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Bradykinin potentiates prostaglandin E_2 release in the human gingival fibroblasts pretreated with interleukin-1β via Ca^{2+} mobilization

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Abstract

Interleukin-1β, a proinflammatory cytokine, causes a slow increase in prostaglandin E_2 release. On the other hand, bradykinin, a chemical mediator for inflammation, induces a rapid prostaglandin E_2 release. Simultaneous stimulation with interleukin-1β (200 pg/ml) and bradykinin (1 μM) evoked a moderately synergistic increase in prostaglandin E_2 release in human gingival fibroblasts. However, in the human gingival fibroblasts pretreated with interleukin-1β, bradykinin drastically enhanced prostaglandin E_2 release. NS-398, a specific inhibitor of cyclooxygenase-2, inhibited not only interleukin-1β-induced prostaglandin E_2 release but also bradykinin-induced prostaglandin E_2 release in the human gingival fibroblasts pretreated with interleukin-1β. Transcriptional and translational inhibitors such as actinomycin D, cycloheximide, and dexamethasone also suppressed the interleukin-1β-induced prostaglandin E_2 release and the bradykinin-induced prostaglandin E_2 release in interleukin-1β-pretreated human gingival fibroblasts. In the fibroblasts pretreated with interleukin-1β, Ca^{2+}-mobilizing reagents such as ionomycin and thapsigargin mimicked the potentiating effect of bradykinin on prostaglandin E_2 release. These results suggest that interleukin-1β- and bradykinin-induced prostaglandin E_2 release is dependent on cyclooxygenase-2 and the potentiated effect of bradykinin in the human gingival fibroblasts primed with interleukin-1β is caused by Ca^{2+} mobilization.

Keywords: Bradykinin; Interleukin-1β; Prostaglandin E_2; Ca^{2+} mobilization; Gingival fibroblast, human

1. Introduction

Prostaglandin E_2 is widely distributed in various organs and exerts effects on various biological activities (Shimizu and Wolfe, 1990). In inflammation processes, prostaglandin E_2 is considered likely to play crucial roles, since chemical mediators invoke prostaglandin E_2 synthesis in fibroblasts (Unemori et al., 1994), endothelial cells (Bottoms et al., 1985), monocytes (Nichols et al., 1987), and neutrophils (Doerfler et al., 1989) at inflammation sites. The rate of production of prostanoids, including prostaglandin E_2, is determined in part by the levels of cyclooxygenase. Two forms of cyclooxygenase have been characterized: a constitutively expressed form, cyclooxygenase-1, and an inducible form, cyclooxygenase-2 (DeWitt, 1991; Geng et al., 1995). Cyclooxygenase-1 may be responsible for basal prostanoid biosynthesis and may be necessary for maintenance of physiological functions and cytoprotection. In contrast, cyclooxygenase-2, which is rapidly induced in inflammatory states, may produce the prostanoids involved in immune and/or inflammatory responses.

Bradykinin is a small peptide with a potent ability to induce pain, vasodilation, and increase of vascular permeability, and is implicated in the pathogenesis of inflammation (Regoli and Barabe, 1980). It has been suggested that proinflammatory properties of bradykinin are mediated by inducing the synthesis and release of arachidonic acid and the prostanoids in a variety of target cells and tissues. We have previously shown that bradykinin rapidly induces prostaglandin E_2 release coupled to Ca^{2+} influx from an extracellular site in human gingival fibroblasts (Yokota et al., 1994). In addition, bradykinin stimulated bone resorp-

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tion in vitro by a mechanism dependent on prostanoid (Gustafson and Lerner, 1984), suggesting that bradykinin may be involved in chronic inflammatory diseases such as periodontitis and rheumatoid arthritis. In the inflammatory process, the formation of prostanoids is regulated not only by bradykinin, but also by several cytokines. Bradykinin has been considered to invoke a greater response in inflamed tissues than in non-inflamed tissues, implying that the effect of bradykinin is potentiated in the cells primed by cytokines. Interleukin-1β is a cytokine implicated in inflammatory and immunologic responses. Interleukin-1β takes part in inflammatory processes, including secretion of collagenase, synthesis of interleukin-6, and prostaglandin E_2 production, in a variety of cells (Dayer et al., 1986; O'Neill and Lewis, 1989; Guerne et al., 1989; Conquer et al., 1992; Endo et al., 1995). In this study, we investigated the effect of bradykinin on prostaglandin E_2 release in the human gingival fibroblasts pretreated with interleukin-1β. Our data show bradykinin induced prostaglandin E_2 release is enhanced in the human gingival fibroblasts primed with interleukin-1β via Ca^{2+} mobilization.

2. Materials and methods

2.1. Materials

α-Minimal essential medium (α-MEM), penicillin and streptomycin, trypsin EDTA and fetal calf serum were purchased from Gibco (Grand Island, NY, USA). Interleukin-1β was purchased from Genzyme (Cambridge, MA, USA). Bradykinin was obtained from Peptide Institute (Osaka, Japan). Prostaglandin E_2 enzyme-linked immunoassay kits was obtained from Cayman Chemical (Ann Arbor, MI, USA). NS-398 was obtained from BIOMOL (Plymouth, PA, USA). All other reagents were from Wako Pure Chemical (Osaka, Japan).

2.2. Cell culture

Human gingival fibroblasts were obtained from explants of healthy human gingival connective tissue according to the method of Somerman et al. (1988). The cells were maintained in α-MEM containing 10% fetal calf serum, 100 U/ml of penicillin, and 100 μg/ml of streptomycin at 37°C under 5% CO_2 in air. When the cells surrounding the tissue explants were confluent, they were subcultured with 0.05% trypsin, 0.53 mM EDTA in Hank's balanced salt solution, and transferred to tissue culture flasks (Ogata et al., 1995a; Niisato et al., 1996).

2.3. Measurement of prostaglandin E_2

Human gingival fibroblasts were cultured in 12-well culture plates in α-MEM containing 10% fetal calf serum. When the cells were confluent, the medium was changed to serum-free α-MEM for 18 h, and interleukin-1β and the other compounds to be tested were added. At the indicated time intervals, medium was collected and the amount of prostaglandin E_2 was determined by enzyme immunoassay with acetylcholinesterase-labeled prostaglandin E_2 as a tracer, using a commercially available kit (Ogata et al., 1995a,b; Niisato et al., 1996).

2.4. Intracellular Ca^{2+} determination

Human gingival fibroblasts were cultured on thin, circular glass plates (9 mm in diameter) in α-MEM containing 10% fetal calf serum. Confluent fibroblasts were preincubated with 2 μM 1-[6-amino-2-(5-carboxy-2-oxazolyl)-5-benzofuranyloxy]-2-(2-amino-5-methylphenoxy)-ethane-*N*, *N*, *N*′, *N*′-tetraacetic acid, pentaacetoxymethyl ester (fura-2/AM) in α-MEM for 30 min at 37°C. After they were loaded with fura-2/AM, the glass plates with the attached cells were washed twice and put into quartz cuvettes containing Krebs–Ringer–HEPES solution [120 mM NaCl, 5 mM KCl, 1 mM $MgSO_4$, 0.96 mM NaH_2PO_4, 0.2% glucose, 0.1% bovine serum albumin, 1 mM $CaCl_2$, and 20 mM HEPES (pH 7.4)] for the determination of intracellular Ca^{2+} ($[Ca^{2+}]_i$). The fluorescence of fura-2-loaded cells was measured with a CAF-110 spectrofluorometer (Nihon Bunkou, Japan) with excitation at 340 and 380 nm and emission at 500 nm. $[Ca^{2+}]_i$ was calculated from the ratio of fluorescence intensities (Grynkiewicz et al., 1985).

3. Results

3.1. Interleukin-1β-induced prostaglandin E_2 release

To assess the effect of interleukin-1β on prostaglandin E_2 release in human gingival fibroblasts, fibroblasts were incubated with 200 pg/ml interleukin-1β for various periods (Fig. 1A). Prostaglandin E_2 release was clearly detected 6 h after stimulation, and increased up to 24 h. On the other hand, prostaglandin E_2 levels released from human gingival fibroblasts incubated without interleukin-1β for 6 and 24 h were very low, 0.36 ± 0.04 and 0.43 ± 0.08 ng/3×10^5 cells, respectively. When the fibroblasts were incubated with interleukin-1β at various concentrations for 6 h, prostaglandin E_2 was released in a concentration-dependent manner, and reached a maximum at 200 pg/ml, as shown in Fig. 1B.

3.2. Bradykinin-stimulated prostaglandin E_2 release in human gingival fibroblasts pretreated with interleukin-1β

As shown in Table 1, stimulation by bradykinin (1 μM) for 6 h also resulted in an increase in prostaglandin E_2 release from human gingival fibroblasts. However, the

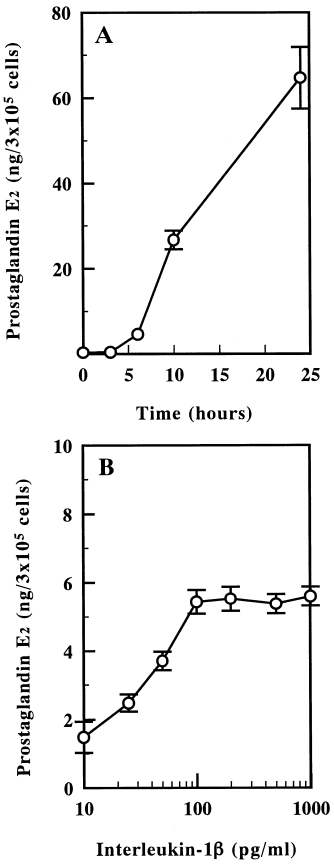

Fig. 1. Time-(A) and dose-(B) dependencies of interleukin-1β-induced prostaglandin E_2 release in human gingival fibroblasts. Human gingival fibroblasts were incubated with interleukin-1β (200 pg/ml) for the indicated times (A), and with the indicated concentrations of interleukin-1β for 6 h (B). At the end of the incubation, prostaglandin E_2 levels in the media were measured by enzyme immunoassay. Values are expressed as means ± S.E.M. of three to five independent experiments.

level of prostaglandin E_2 released was similar to that induced by the stimulation with bradykinin for 30 min (3.2 ± 0.6 ng/3×10^5 cells). We have previously shown that bradykinin rapidly stimulated prostaglandin E_2 release from human gingival fibroblasts (Yokota et al., 1994). These data imply that bradykinin causes prostaglandin E_2 release by a rapidly acting mechanism. Interleukin-1β (200 pg/ml) also stimulated prostaglandin E_2 release in human gingival fibroblasts as shown in Fig. 1 and Table 1. The level of prostaglandin E_2 induced by the simultaneous stimulation with interleukin-1β (200 pg/ml) and bradykinin (1 μM) for 6 h was higher than that induced by separate stimulation with each drug as shown in the previous report (Sundqvist and Lerner, 1996). However, when human gingival fibroblasts were preincubated with 200 pg/ml interleukin-1β for 6 h and then stimulated with 1 μM bradykinin for 30 min, prostaglandin E_2 release was markedly augmented (Table 1). In the human gingival fibroblasts pretreated with interleukin-1β for 6 h, bradykinin potentiated prostaglandin E_2 release in a time-dependent manner from 5 to 30 min (Fig. 2). Fig. 3 shows the stimulation of prostaglandin E_2 release induced by bradykinin (1 μM) for 30 min in the human gingival fibroblasts pretreated with interleukin-1β (200 pg/ml) for various periods. In the fibroblasts pretreated with interleukin-1β for 3, 6, 10 and 24 h, bradykinin increased interleukin-1β-induced prostaglandin E_2 release by about 40-, 9-, 2.5-, and 1.2-fold, respectively. These results indicate that bradykinin synergistically potentiates prostaglandin E_2 release in the interleukin-1β-pretreated human gingival fibroblasts. The potentiation of prostaglandin E_2 release by bradykinin occurred even in the human gingival fibroblasts washed to remove interleukin-1β after the preincubation (data not shown).

Table 1
Synergistic effect of interleukin-1β and bradykinin on prostaglandin E_2 release in human gingival fibroblasts
Human gingival fibroblasts were incubated with vehicle (Control), bradykinin (1 μM), interleukin-1β (200 pg/ml), interleukin-1β (200 pg/ml), and bradykinin (1 μM) (Interleukin-1β/Bradykinin) for 6 h, or interleukin-1β (200 pg/ml) for 6 h and then stimulated with bradykinin (1 μM) for 30 min (Interleukin-1β + Bradykinin). After the stimulation, prostaglandin E_2 levels in the media were measured by enzyme immunoassay. Data are means ± S.E.M. of triplicate determinations and are representative of three separate experiments.

Treatment	Prostaglandin E_2 release (ng/3×10^5 cells)
Control	0.44 ± 0.08
Bradykinin	3.00 ± 0.05
Interleukin-1β	4.70 ± 0.93
Interleukin-1β/Bradykinin	23.08 ± 3.51
Interleukin-1β + Bradykinin	46.98 ± 5.81

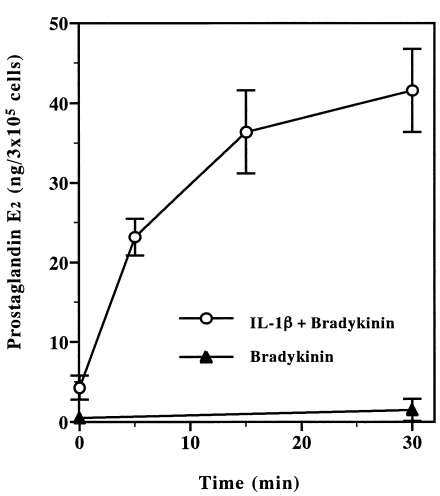

Fig. 2. Bradykinin-stimulated prostaglandin E_2 release in the human gingival fibroblasts pretreated with or without interleukin-1β. Human gingival fibroblasts were incubated with (○) or without (●) interleukin-1β (IL-1β, 200 pg/ml) for 6 h and subsequently stimulated with bradykinin (1 μM) for varying times. At the end of the incubation, the prostaglandin E_2 level in the medium was measured by enzyme immunoassay. Values are means ± S.E.M. of three to five independent experiments.

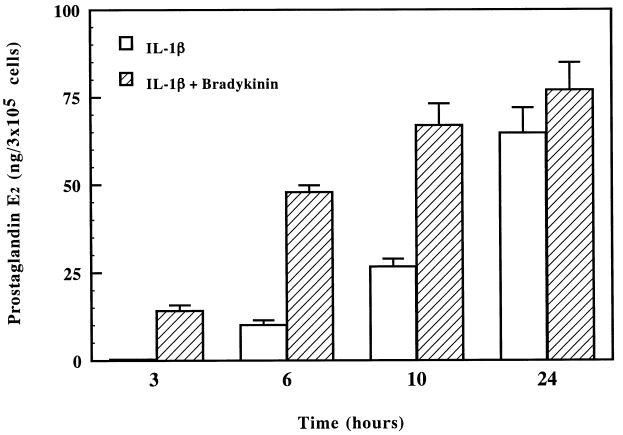

Fig. 3. Change of potentiating effect of bradykinin on prostaglandin E_2 release depended on pretreating time with interleukin-1β. Human gingival fibroblasts were pretreated with interleukin-1β (IL-1β, 200 pg/ml) for 3, 6, 10 or 24 h and then subsequently incubated without (open bars) or with (hatched bars) bradykinin (1 μM) for 30 min. After the incubation, prostaglandin E_2 levels in the media were measured by enzyme immunoassay. Values are means ± S.E.M. of three to five independent experiments.

3.3. Effect of a cyclooxygenase-2 inhibitor on prostaglandin E_2 release induced by interleukin-1β plus bradykinin

To elucidate which cyclooxygenase enzyme regulates prostaglandin E_2 release induced by interleukin-1β or by bradykinin followed by interleukin-1β, we investigated the effect of a specific inhibitor of cyclooxygenase-2, NS-398. As shown in Table 2, NS-398 (10 μM) completely inhibited interleukin-1β-induced prostaglandin E_2 release. In the human gingival fibroblasts pretreated with interleukin-1β, bradykinin induced prostaglandin E_2 release was inhibited in the presence of NS-398, although the prostaglandin E_2 level was a bit higher than the control level. In the fibroblasts without pretreatment with interleukin-1β, NS-398 had no effect on bradykinin-induced PGE_2 release (data not shown). These results suggest that cyclooxygenase-2 is contributed to prostaglandin E_2 release induced by interleukin-1β and by bradykinin followed by interleukin-1β.

3.4. Effects of transcriptional and translational inhibitors on prostaglandin E_2 release induced by interleukin-1β plus bradykinin

We next investigated the effects of transcriptional and translational inhibitors. Table 3 summarizes the effect of the inhibitors on prostaglandin E_2 release induced by interleukin-1β and bradykinin after interleukin-1β pretreatment. When HGF were incubated with interleukin-1β (200 pg/ml) for 6 h in the presence of 1 μM actinomycin D or 100 μM cycloheximide, the prostaglandin E_2 release induced by interleukin-1β was significantly reduced. The bradykinin-induced prostaglandin E_2 release in the human gingival fibroblasts pretreated with interleukin-1β was completely inhibited by actinomycin D and partially by cycloheximide. These results suggest that interleukin-1β- and bradykinin-induced prostaglandin E_2 release is coupled to protein synthesis, perhaps through cyclooxygenase-2 transcriptional and translational pathways. Dexamethasone (100 nM) also inhibited interleukin-1β- and bradykinin-induced prostaglandin E_2 release. Because dexamethasone has been reported to be an inhibitor of cyclooxygenase-2 expression (Mitchell et al., 1994; Szczepanski et al., 1994), this result also suggests that cyclooxygenase-2 expression contributes to the prostaglandin E_2 release induced by bradykinin in the fibroblasts pretreated with interleukin-1β.

Table 2
Inhibition of interleukin-1β-induced prostaglandin E_2 release and bradykinin induced prostaglandin E_2 release in interleukin-1β-pretreated human gingival fibroblasts by a cyclooxygenase-2 inhibitor
Human gingival fibroblasts were incubated with interleukin-1β (200 pg/ml) for 6 h and then stimulated with vehicle (Interleukin-1β + Vehicle) or bradykinin (1 μM) (Interleukin-1β + Bradykinin) for 30 min in the absence or presence of NS 398 (10 μM). After the stimulation, the prostaglandin E_2 level in the medium was measured by enzyme immunoassay. Data are means ± S.E.M of fourfold determinations and are representative of four separate experiments.

Stimulation	Prostaglandin E_2 release ($ng/3\times10^5$ cells)	
	Without NS-398	With NS-398
Control	0.42 ± 0.06	0.40 ± 0.03
Interleukin-1β + Vehicle	22.25 ± 1.83	0.36 ± 0.07
Interleukin-1β + Bradykinin	69.35 ± 3.32	2.00 ± 0.49

Table 3
Inhibition of interleukin-1β-induced prostaglandin E_2 release and bradykinin induced prostaglandin E_2 release in interleukin-1β-pretreated human gingival fibroblasts by transcription and translation inhibitors
Human gingival fibroblasts were incubated with interleukin-1β (200 pg/ml) for 6 h and then stimulated with vehicle (Interleukin-1β + Vehicle) or bradykinin (1 μM) (Interleukin-1β + Bradykinin) for 30 min in the presence of actinomycin D (1 μM), cycloheximide (100 μM), or dexamethasone (100 nM). After the stimulation, the prostaglandin E_2 level in the medium was measured by enzyme immunoassay. The level of prostaglandin E_2 induced by interleukin-1β or bradykinin after pretreatment by interleukin-1β was taken as 100% prostaglandin E_2 release. Data are means ± S.E.M. of triplicate determinations and are representative of three separate experiments.

Inhibitors	Prostaglandin E_2 release (% of control value)	
	Interleukin-1β Vehicle	Interleukin-1β + Bradykinin
Actinomycin D	3.74 ± 0.73	2.21 ± 0.45
Cycloheximide	4.41 ± 0.93	27.70 ± 1.61
Dexamethasone	6.52 ± 1.30	4.58 ± 0.90

3.5. Role of Ca^{2+} mobilization in the increase of prostaglandin E_2 release by bradykinin

We have previously demonstrated that bradykinin induces a rapid increase in $[Ca^{2+}]_i$ in human gingival fibroblasts (Lerner et al., 1992a; Yokota et al., 1994). Therefore, the effect of the Ca^{2+}-mobilizing reagent ionomycin on prostaglandin E_2 release in human gingival fibroblasts pretreated with interleukin-1β was investigated. Ionomycin (1 μM) showed the same effect on prostaglandin E_2 release as bradykinin in the human gingival fibroblasts not pretreated with interleukin-1β (data not shown). In the fibroblasts pretreated with interleukin-1β, ionomycin mimicked the potentiating effect of bradykinin on prostaglandin E_2 release (Fig. 4). When the extracellular Ca^{2+} was chelated with 1 mM EGTA, bradykinin- and ionomycin-induced prostaglandin E_2 release was partially reduced. The incompleteness of the reduction was probably due to Ca^{2+} release from intracellular Ca^{2+} pools. Thapsigargin induced the same effect on prostaglandin E_2 release (data not shown). We checked the effects of bradykinin and ionomycin on the levels of $[Ca^{2+}]_i$ in fura-2-loaded human gingival fibroblasts pretreated with or without interleukin-1β. The peak levels of $[Ca^{2+}]_i$ in human gingival fibroblasts treated with bradykinin and ionomycin were not affected by the interleukin-1β pretreatment (data not shown). Taken together, these results suggest that Ca^{2+} mobilization consisted of release from intracellular Ca^{2+} pools, and that Ca^{2+} influx from extracellular sites is necessary for the bradykinin-induced potentiation of prostaglandin E_2 release in human gingival fibroblasts.

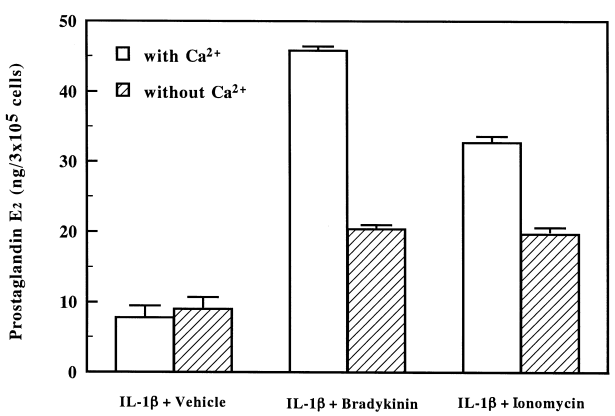

Fig. 4. Effect of extracellular Ca^{2+} on prostaglandin E_2 release induced by bradykinin and ionomycin in human gingival fibroblasts pretreated with interleukin-1β. Human gingival fibroblasts were pretreated with interleukin-1β (IL-1β, 200 pg/ml) for 6 h, and further incubated with vehicle, bradykinin (1 μM) or ionomycin (1 μM) for 30 min in the presence (open bars) or the absence (hatched bars) of extracellular Ca^{2+}. In the cultures without extracellular Ca^{2+}, EGTA (1 mM) was added in the medium. At the end of the incubation, prostaglandin E_2 levels in the media were measured by enzyme immunoassay. Values are expressed as the means ± S.E.M. of three to five independent experiments.

4. Discussion

In this report, we have demonstrated that bradykinin synergistically potentiates prostaglandin E_2 release in interleukin-1β-pretreated human gingival fibroblasts and the effect of bradykinin is linked to the increase in $[Ca^{2+}]_i$. The synergistic effect of interleukin-1β and bradykinin on prostaglandin E_2 release has been demonstrated in human synovial cells (Angel et al., 1994; Bathon et al., 1989, 1996; O'Neill and Lewis, 1989). In the synovial cells, bradykinin a relatively small effect on prostaglandin E_2 release. However, bradykinin clearly induced a 2-fold increase in prostaglandin E_2 release from the human synovial cells pretreated with interleukin-1β. Therefore, bradykinin has been considered to be a modulator of the effect of interleukin-1β on prostaglandin E_2 release in human synovial cells (Angel et al., 1994; Bathon et al., 1989, 1996; O'Neill and Lewis, 1989). The synergistic effect of interleukin-1β and bradykinin on prostaglandin E_2 release has also been reported in human dental pulp fibroblasts (Sundqvist and Lerner, 1996). In the cells, a simultaneous treatment with bradykinin and interleukin-1β for 2–24 h synergistically stimulated prostaglandin E_2 release. Furthermore, a subsequent treatment with bradykinin for 24 h in the pulp fibroblasts preincubated with interleukin-1β for 24 h evoked a synergistic release of prostaglandin E_2. In human gingival fibroblasts, simultaneous stimulation with interleukin-1β and bradykinin caused greater release of prostaglandin E_2 than that induced by separate stimulation with interleukin-1β or bradykinin (Lerner and Modéer, 1991). Moreover, bradykinin caused a rapid and drastic increase in the level of prostaglandin E_2 released from the human gingival fibroblasts pretreated with interleukin-1β as demonstrated here. The potentiating effect of bradykinin on prostaglandin E_2 release was the largest in the human gingival fibroblasts pretreated for 3 h, and decreased with increasing time of pretreatment with interleukin-1β, suggesting that bradykinin accelerates the prostaglandin E_2 release induced by IL-1β. The potentiating effect of bradykinin also occurred in human gingival fibroblasts washed after the interleukin-1β-pretreatment. Therefore, bradykinin appears to modulate prostaglandin E_2 release in the interleukin-1β-pretreated or primed human gingival fibroblasts.

Actinomycin D and cycloheximide, transcriptional and translational inhibitors, respectively, inhibited the interleukin-1β-induced prostaglandin E_2 release. Prostaglandin E_2 production is regulated by a constitutive enzyme, cyclooxygenase-1, and an inducible enzyme, cyclooxygenase-2 (DeWitt, 1991). The expression of cyclooxygenase-2 mRNA is modulated by many extracellular stimuli such as mitogens, cytokines and hormones, whereas cyclooxygenase-1 expression is not affected by such stimuli (Geng et al., 1995). Interleukin-1β regulates the expression of cyclooxygenase-2 in the many cell types such as synovial cells (Knott et al., 1994), macrophages (Thivierge

and Rola-Pleszczynski, 1995), and mesangial cells (Tetsuka et al., 1996). Therefore, it has been speculated that control of cyclooxygenase-2 transcription and de novo protein synthesis of newly synthesized cyclooxygenase-2 enzyme contributes to interleukin-1β-dependent stimulation of prostaglandin E_2 release.

A primary effect of bradykinin is intracellular Ca^{2+} mobilization in human gingival fibroblasts (Lerner et al., 1992b; Yokota et al., 1994; Yucel-Lindberg et al., 1995; Niisato et al., 1997). We observed here that ionomycin and thapsigargin mimicked the potentiating effect of bradykinin on prostaglandin E_2 release in human gingival fibroblasts pretreated with interleukin-1β, implying that the effect of bradykinin is dependent on an increase in $[Ca^{2+}]_i$. In human gingival fibroblasts, interleukin-1β had no effect on $[Ca^{2+}]_i$, nor did it alter the bradykinin-induced increase in $[Ca^{2+}]_i$ (Lerner et al., 1992b). These results indicate that Ca^{2+} mobilization is important for the potentiation of prostaglandin E_2 release via cyclooxygenase-2 expression in interleukin-1β-primed human gingival fibroblasts. However, the mechanism of the Ca^{2+} dependent effect on cyclooxygenase-2 expression is not yet known. Since arachidonic acid has been reported to stimulate expression of immediate early genes such as c-*fos* and Erg-1 in fibroblasts (Danesch et al., 1994), a Ca^{2+}-dependent phospholipase A_2 activation pathway is a candidate. However, no synergistic interaction between bradykinin and interleukin-1β on the release of arachidonic acid was seen in human gingival fibroblasts (Lerner and Modéer, 1991), suggesting phospholipase A_2 pathway less contributes to the synergistic action of bradykinin and interleukin-1β. On the other hand, phospholipase D has been considered to be involved in the synergistic action of bradykinin and interleukin-1β in human synovial cells (Angel et al., 1994). Furthermore, Ca^{2+} oscillation have recently been demonstrated to regulate the efficiency and specificity of gene expression (Dolmetsch et al., 1998; Li et al., 1998). To elucidate whether these mechanisms are involved in the effects of bradykinin on human gingival fibroblasts primed with interleukin-1β, further investigation is necessary.

In conclusion, we have demonstrated the role of bradykinin as a modulator in interleukin-1β-primed human gingival fibroblasts. We showed that bradykinin stimulates intracellular Ca^{2+} mobilization and thereby potentiates cyclooxygenase-2 dependent prostaglandin E_2 release in interleukin-1β primed human gingival fibroblasts. These responses may occur in the periodontal pocket and contribute to inflammatory diseases such as periodontitis.

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