293T cells, we showed that PGE_2 inhibits extracellular signal-regulated kinase (ERK) phosphorylation induced by a PAR2-activating peptide (PAR2-AP). AH-6809 (an inhibitor of PGE_2 receptors 1 [EP1] and 2 [EP2]), but not ONO-AE3-208 (a PGE_2 receptor 4 [EP4] inhibitor), reversed the inhibitory effects of PGE_2 on PAR2-AP-induced ERK phosphorylation. Studies on PAR2 expression revealed that PGE_2 suppressed cell surface expression of PAR2 and induced internalization of PAR2, and not PAR4, in N2a mouse neuroblastoma cells that were transiently transfected with either PAR2 or PAR4. Furthermore, forskolin, an adenylate cyclase activator, induced PAR2 internalization and inhibited PAR2-AP-induced phosphorylation of ERK. Because EP2 (not EP1) also increases intracellular cyclic AMP, we conclude that PGE_2 inhibited PAR2-dependent signal transduction by inducing the internalization of PAR2 through an EP2-dependent increase in intracellular cyclic AMP. This novel regulatory pathway in which PAR2 function is regulated by PGE_2 will broaden our understanding of PAR2-dependent inflammation and could provide novel strategies for drug development.

KEY WORDS: cyclic AMP, receptor internalization, prostaglandin E_2 (PGE_2), proteinase-activated receptor 2 (PAR2).

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Proteinase-activated receptors (PARs) mediate inflammatory responses. They are widely expressed G-protein-coupled receptors (GPCRs), which are activated by serine protease-mediated cleavage of the extracellular N-terminal domain. The PAR family consists of 4 members: PAR1, PAR2, PAR3 and PAR4. In particular, PAR2 is well studied and has been shown to increase inflammatory cytokine production in many types of cells [10, 13, 28]. Infiltration of immune cells has been shown to be inhibited in PAR2-deficient mice; these mice had decreased allergic inflammation of the airway and neuroinflammation [19, 26]. In pancreatitis, PAR2 is activated by leaked trypsin, which mediates both pro-inflammatory and anti-inflammatory responses [12, 13]. Furthermore, PAR2 is involved in colitis, skin inflammation, and neurogenic inflammation [22]. Thus, PAR2 is an attractive target for therapeutic drug development, and further studies on the mechanism by which PGE_2 regulates PAR2 are therefore warranted.

PAR activation induces cyclooxygenase (COX) expression in many types of cells, which in turn induces the synthesis of various prostaglandins including prostaglandin E_2 (PGE_2) [3]. PGE_2 is produced by almost every cell type, and it activates the EP receptor in an autocrine and/or paracrine manner. The EP receptor has 4 subtypes: EP1, EP2, EP3 and EP4. Each subtype activates a different signaling pathway through a different G protein. For example, stimulation of EP1 induces Ca^{2+} influx and protein kinase C activation via G_q/G_{11} protein. On the other hand, EP2 and EP4 couple to G_s protein and activate adenylate cyclase, resulting in an increase in cyclic AMP (cAMP) expression. EP3 stimulation decreases cAMP production via G_i protein [30]. Therefore, PGE_2 exerts both pro-inflammatory and anti-inflammatory effects via different types of EP receptors. This finding is consistent with that of PGE_2 involvement in both the early and late phases of inflammation. In the early phase, PGE_2 induces local increases in blood flow, edema formation, and pain sensitization [7, 11, 35]. In the late phase, however, PGE_2 represses immune cell activation and inflammatory mediator production [17, 24, 32, 33]. These late anti-inflammatory effects of PGE_2 are particularly important, since enhancement of these effects could be therapeutically beneficial.

PAR1 expression is upregulated during inflammatory lung disease, in which case it then regulates the production of pro-inflammatory and profibrotic mediators [4, 9]. On the other hand, PAR1 activation also increases PGE_2 production, which plays an important role as an anti-inflammatory mediator partly by downregulating PAR1 expression [20, 27, 29]. PGE_2 is also produced by the activation of PAR2 and plays an anti-inflammatory role in lung disease [8]. In contrast to PAR1, however, the effects of PGE_2 on PAR2 are still unclear. In this study, we found that PGE_2 inhibited PAR2 signal transduction by decreasing PAR2 surface expression.

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MATERIALS AND METHODS

Cell lines and culture: Human embryonal kidney 293 (HEK293T) cells and Neuro 2a mouse neuroblastoma (N2a) cells were grown in Dulbecco's modified Eagle's medium (DMEM; Sigma-Aldrich, St. Louis, MO, U.S.A.) supplemented with 10% fetal bovine serum (FBS; JR Scientific, Woodland, CA, U.S.A.) and 1% antibiotic–antimycotic solution (Life Technologies, Carlsbad, CA, U.S.A.). To prepare N2a cells for transfection, cells were plated on 24-well plates (25,000 cells per well) and incubated overnight. Cells were then incubated with a plasmid (0.5 μg) and Arrest-In transfection reagent (2.5 μl; Thermo Scientific, Woburn, MA, U.S.A.) in FBS-free DMEM (250 μl) for 18 hr, following the manufacturer's protocol.

Generation of HEK293T cells stably expressing FLAG-PAR2-mCherry: The seYFP (Venus) and C-terminal multi cloning site (*Bam*HI, *Not*I, and *Eco*RI) in pLV-Venus was replaced with mCherry and an N-terminal multi cloning site (*Bam*HI, *Not*I, and *Eco*RI) (pLV-mCC) [15]. Human PAR2 with N-terminus FLAG-tag was PCR amplified using pcDNA3.1 FLAG-human PAR2-HA [5] as a template and subcloned into *Bam*HI/*Eco*RI sites of pLV-mCC. A lentivirus-producing cell line was generated by transfecting Lenti-X 293T cells with Lipofectamine LTX (Life Technologies), 3 μg of lentiviral plasmid, 2.3 μg of packaging plasmid (psPAX2), and 1.3 μg of VSVG coat protein plasmid (pDM2.G). Viral supernatants were collected after 48 hr, filtered (0.22 μm), and used to treat 293T cells for 8 hr.

Western blotting: Cells were lysed in a buffer containing 50 mM Tris (pH 8.0), 5 mM ethylenediamine tetraacetic acid (EDTA), 5 mM ethylene glycol tetraacetic acid (EGTA), 1% Triton X-100, 1 mM sodium orthovanadate, 20 mM sodium pyrophosphate and Roche complete protease inhibitor mixture (1 tablet/50 m*l*; Roche, Indianapolis, IN, U.S.A.). The extracted protein was separated by SDS-polyacrylamide gel electrophoresis (SDS-PAGE) and transferred onto a PVDF membrane (Bio-Rad Laboratories, Hercules, CA, U.S.A.). The membrane was blocked with 2% skim milk in Tris-buffered saline (TBS) containing 0.05% Tween20, and treated with antibodies. The immunoreactive bands were detected using a chemiluminescence (ECL) detection kit (GE Healthcare, Freiburg, Germany), and the images were captured with LAS-3000 mini imager (Fujifilm, Tokyo, Japan). Band densities were quantified using ImageJ densitometry analysis software (National Institutes of Health, Bethesda, MD, U.S.A.).

Flow Cytometry: HEK293T cells stably expressing FLAG-PAR2-mCherry (FLAG-PAR2-mC HEK293T) were treated with or without PGE_2 for 30 min, suspended in PBS-EDTA (0.5%), and plated in a 96-well plate for immunocytochemical staining. Cells were then treated with Alexa Fluor 488 conjugated anti-FLAG rabbit IgG antibody (Cell Signaling Technology, Beverly, MA, U.S.A.) or rabbit isotype control antibody (Cell Signaling Technology) in FACS buffer (2% FBS and 1% sodium azide in PBS) for 30 min on ice. The fluorescence intensity of mCherry in 6,000 FLAG-PAR2-mC HEK293T cells was measured using a CyFlow space flow cytometer (Partec, Münster, Germany). The cells that showed higher fluorescence levels than the maximum fluorescence levels shown by the cells stained with the isotype control antibody were defined as FLAG-positive cells.

Confocal microscopy: Confocal microscopy studies were then adopted in order to investigate the intracellular distribution of PAR2 and PAR4. In the preliminary study, it was difficult to determine the endogenous expression of PAR2 and PAR4, because of the low-selectivity of commercially available antibody for these receptors. Therefore, we used N2a cells that were transiently transfected to express the FLAG-labeled protein. N2a cells, transiently expressing FLAG-PAR2-HA or FLAG-PAR4, were cultured on cover glass for 4 hr before drug treatments. After being treated with various drugs, the cells were fixed with 4% paraformaldehyde in TBS for 20 min and then treated with 0.3% Triton X-100 for 10 sec. Cells were then blocked with a solution containing 3% bovine serum albumin fraction V (BSA; Roche) in TBS for 30 min. Fixed cells were incubated overnight with rabbit anti-FLAG antibody (Sigma-Aldrich) at 4°C. After washes, cells were incubated with Alexa Fluor 488 conjugated anti-rabbit IgG antibody (Life Technologies) for 1 hr at room temperature. Fluorescent images of the cells were visualized with a LSM510 confocal microscope (Carl Zeiss, Inc., Thornwood, CA, U.S.A.). In order to quantify the surface expression of PAR2 on the plasma membrane, two-dimensional images of the cells were scanned using optical sections with a diameter of 0.8–1.2 μm across the Z-axis through the middle of the cells. The cells in 3 views from each cover glass (50–300 cells) were observed. The states of receptor expression were classified into 3 patterns as following; Localized cell surface: receptors were sharply labeled at the cell membrane and hardly detected in the cytosolic region (Fig. 1A); Partially internalized: receptors were evenly distributed on the cell surface and cytosolic region (Fig. 1B); Completely internalized: receptors were labeled only in the cytosolic region (Fig. 1C).

Intracellular cAMP detection: Intracellular cAMP was detected using a cAMP-Glo assay kit (Promega, Madison, WI, U.S.A.). In brief, 10,000 N2a cells were plated on a 96-well plate and incubated overnight. After washing, N2a cells were treated with PGE_2 or forskolin in 30 μl of induction buffer (100 μM RO 20–1724 and 500 μM 3-isobutyl-1-methylxanthin in FBS-free DMEM) for 1 hr. The induction buffer was incubated with cAMP-Glo Lysis Buffer for 15 min, and cAMP-Glo Detection Solution for 20 min. Finally, the medium was incubated with Kinase-Glo Reagent for 10 min and chemiluminescence was then detected using the DTX800 (Beckman Coulter, Fullerton, CA, U.S.A.).

Antibodies, plasmid and reagents: The following antibodies were used for this study: phospho-ERK p44/42 (Cell Signaling Technology), and tubulin (Thermo Scientific). pcDNA3.1 FLAG-human PAR2-HA and pBJKS FLAG-human PAR4 were kindly provided from Dr. Nigel W. Bunnett (University of California, San Francisco, CA, U.S.A.) and Dr. Shaun R. Coughlin (University of California, San Francisco, CA, U.S.A.), respectively [5]. AH-6809 was purchased from Sigma-Aldrich. SLIGRL-NH2 (PAR2-

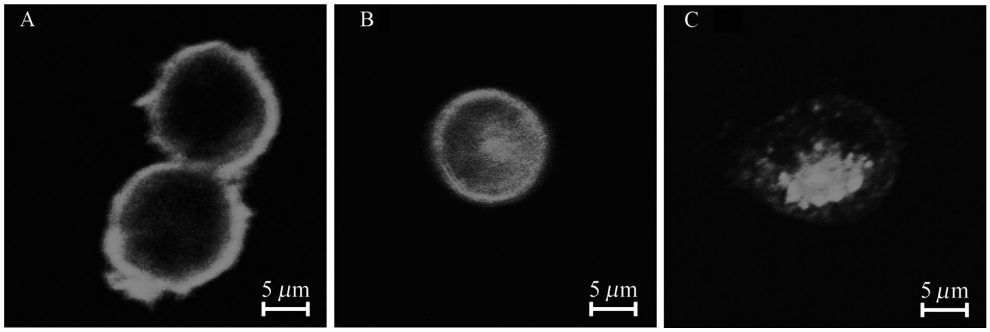

Fig. 1. PAR2 localization patterns obtained using confocal microscopy. N2a cells transiently expressing FLAG-PAR2 or FLAG-PAR4 were stained with anti-FLAG antibody and Alexa Fluor 488 conjugated antibody, and categorized by confocal microscopy analysis. Representative images of categorized cells, localized cell surface (A), partially internalized (B) and completely internalized (C).

activating peptide; PAR2-AP) was purchased from Bachem AG (Bubendorf, Germany). ONO-AE3-208 was donated from Fuso Pharmaceutical Industries, Ltd. (Osaka, Japan) and Ono Pharmaceutical Co. (Osaka, Japan), respectively. All other chemicals were purchased from Wako Pharmaceuticals (Osaka, Japan).

Statistical analysis: Results of the statistical analysis are expressed as mean ± standard error of the mean (SEM). Statistical evaluation of data was performed using paired or unpaired Student's *t*-tests for comparisons between 2 groups, and 1-way analysis of variance (ANOVA) followed by Student-Newman-Keuls test was used for comparisons between more than 3 groups. For all analyses, $P<0.05$ was considered to indicate statistical significance.

RESULTS

Effect of PGE_2 on PAR2-AP-dependent ERK phosphorylation: To assess the effect of PGE_2 on PAR2 signal transduction, we investigated PAR2-AP-induced ERK phosphorylation in HEK293T cells by western blotting. The level of ERK phosphorylation was significantly increased with 5 min of PAR2-AP (5 μM) treatment (Fig. 2). In HEK293T cells pre-treated with PGE_2 (3 μM) for 30 min, PAR2-AP-induced ERK phosphorylation was significantly inhibited (Fig. 2).

EP receptors regulate the effects of PGE_2 on PAR2 signal transduction: We next investigated which EP receptor subtype is involved in the inhibitory effect of PGE_2 on PAR2 signal transduction. To clarify this point, we used AH-6809 (EP1 and EP2 antagonist) and ONO-AE3-208 (EP4 antagonist). The inhibitory effect of PGE_2 (3 μM) on PAR2-AP (5 μM)-induced phosphorylation of ERK was completely reversed by pretreatment with AH-6809 (20 μM) for 20 min (Fig. 3). However, pre-treatment with ONO-AE3-208 (3 μM) did not significantly alter the inhibitory effects of PGE_2 on PAR2-AP-induced ERK phosphorylation. Treatment with AH-6809 or ONO-AE3-208 did not affect baseline ERK phosphorylation (data not shown). These results suggest that EP1 or EP2 receptors, and not EP4 receptors, are involved in the inhibitory effect of PGE_2 on PAR2 signal transduction.

Effect of PGE_2 on cell surface expression of PAR2 and PAR4: PGE_2 decreased the cell surface expression of PAR1 [29]. To study the inhibitory mechanism of PGE_2 on PAR2 signal transduction, we investigated the expression levels of PAR2 on the cell surface using flow cytometric analysis of HEK293T cells stably expressing FLAG-PAR2-mC. The fluorescence intensity of FLAG-positive cells was detectable using this method (Fig. 4A). As shown in Fig. 4B and 4C, treatment with PGE_2 (3 μM) for 30 min significantly decreased the cell surface expression of PAR2. The cell surface expression of PAR2 was also investigated with confocal microscopy by using cells transiently expressing FLAG-tagged

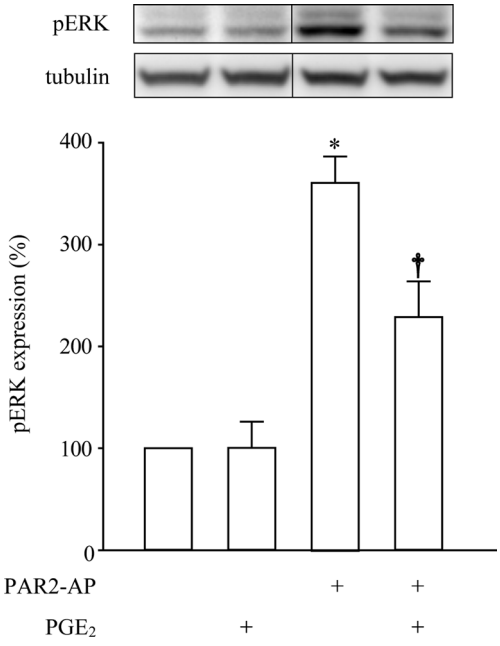

Fig. 2. PGE_2 inhibits PAR2-AP-induced ERK phosphorylation. HEK293T cells were treated with PGE_2 (3 μM) for 30 min, followed by treatment with or without PAR2-AP (5 μM) for 5 min. ERK-phosphorylation and tubulin were measured by western blotting. *$P<0.05$ vs. control (no treatment). †$P<0.05$ vs. PAR2-AP treatment. N=4.

PAR2. As a comparison, cells transiently expressing FLAG-tagged PAR4 were also observed. Because it is easier to evaluate the localization of receptors in round shaped cells, we used N2a cells. In non-stimulated transfected N2a cells, more than 70% of PAR2 and PAR4 were expressed on the cell surface (Fig. 5). The expression pattern of these receptors did not change for at least 24 hr in these transfected cells (data not shown). After the treatment with PGE_2 (3 μM), PAR2 internalization was observed after 1 hr of treatment and lasted 24 hr; conversely, the expression pattern of PAR4 was not changed with PGE_2 treatment (Fig. 5). These results suggest that PAR2, and not PAR4, on the cell surface of N2a cells was internalized following PGE_2 treatment.

Effect of forskolin on cell surface expression and function of PAR2: We showed in Fig. 3, that the EP1 or EP2 receptors were involved in the inhibitory effect of PGE_2. It is well known that the activation of EP2 increases intracellular cAMP but not EP1 [23]. It was also reported that another type of PAR, PAR1, is downregulated by PGE_2 via EP2 [29]. Therefore, we investigated if cAMP was involved in the PGE_2 dependent inhibitory effect on PAR2 expression and localization by using forskolin, an activator of adenylate cyclase. We found that PGE_2 (3 μM) and forskolin (0.3 μM) increase intracellular cAMP levels in N2a cells. (Fig. 6A). When N2a cells transiently expressing FLAG-tagged receptors were treated with forskolin (0.3 μM) for 1, 7 and 24 hr, PAR2 internalization was observed 1 hr after the treatment with forskolin and the internalization lasted 24 hr; as

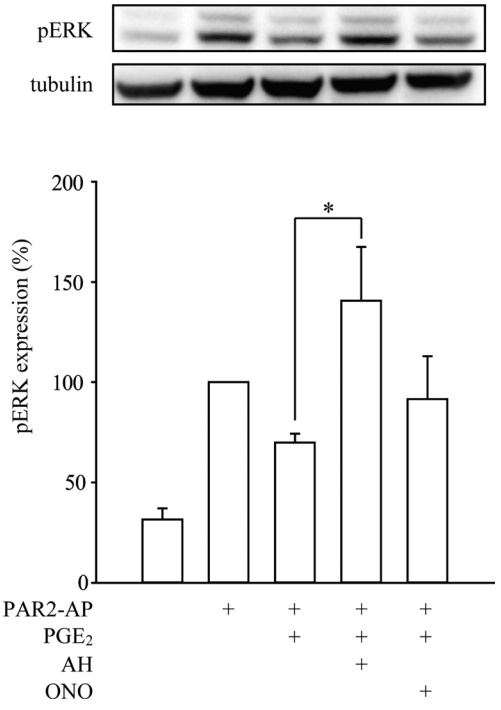

Fig. 3. AH-6809 reverses the inhibitory effect of PGE_2 on ERK phosphorylation. HEK293T cells were pre-treated with AH-6809 (AH; 20 μM) or ONO-AE3-208 (ONO; 3 μM) for 20 min, followed by treatment with PGE_2 (3 μM) for 30 min. Cells were then stimulated with PAR2-AP (5 μM) for 5 min. ERK-phosphorylation and tubulin were measured by western blotting. * P<0.05 vs. PGE_2 and PAR2-AP treatment. N=4.

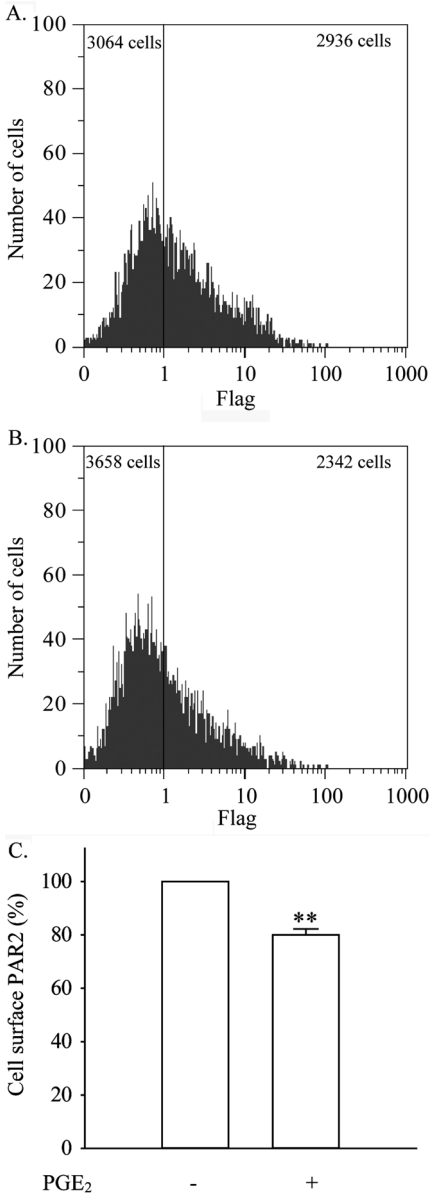

Fig. 4. PGE_2 decreases cell surface expression of PAR2. FLAG-PAR2-mC HEK293T cells were treated with PGE_2 (3 μM) for 30 min. Cell surface expression of PAR2 was determined by flow cytometry. FLAG-PAR2-mC HEK293T cells were stained with anti-FLAG antibody conjugated with Alexa Fluor 488. Representative images from control cells (A) and PGE_2-treated cells (B) are shown. (C) The number of FLAG-positive cells in the control without PGE_2 was defined as 100%. ** P<0.01 vs. control. N=3.

expected, the expression of PAR4 was not changed (Fig. 6B and 6C). These results suggest that forskolin induced PAR2 internalization and not PAR4, similar to our observations using PGE_2.

Finally, to confirm the role of intracellular cAMP in PAR2 function, we investigated the effect of forskolin on PAR2-AP-induced ERK phosphorylation in HEK293T cells. When HEK293T cells were pre-treated with forskolin (0.3 μM) for 30 min, PAR2-AP-induced ERK phosphorylation was significantly inhibited (Fig. 6D). Treatment with forskolin alone did not affect the resting phosphorylation level of ERK. These data suggest that PGE_2 inhibits PAR2 signal transduction through a cAMP dependent system.

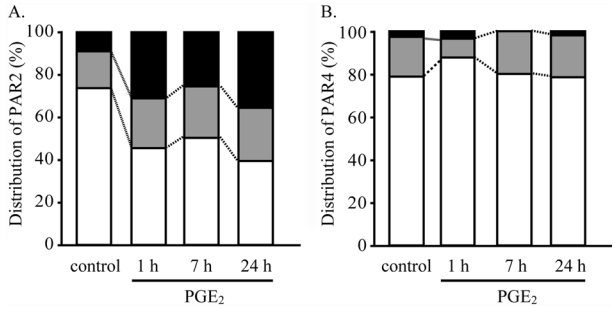

Fig. 5. PGE_2 induces PAR2 and not PAR4 internalization. N2a cells transiently expressing FLAG-PAR2 (A) or FLAG-PAR4 (B) were treated with PGE_2 (3 μM) for the indicated time points. The cell surface expression of receptors was categorized as described in Fig. 1; localized cell surface (white), partially internalized (gray) and completely internalized (black). The number of whole counted cells was evaluated as 100%. N=3–6.

DISCUSSION

The contribution of PAR2 to inflammation is complex, since PAR2 has pro-inflammatory roles [31] and anti-inflammatory roles [22]. Previous data have demonstrated that PAR1 activation induces PGE_2 production, and PGE_2 regulates PAR1 expression through a negative feedback loop [20, 27, 29]. However, the effects of PGE_2 on PAR2-signal transduction have not been clearly elucidated. ERK is one of the most abundant kinases in the cell and is phosphorylated by the activation of PAR2 [18]. In this study, we found that PGE_2 inhibited PAR2-dependent ERK phosphorylation (Fig. 2).

Our experiments show that the treatment of HEK293T cells with PGE_2 inhibited PAR2 activation within a short incubation period (Fig. 2), suggesting that suppression of

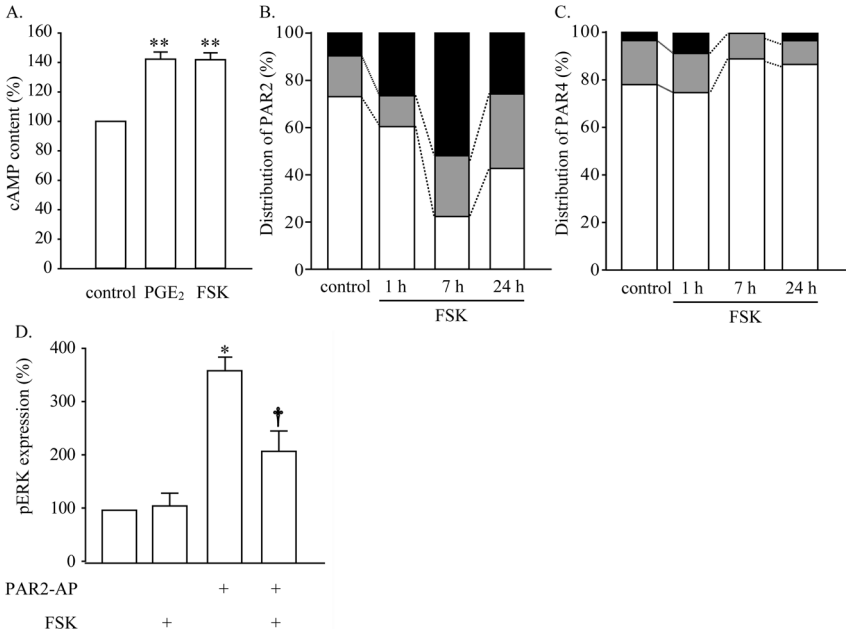

Fig. 6. Forskolin mimics the inhibitory effect of PGE_2 on PAR2. N2a cells were treated with PGE_2 (3 μM) or forskolin (0.3 μM) for 1 hr, at which point intracellular cAMP levels were measured (A). N2a cells transiently expressing FLAG-PAR2 (B) or FLAG-PAR4 (C) were treated with forskolin (0.3 μM) for the indicated time points. The cell surface expression of receptors was categorized as described in Fig. 1; localized cell surface (white), partially internalization (gray), and complete internalization (black). The number of whole counted cells was evaluated as 100%. ERK-phosphorylation was measured by western blotting (D). HEK293T cells were treated with forskolin (FSK; 0.3 μM) for 30 min, followed by the treatment with or without PAR2-AP (5 μM) for 5 min (D). *P<0.05 vs. control. **P<0.01 vs. control. †P<0.05 vs. PAR2-AP treatment. N=3–6.

PAR2 mRNA expression did not contribute to PGE_2-induced inhibition. Therefore, we hypothesized that PGE_2 regulated localization of PAR2 protein. The decrease in PAR2 expression was confirmed by confocal microscopy. In addition, we classified the states of PAR2 expression using flow cytometric analysis. These results suggest that PGE_2-mediated PAR2 internalization contributes to the inhibition of PAR2-signal transduction. PGE_2 increases cAMP levels in many cell types, and it has been reported that PGE_2 inhibits ERK phosphorylation in a cAMP-dependent manner [34]. In our experiments, both of PGE_2 and forskolin, increased intracellular cAMP levels (Fig. 6A). Furthermore, forskolin induced not only the internalization of PAR2, but also the inhibition of ERK-phosphorylation, similar to PGE_2 (Fig. 6B–D). These results suggest that cAMP increase is involved in the PAR2 regulation by PGE_2.

Intracellular cAMP is positively and negatively regulated by G_s and G_i proteins, respectively. In EP receptors, EP2 and EP4 are coupled with G_s, and EP3 is coupled with G_i [30]. As shown in Fig. 3, the inhibition of PAR2-AP-induced ERK phosphorylation by PGE_2 was reversed by treatment with AH-6809, an EP1 and EP2 antagonist. It has also been reported that HEK293T cells express EP1, EP2 and EP3 but not EP4 [1]. These results suggest that PGE_2 negatively regulates PAR2-signal transduction through EP2.

In the presence of AH-6809 and PGE_2, PAR2-AP-induced ERK phosphorylation levels were increased compared to those in the cells with PAR2-AP alone. In this experiment, PAR2-AP was treated for only 5 min, so it was unlikely that PAR2-AP upregulated sufficient expression of COX-2 (inducible COX) protein. However, it has been reported that the stimulation of PAR2 induces activation of COX and release of PGE_2 from airway smooth muscle cells and epithelial cells [8]. Therefore, it is possible that PAR2 activation produces PGE_2 and negatively regulates PAR2 functions.

Sokolova *et al.* [29] reported that PGE_2-induced downregulation of PAR1 is dependent on intracellular cAMP and protein kinase A (PKA) activation [16]. On the other hand, PKA-independent actions of cAMP have been described in several types of cells [25]. Furthermore, cAMP induces internalization of receptors and increases β-arrestin protein expression, which can occur independently of PKA [6, 14]. Therefore, further experiments are required to clarify the exact mechanism by which PGE_2 downregulates PAR2.

PGE_2 induces the production of proteases, which may then induce PAR2 internalization [2, 36]. It is possible that proteases produced by PGE_2 are involved in the downregulation of PAR2. PAR2 activated by agonist proteases or PAR2-AP is internalized. PAR2 downregulation is mediated by C-terminal phosphorylation of the receptor by GPCR kinase followed by association with an adaptor protein like β-arrestin. The adaptor protein recruits clathrin, which induces endosome formation, resulting in internalization. Finally, endosomal PAR2 is ubiquitylated and sorted to the lysosome. In these processes, both the activation and internalization of PAR2 activate ERK signaling [2]. As shown in Fig. 2, treatment with PGE_2 alone did not affect ERK phosphorylation. These results suggest that PGE_2-induced PAR2 internalization is not mediated by the production of proteases and also suggests that PGE_2-induced PAR2 internalization could not activate ERK signaling. Although β-arrestin is the key protein which triggers PAR2 internalization and ERK signaling, Ricks and Trejo [21] reported that PAR2 internalization was induced through a β-arrestin-independent mechanism. PGE_2 may also induce PAR2 internalization in a β-arrestin independent process.

As shown in Fig. 5 and Fig. 6, contrary to PAR2, cell surface expression of PAR4 was not changed by treatment with either PGE_2 or forskolin. These results suggest that PGE_2 may not affect all PARs equally. Similar to PAR2, PAR4 internalization is induced by a PAR4 agonist, but the adaptor protein which triggers PAR4 internalization is still unknown [2]. The difference in the adaptor proteins used by PAR2 and PAR4 may contribute to the specificity of PGE_2 mediated internalization.

We conclude that PGE_2 inhibits PAR2 signal transduction through EP2 and the downregulation of PAR2. These data help to increase our understanding of PGE2 mediated regulation of PAR2 activity and could provide new strategies for drug development.

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