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Original

Involvement of protein kinase C in IL-1β-induced expression of cyclooxygenase-2 in human gingival fibroblasts

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Abstract: Interleukin-1β (IL-1β) stimulates expression of the highly inducible enzyme cyclooxygenase-2 (COX-2) via activation of nuclear factor kappaB (NFκB), and consequently provokes prostaglandin E_2 (PGE_2) synthesis, which induces inflammatory responses. In this study, the contribution of protein kinase C (PKC) to IL-1β-induced PGE_2 synthesis in human gingival fibroblasts was investigated. The PKC activator phorbol 12-myristate 13-acetate (PMA) stimulated PGE_2 release and COX-2 mRNA expression, as shown in human gingival fibroblasts stimulated by IL-1β. However, PMA showed only a weak effect on the formation of COX-2-NFκB DNA-protein complex, whereas IL-1β had a clearly stimulatory effect. In cells in which PMA-dependent PKC was down-regulated, PMA failed to induce the formation of NFκB DNA-protein complex and reduced the release of PMA-induced PGE_2, whereas IL-1β stimulated the formation of COX-2-NFκB DNA-protein complex and PGE_2 release. The atypical PKC (aPKC) inhibitor Gö6983 clearly suppressed the formation of COX-2-NFκB DNA-protein complex and PGE_2 release stimulated by IL-1β but not the inhibitor of conventional PKC (cPKC) and the novel PKC (nPKC) inhibitor Gö6976. These observations suggest that aPKC is involved in IL-1β-induced PGE_2 synthesis, which is controlled by transcription of the COX-2 gene via the NFκB-dependent pathway in human gingival fibroblasts. (J Oral Sci 51, 417-423, 2009)

Keywords: protein kinase C; COX-2; NFκB; PGE_2; gingival fibroblasts.

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Introduction

In human gingival fibroblasts, augmentation of prostaglandin E_2 (PGE_2) synthesis and release has been considered to be a prominent feature of inflammatory reactions (1,2), since elevated levels of PGE_2 are detected in inflamed gingival tissues and crevicular fluid, especially from periodontal sites exhibiting recent attachment loss (3). Cyclooxygenase (COX) is a rate-limiting enzyme of prostanoid biosynthesis and exists as a constitutive isoform (COX-1), responsible for physiological levels of prostaglandins and as an inducible COX-2 encoded by an immediate early gene, which is rapidly induced after pro-inflammatory stimuli. COX-2 is expressed by inflammatory cells and is responsible for the high levels of prostaglandins in acute and chronic inflammation (4,5). Interleukin-1β (IL-1β), a multifunctional pro-inflammatory cytokine, has been demonstrated to play an important role in the expressional regulation of many genes involved in the inflammatory process (6,7). We have previously demonstrated that IL-1β and TNF-α stimulate the expression of COX-2 mRNA and PGE_2 release in human gingival fibroblasts (8,9).

It has been shown that the IL-1β-mediated transcription of COX-2 is regulated by many factors, such as extracellular signal-regulated protein kinase (ERK) (10), the NFκB signaling pathway (11) and protein kinase C (PKC) (12-14). However, the signaling pathways of IL-1β-induced

COX-2 expression are very complex. Therefore, it is important to clarify the signaling process of IL-1β-mediated COX-2 expression in human gingival fibroblasts. In this study, the roles of PKC and transcriptional activity of NFκB in IL-1β-mediated COX-2 expression and PGE_2 release in human gingival fibroblast were investigated using several inhibitors for the PKC family.

Materials and Methods

Materials

IL-1β was purchased from R & D Systems (Minneapolis, MN). Dulbecco's Modified Eagle Medium (D-MEM) and a One-Step Reverse transcription-polymerase chain reaction (RT-PCR) with Platinum Taq were purchased from Invitrogen (Carlsbad, CA). RNeasy was obtained from QIAGEN (Tokyo, Japan). A PGE_2 enzyme immunoassay kit was obtained from Cayman (Ann Arbor, MI). Phorbol 12-myristate 13-acetate (PMA) was purchased from Sigma (St. Louis, MO). Other reagents were purchased from Wako Pure Chemical Industries (Osaka, Japan).

Cell culture

D-MEM supplemented with 10% FBS, 100 U/ml penicillin and 100 μg/ml streptomycin was used for cell culture. Samples of normal gingival tissue were obtained from three healthy individuals undergoing third-molar extraction. Human gingival fibroblasts were cultured using the methods described previously (15). When the cells surrounding the tissue explants had become confluent, they were detached with 0.05% trypsin and 0.02% EDTA in Hanks' balanced salt solution, and transferred to a tissue culture flask for subculture. The cells were used for the experiments between the 5th and 10th passages. This study was approved by the ethics committee of Nihon University School of Dentistry at Matsudo (No. EC 03-041).

RT-PCR

Total RNA was isolated using RNeasy. RT-PCR was performed using Super Script One-Step RT-PCR with Platinum Taq System. Primers were synthesized on the basis of the reported human cDNA sequence for COX-2 and glyceraldehyde-3-phosphate dehydrogenase (GAPDH) (16,17). Sequence of the primers used for RT-PCR were as follows: COX-2, 5′-ATGAGATTGTGGAAAAATTGCT-3′ (forward) and 5′-GATCATCTCTGCCTGAGTATC-3′ (reverse); GAPDH, 5′-CCACCCATGGCAAATTCCATGGCA (forward) and; 5′-TCTAGACGGCAGGTCAGGTCCACC-3′ (reverse). The PCR reactions were performed in a final volume of 50 μl, which contained 25 μl of reaction mixture, 1 μg total RNA, 10 μM each forward and reverse primer, and 1 μl TaqMix. A two-step cycling program was used, and this consisted of an initial template melting step at 94°C for 2 min, denaturation at 94°C for 30 s, annealing at 55°C for 30 s, extension at 72°C for 30 s (22 cycles) and a final extension at 72°C for 10 min (18). After amplification, 10 μl of each reaction mixture was subjected to 2% agarose gel electrophoresis (0.5× Tri-borate buffer) and visualized by ethidium bromide staining under UV light. The relative amount of PCR product was normalized to the corresponding amount of GAPDH PCR product. The PCR products for COX-2 and GAPDH were 310 bp and 598 bp, respectively.

Measurement of PGE_2

Human gingival fibroblasts were cultured in 24-well culture plates in D-MEM containing 10% FCS. When the cells had become confluent, they were deprived of serum for 18 h to prevent further extracellular stimulation, and then IL-1β or PMA was added. The medium was collected after various time intervals and the amount of PGE_2 was determined by enzyme immunoassay using a commercially available kit (18).

Gel mobility shift assay

Confluent human gingival fibroblasts in T-75 flasks incubated for 60 min with IL-1β or PMA in D-MEM without serum were used to prepare nuclear extracts. Nuclear protein was extracted by the method of Digman et al. (19) with the addition of extra proteinase inhibitors (the extraction buffer was 0.42 M NaCl, 1.5 mM $MgCl_2$, 0.2 mM EDTA, 1 mM dithiothreitol (DTT), 0.25% (V/V) glycerol, 0.5 mM phenylmethylsulfonyl fluoride, 2 μg/ml leupeptin, 2 μg/ml pepstatin, and 1μg/ml apotinin, pH 7.9). Double-stranded oligonucleotides corresponding to the NFκB motif (COX-2- NFκB; -230 to -207) (5′-GAGAGTGGGGACTACCCCCTCTGC) in the human COX-2 gene promoter (20) were end-labeled with [γ-^{32}P]ATP using T4 polynucleotide kinase, and the product at 0.1 pM was incubated at 21°C for 20 min in binding buffer (50 mM KCl, 0.5 mM EDTA, 10 mM Tris-HCl (pH 7.9), 1 mM DTT, 0.04% Nonidet P-40, 5% glycerol and 1 μg poly(dI-dC)) with nuclear protein extracts (3 μg). In each case the reaction mixtures were analyzed on 5% non-denaturing acrylamide gels followed by evaluation with an image analyzer (21).

Down-regulation of PKC

Human gingival fibroblasts were treated with PMA (100 nM) for 18 h to induce depletion of PKC, as described previously (22).

Statistical analysis

All values are expressed as means ± S.E.M. Statistical analysis was performed using the PRISM software package. The results were evaluated by Dunnett's post-test and Welch's *t*-test. Values were considered significant at $P < 0.01$.

Results

First, the effect of the PKC activator PMA on PGE_2 release from human gingival fibroblasts was examined and compared with the effect of IL-1β. When human gingival fibroblasts were stimulated with PMA (100 nM) for 12 h, PGE_2 release was increased, as induced by IL-1β (1 ng/ml) (Fig. 1).

We have previously demonstrated that IL-1β-mediated PGE_2 release is a consequence of COX-2 mRNA induction in human gingival fibroblasts (8). Therefore, the effect of PMA on COX-2 mRNA expression was examined by RT-PCR. As Fig. 2 shows, PMA stimulated the expression of COX-2 mRNA to an extent comparable to that of IL-1β in human gingival fibroblasts treated with drugs for 6 h.

NFκB is a critical transcription factor involved in the transcriptional control of COX-2 gene expression (6). To examine the binding of the NFκB sequence to nuclear proteins, COX-2-NFκB oligonucleotide was end-labeled and incubated with aliquots of nuclear proteins extracted from human gingival fibroblasts treated with IL-1β or PMA for 60 min. IL-1β clearly stimulated the formation of COX-2-NFκB DNA-protein complex, but less PMA, as Fig. 3 summarizes. On the other hand, the effects of IL-1β and PMA on the formation of COX-2-NFκB DNA-protein complex were examined in cells where PMA-sensitive PKC activity was down-regulated. As Fig. 3 shows, IL-1β stimulated the formation of COX-2-NFκB DNA-protein complex, but PMA failed to do so in the PKC-downregulated cells. In the PKC-downregulated cells, IL-1β-stimulated PGE_2 release occurred, but PMA-induced PGE_2 release was reduced, as shown in Fig. 4. These observations suggest that PMA-sensitive PKC or PKC itself is less involved in COX-2 expression via NFκB activation induced by IL-1β in human gingival fibroblasts.

We then examined the effect of PKC inhibitors. The PKC family consists of at least 10 members, which are divided into three groups on the basis of their structural characteristics and cofactor requirements: the classical PKC (cPKC), the novel PKC (nPKC) and the atypical PKC (aPKC) isoforms (26). As Fig. 5 shows, Gö6983 (10 μM), an aPKC inhibitor (27), clearly inhibited the formation of COX-2-NFκB DNA-protein complex induced by IL-1β, whereas Gö6976 (10 μM), an inhibitor of cPKC and nPKC (28,29), did not. Next, the effects of inhibitors on IL-1β-stimulated PGE_2 release were examined. As Fig. 6 shows, IL-1β-induced PGE_2 release was clearly reduced in the presence of Gö6983, but not in the presence of Gö6976.

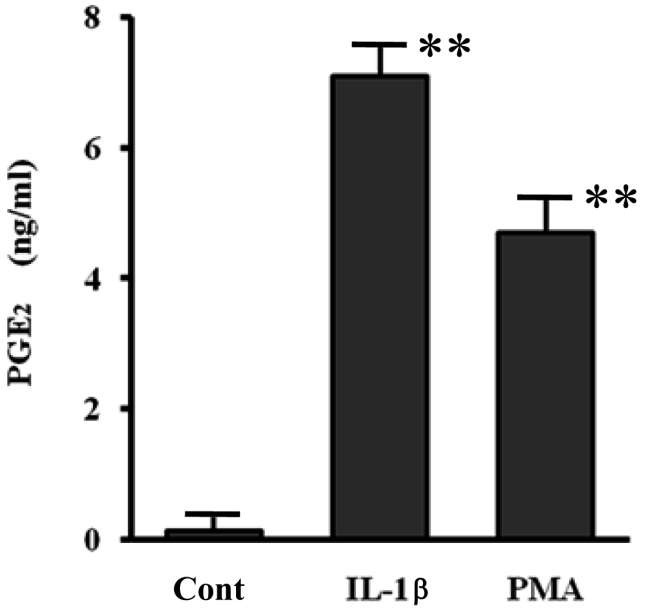

Fig. 1 IL-1β- or PMA-induced PGE_2 release. Human gingival fibroblasts were treated with IL-1β (1 ng/ml) or PMA (100 nM) for 12 h. After incubation, PGE_2 levels in the media were measured by an enzyme immunoassay. Values are expressed as means ± S.E.M. of 4-6 independent experiments. ** $P < 0.01$, significant differences from the control using analysis of variance followed by Dunnett's post-test.

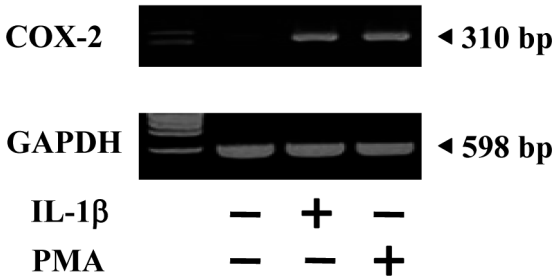

Fig. 2 IL-1β- or PMA-induced COX-2 mRNA expression. Total RNA was isolated from human gingival fibroblasts treated with IL-1β (1 ng/ml) or PMA (100 nM) for 6 h. The mRNA levels of COX-2 and GAPDH were assessed by RT-PCR. The PCR products for COX-2 and GAPDH, 310 and 598 bps, respectively, were detected. Results are representative of three independent experiments.

These results suggest that aPKC is involved in transcriptional control of COX-2 via NFκB activation, which consequently regulates IL-1β-induced PGE_2 synthesis in human gingival fibroblasts.

Discussion

The pro-inflammatory cytokine IL-1β enhances PGE_2 synthesis in human gingival fibroblasts. However, the signaling pathways implicated in IL-1β-induced PGE_2 synthesis are not well understood. In this study, the involvement of PKC in IL-1β-induced PGE_2 release via COX-2 expression in human gingival fibroblasts was demonstrated.

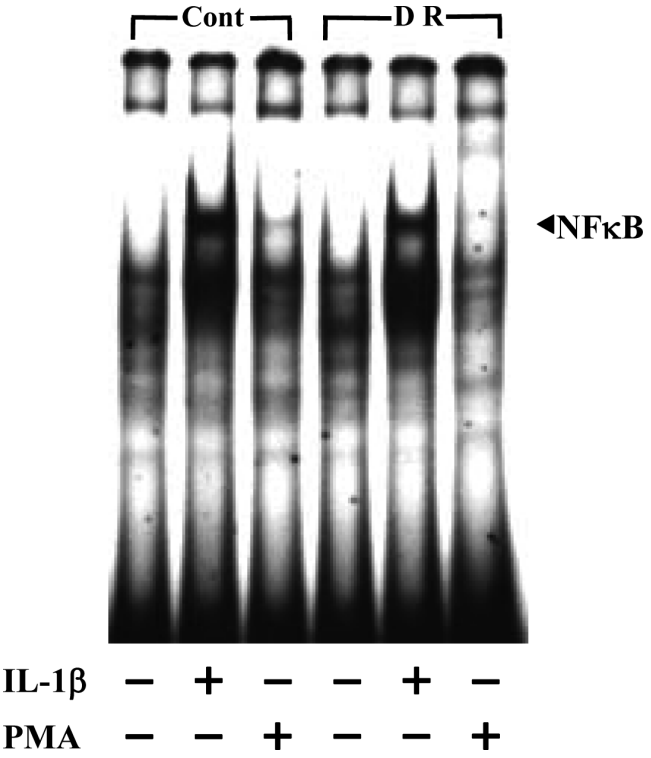

Fig. 3 IL-1β- and PMA-induced DNA binding activity recognizing the NFκB sequence in normal and PKC-down-regulated human gingival fibroblasts. Human gingival fibroblasts were treated with (DR) or without (Cont) PMA (100 nM) for 18 h to induce the down-regulation of PKC. Then nuclear protein extracts were isolated from the cells further treated with IL-1β (1 ng/ml) or PMA (100 nM) for 60 min. Radiolabeled double-stranded COX-2-NFκB was incubated for 20 min at 21°C with a nuclear protein extract (5 μg). DNA-protein complexes were separated on 5% polyacrylamide in low-strength Tris borate buffer, dried under vacuum, and exposed to an imaging plate for quantitation using an image analyzer. Results are representative of three independent experiments.

In human gingival fibroblasts, PMA, a known direct PKC activator, mimicked the effect of IL-1β on COX-2 mRNA expression and PGE_2 release. These results suggest that PMA-activated PKC is involved in PGE_2 release via COX-2 expression.

The NFκB transcription factors play key roles in regulating the expression of a variety of genes involved in immune and inflammatory responses, cell proliferation, and apoptosis (27). In human gingival fibroblasts, pro-inflammatory cytokines such as IL-1β and TNF-α, which play a major role in inflammation, rapidly induce NFκB activation and cause up-regulation of NFκB-dependent genes, including COX-2 (8,9). However, PMA had a less marked stimulatory effect on the formation of COX-2-NFκB-DNA complex, whereas IL-1β had a clearly stimulatory effect. In the PMA-dependent PKC-down-regulated cells, PMA failed to induce the formation of COX-2-NFκB-DNA complex, whereas IL-1β induced sufficient stimulation. These observations suggest that PMA-stimulated PKC or PKC itself is less involved in COX-2 expression via NFκB activation induced by IL-1β although the PMA-sensitive PKC pathway appears to contribute to

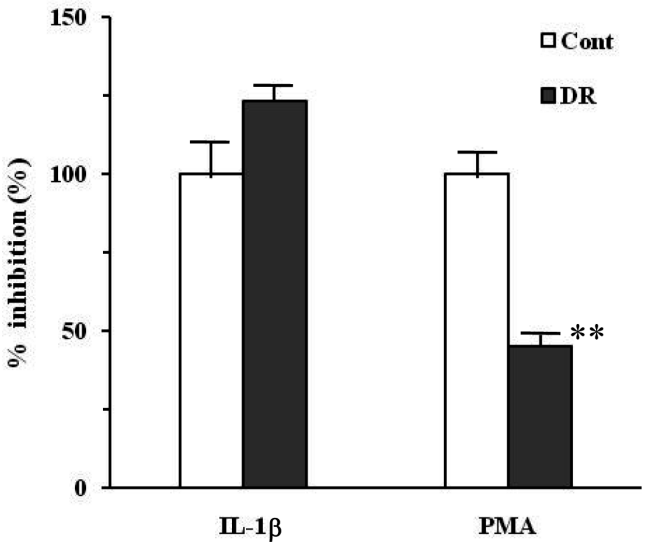

Fig. 4 IL-1β- or PMA-induced PGE_2 release in PKC-down-regulated human gingival fibroblasts. Human gingival fibroblasts were treated with (DR) or without (Cont) PMA (100 nM) for 18 h to induce down-regulation of PKC, then treated with IL-1β (1 ng/ml) or PMA (100 nM) for 6 h. After incubation, PGE_2 levels in the media were measured by an enzyme immunoassay. Values are represented as percentages of the control. Values are expressed as means ± S.E.M. of 4-6 independent experiments. ** $P < 0.01$, significant differences from the control using analysis of variance followed by Welch's *t*-test.

PGE_2 release via COX-2 mRNA expression induced by factors other than IL-1β.

The PKC family is composed of at least 10 members, which are divided into three subgroups based on their structural characteristics and cofactor requirements. They include the classical PKC (cPKC; α, βI, βII and γ), the novel PKC (nPKC; δ, ε, η and θ), and the atypical PKC (aPKC; ζ and ι/λ) isoforms (28). All isoforms require phosphatidylserine, a component of the phospholipid bilayer, for their activation. The cPKCs are Ca^{2+}-sensitive and also require diacylglycerol (DAG) or tumor-promoting phorbol esters for their activation. The nPKCs are Ca^{2+}-independent but still require DAG or phorbol esters (29, 30). The aPKCs are also Ca^{2+}-independent, and phosphatidylserine is sufficient for their maximal activity (31).

In this study, it was demonstrated that PMA has a very weak effect on the formation of COX-2-NFκB-DNA complex in human gingival fibroblasts. Furthermore, the IL-1β response is Ca^{2+}-independent in human gingival fibroblasts, because IL-1β had no effect on Ca^{2+} mobilization (32). Therefore, the possibility that Ca^{2+}- and PMA-independent PKC contributes to the formation of COX-2-NFκB-DNA complex induced by IL-1β was considered. In fact, the effect of IL-1β on the formation of COX-2-NFκB-DNA complex was inhibited by the aPKC inhibitor Gö6983, but not by the inhibitor of cPKC and nPKC, Gö6976. Taken together, it is conceivable that aPKC is involved in the effect of IL-1β on formation of the COX-2-NFκB-DNA complex.

Even in cells where PMA-sensitive PKC was down-regulated, IL-1β stimulated the formation of COX-2-NFκB-DNA complex and simultaneously induced PGE_2 release. Furthermore, in the presence of the aPKC inhibitor, the effect of IL-1β on formation of the COX-2-NFκB-DNA complex was reduced, simultaneously with that on PGE_2 release. These observations suggest that aPKC contributes to COX-2 expression via NFκB activation induced by IL-1β and subsequently regulates PGE_2 synthesis and release in human gingival fibroblasts.

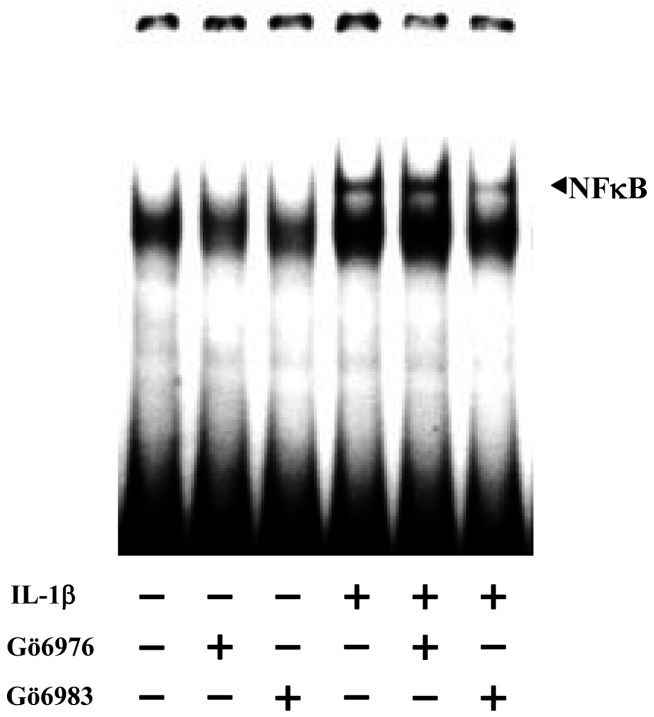

Fig. 5 The effect of PKC inhibitors on IL-1β-induced DNA-complex formation. Human gingival gingival fibrolasts were preincubated with PKC inhibitors (Gö6976 10 μM, Gö6983 10 μM) for 30 min, and then incubated with IL-1β (1 ng/ml) for 60 min. Nuclear protein extracts were obtained from the cells. Radiolabeled double-stranded COX-2-NFκB was incubated for 20 min at 21°C with the nuclear protein extract (5 μg). DNA-protein complexes were separated on 5% polyacrylamide in low-strength Tris borate buffer, dried under vacuum, and exposed to an imaging plate for quantitation using an image analyzer. Results are representative of three independent experiments.

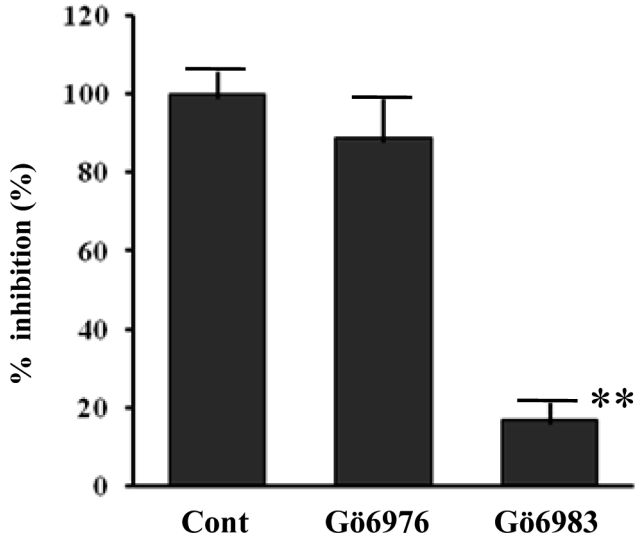

Fig.6 Effect of PKC inhibitors on IL-1β-induced PGE_2 release. Human gingival fibroblasts were preincubated with PKC inhibitors (Gö6976 10 μM, Gö6983 10 μM) for 30 min, and then incubated with IL-1β (1 ng/ml) in for 6 h. After incubation, PGE_2 levels in the media were measured by an enzyme immunoassay. Values are represented as a percentage of IL-1β. Values are expressed as means ± S.E.M. of 4-6 independent experiments. ** $P < 0.01$, significant differences from the control using analysis of variance followed by Dunnett's test.

aPKC consists of ζ and ι/λ isoforms (33). Recently, it has been reported that PKCζ is involved in COX-2 expression in smooth muscle cells and platelets (22,34). Further experiments will be required to confirm which isozyme predominates in the transactivation of the COX-2 gene in response to IL-1β in human gingival fibroblasts.

In conclusion, it has been demonstrated that aPKC is involved in the effect of IL-1β on PGE_2 synthesis via the formation of COX-2-NFκB-DNA complex in human gingival fibroblasts, which appears to be related to inflammation in gingival tissues.

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References

1. Yokota Y, Niisato N, Ogata Y, Moriwaki K, Furuyama S, Sugiya H (1994) Bradykinin stimulates Ca^{2+} release from inositol 1,4,5-trisphosphate sensitive pool, which is insufficient for prostaglandin E_2 release in human gingival fibroblasts. Biomed Res 15, 391-397.
2. Niisato N, Ogata Y, Furuyama S, Sugiya H (1996) Histamine H_1 receptorinduced Ca^{2+} mobilization and prostaglandin E_2 release in human gingival fibroblasts. Biochem Pharmacol 52, 1015-1023.
3. Offenbacher S, Farr DH, Goodson JM (1981) Measurement of prostaglandin E in crevicular fluid. J Clin Periodontol 8, 359-367.
4. Herschman HR (1996) Prostaglandin synthase 2. Biochim Biophys Acta 1299, 125-140.
5. Smith WL, DeWitt DL, Garavito RM (2000) Cyclooxygenases: structural, cellular, and molecular biology. Annu Rev Biochem 69, 145-182.
6. Newton R, Kuitert LM, Bergmann M, Adcock IM, Barnes PJ (1997) Evidence for involvement of NFκB in the transcriptional control of COX-2 gene expression by IL-1β. Biochem Biophys Res Commun 237, 28-32.
7. Subbaramaiah K, Hart JC, Norton L, Dannenberg AJ (2000) Microtubule-interfering agents stimulate the transcription of cyclooxygenase-2. Evidence for involvement of ERK1/2 AND p38 mitogen-activated protein kinase pathways. J Biol Chem 275, 14838-14845.
8. Nakao S, Ogata Y, Shimizu-Sasaki E, Yamazaki M, Furuyama S, Sugiya H (2000) Activation of NFκB is necessary for IL-1β-induced cyclooxygenase-2 (COX-2) expression in human gingival fibroblasts. Mol Cell Biochem 209, 113-118.
9. Nakao S, Ogata Y, Shimizu E, Yamazaki M, Furuyama S, Sugiya H (2002)Tumor necrosis factor alpha (TNF-α)-induced prostaglandin E2 release is mediated by the activation of cyclooxygenase-2 (COX-2) transcription via NFκB in human gingival fibroblasts. Mol Cell Biochem 238, 11-18.
10. Mifflin RC, Saada JI, Di Mari JF, Adegboyega PA, Valentich JD, Powell DW (2002) Regulation of COX-2 expression in human intestinal myofibroblasts: mechanisms of IL-1-mediated induction. Am J Physiol Cell Physiol 282, C824-834.
11. Yang CM, Chien CS, Hsiao LD, Luo SF, Wang CC (2002) Interleukin-1β-induced cyclooxygenase-2 expression is mediated through activation of p42/44 and p38 MAPKS, and NFκB pathways in canine tracheal smooth muscle cells. Cell Signal 14, 899-911.
12. Lin CH, Sheu SY, Lee HM, Ho YS, Lee WS, Ko WC, Sheu JR (2000) Involvement of protein kinase C-γ in IL-1β-induced cyclooxygenase-2 expression in human pulmonary epithelial cells. Mol Pharmacol 57, 36-43.
13. Molina-Holgado E, Ortiz S, Molina-Holgado F, Guaza C (2000) Induction of COX-2 and PG E_2 biosynthesis by IL-1β is mediated by PKC and mitogen-activated protein kinases in murine astrocytes. Br J Pharmacol 131, 152-159.
14. Di Mari JF, Mifflin RC, Adegboyega PA, Saada JI, Powell DW (2003) IL-1α-induced COX-2 expression in human intestinal myofibroblasts is dependent on a PKCζ-ROS pathway. Gastroenterology 124, 1855-1865.
15. Somerman MJ, Archer SY, Imm GR, Foster RA (1988) A comparative study of human periodontal ligament cell and gingival fibroblasts in vitro. J Dent Res 67, 66-70.
16. Hla T, Neilson K (1992) Human cyclooxygenase-2 cDNA. Proc Natl Acad Sci 89, 7384-7388.
17. Tokunaga K, Nakamura Y, Satake K, Fujimori K, Ohkubo M, Sawada K, Sakiyama S (1987) Enhanced expression of a glyceraldehydes-3-phosphate dehydrogenase gene in human lung cancers. Cancer Res 47, 5616-5619.
18. Nakao S, Ogata Y, Yamamoto Y, Furuyama S, Sugiya H (2004) Platelet-derived growth factor-induced archidonic acid release for enhancement of prostaglandin E_2 synthesis in human gingival fibroblasts pretreated with interleukin-1β. J Cell

Biochem 92, 579-590.
19. Dignam JD, Lebovitz RM, Roeder RG (1983) Accurate transcription initiation by RNA polymerase II in a soluble extract from isolated mammalian nuclei. Nucleic Acids Res 11, 1475-1489.
20. Inoue H, Umesono K, Nishimori T, Hirata Y, Tanabe T (1999) Glucocorticoid-mediated suppression of the promoter activity of the cyclooxygenase-2 gene is modulated by the expression of its receptor in vascular endothelial cells. Biochem Biophys Res Commun 254, 579-590.
21. Samoto H, Shimizu E, Matsuda-Honjyo Y, Saito R, Nakao S, Yamazaki M, Furuyama S, Sugiya H, Sodek J, Ogata Y (2003) Prostaglandin E_2 stimulates bone sialoprotein (BSP) expression through cAMP and fibroblast growth factor 2 response elements in the proximal promoter of the rat BSP gene. J Biol Chem 278, 28659-28667.
22. Duggan SV, Lindstrom T, Iglesias T, Bennett PR, Mann GE, Bartlett SR (2007) Role of atypical protein kinase C isozymes and NFκB in IL-1β-induced expression of cyclooxygenase-2 in human myometrial smooth muscle cells. J Cell Physiol 210, 637-643.
23. Ohno S, Nishizuka Y (2002) Protein kinase C isotypes and their specific functions: prologue. J Biochem 132, 509-511.
24. Cussac D, Newman-Tancredi A, Pasteau V, Millan MJ (1999) Human dopamine D3 receptors mediate mitogen-activated protein kinase activation via a phosphatidylinositol 3-kinase and an atypical protein kinase C-dependent mechanism. Mol Pharmacol 56, 1025-1030.
25. Martiny-Baron G, Kazanietz MG, Mischak H, Blumberg PM, Kochs G, Hug H, Marmé D, Schächtele C (1993) Selective inhibition of protein kinase C isozymes by the indolocarbazole Gö 6976. J Biol Chem 268, 9194-9197.
26. Gschwendt M, Dieterich S, Rennecke J, Kittstein W, Mueller HJ, Johannes FJ (1993) Inhibition of protein kinase Cμ by various inhibitors. Differentiation from protein kinase C isoenzymes. FEBS Lett 26, 77-80.
27. Baldwin AS Jr (1996) The NFκB and I κB proteins: new discoveries and insights. Annu Rev Immunol 14, 649-683.
28. Steinberg SF (2008) Structural basis of protein kinase C isoform function. Physiol Rev 88, 1341-1378.
29. Farhadi A, Keshavarzian A, Ranjbaran Z, Fields JZ, Banan A (2006) The role of protein kinase C isoforms in modulating injury and repair of the intestinal barrier. J Pharmacol Exp Ther 316, 1-7.
30. Das Evcimen N, King GL (2007) The role of protein kinase C activation and the vascular complications of diabetes. Pharmacol Res 55, 498-510.
31. Moscat J, Rennert P, Diaz-Meco MT (2006) PKCζ at the crossroad of NFκB and Jak1/Stat6 signaling pathways. Cell Death Differ 13, 702-711.
32. Nakao S, Ogata Y, Modéer T, Segawa M, Furuyama S, Sugiya H (2001) Bradykinin induces a rapid cyclooxygenase-2 mRNA expression via Ca^{2+} mobilization in human gingival fibroblasts primed with interleukin-1β. Cell Calcium 29, 446-452.
33. Suzuki A, Akimoto K, Ohno S (2003) Protein kinase C λ/ι (PKC λ/ι): a PKC isotype essential for the development of multicellular organisms. J Biochem 133, 9-16.
34. Barry OP, Kazanietz MG, Praticò D, FitzGerald GA (1999) Arachidonic acid in platelet microparticles up-regulates cyclooxygenase-2-dependent prostaglandin formation via a protein kinase C/mitogen-activated protein kinase-dependent pathway. J Biol Chem 274, 7545-7556.