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Interleukin-1β Induces Bradykinin B_2 Receptor Gene Expression through a Prostanoid Cyclic AMP-Dependent Pathway in Human Bronchial Smooth Muscle Cells

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ABSTRACT

We investigated the hypothesis that inflammatory mediators such as interleukin-1β (IL-1β) might be responsible for the hyperreactivity of asthmatic patients to bradykinin. In cultured human bronchial smooth muscle cells, IL-1β elicited a rapid and transient increase in the density of bradykinin B_2 receptors without affecting their affinity for ligands. The increase in B_2 receptors was correlated to an enhancement of inositol phosphate formation elicited by bradykinin, indicating its relevance to the contractile response of smooth muscle cells to bradykinin. The increase in receptor density was related to an increase in B_2 receptor mRNA level corresponding to a 5-fold enhancement of the transcriptional rate and to a lengthened half-life of mRNA. These effects of IL-1β were largely inhibited by indomethacin, suggesting the involvement of a prostanoid pathway in IL-1β transduction process. An increase in prostaglandin E_2 levels preceded the mRNA increase, confirming this involvement. Moreover, IL-1β and prostaglandin E_2 led to cAMP formation. We propose this predominant transduction pathway of IL-1β to stimulate the transcription of the bradykinin B_2 gene in human bronchial smooth muscle cells as a major mechanism involved in the hyperresponsiveness of asthmatic patients to bradykinin.

The nonapeptide BK exerts its effects through two different seven-membrane G protein-coupled receptors, B_1 and B_2. These receptors have been characterized using different analogues of BK (Regoli *et al*., 1990) and have been cloned in human cells (Hess *et al*., 1992; Menke *et al*., 1994). The B_2 receptor is constitutively expressed in many cell types in the lung, including fibroblasts, sensory fibers, and epithelial cells (Mak and Barnes, 1991). The B_1 receptor, which is absent under normal conditions, can be induced by lipopolysaccharides in vascular smooth muscle (DeBlois *et al*., 1989). Bradykinin is generated in the tracheobronchial tree and in the plasma during lung inflammatory diseases such as asthma (Proud and Kaplan, 1988). Kallikrein activity, leading to the synthesis of BK, has been reported in the bronchoalveolar lavage fluid of asthmatic patients (Christansen *et al*., 1987). Bradykinin causes bronchoconstriction in asthmatic patients but not in healthy subjects (Simmonsson *et al*., 1973), suggesting an overexpression of B_2 receptors by the BSMCs in an inflammatory state.

The cytokine IL-1 is a potent contributor to inflammation and is involved in the late asthmatic response (Barnes, 1994). A high level of IL-1β, mainly secreted by macrophages, has been observed in bronchoalveolar lavage fluids from asthmatic patients (Borish *et al*., 1992). The ability of BK to stimulate the release of IL-1β from macrophages has been reported (Tiffany and Burch, 1989), as well as its ability to elicit IL-1β release from isolated lung strips (Paegelow *et al*., 1995). Reciprocally, in another study, Bathon *et al*. (1992) reported that IL-1β up-regulates B_2 receptor number in human synovial cells, but the mechanism by which this increase appears has not been investigated. Airway hyperresponsiveness to BK has been described after intratracheal administration of recombinant human IL-1β in rats (Tsukagoshi *et al*., 1995), suggesting that the up-regulation of BK receptors observed in synoviocytes (Bathon *et al*., 1992) also might occur in lung smooth muscle cells. These observations led us to study the effect of IL-1β on B_2 gene expression on human smooth muscle cells and the underlying transduction mechanisms.

The effects of IL-1 are exerted through a transcriptional regulation, which is proposed to involve NK-κB (Kessler *et al*., 1992). IL-1 stimulation led to the activation of NK-κB

ABBREVIATIONS: NF-κB, nuclear factor-κB; PG, prostaglandin; BSMC, bronchial smooth muscle cell; GAPDH, glyceraldehyde-3-phosphate dehydrogenase; BK, bradykinin; PCR, polymerase chain reaction, SSC, standard saline citrate; SDS, sodium dodecyl sulfate; IP, inositol phosphate; IL-1β, interleukin-1β.

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through the phosphorylation and subsequent degradation of the cytosolic inhibitor Iκ-Bα (Beg *et al.*, 1993; Israel, 1997). IL-1 also activates the mitogen-activated protein kinase pathway; however, the implication of the mitogen-activated protein kinase pathway in the activation of NF-κB has not been clearly resolved (Bird *et al.*, 1992). More recently, the activation of the Iκ-Bα kinase complex by mitogen-activated protein kinase kinase 1, a kinase of the JNK pathway, has been reported after tumor necrosis factor-α stimulation (Lee *et al.*, 1997). The response of cells to IL-1β includes the transcription of genes encoding for key enzymes implicated in prostanoids cascades, with a late production of prostanoids (Croxtall *et al.*, 1996). In the current study, we show that the induction of PGE_2 synthesis also is an early event in the effect of IL-1β, preceding an increase in cAMP and the transcriptional activation of the B_2 receptor gene. The current results indicate that IL-1β induces a transient increase in BK B_2 mRNA and protein levels through a prostanoid cAMP-dependent pathway in human BSMCs.

Materials and Methods

Cell culture and cytokine treatment. Primary human BSMCs (Clonetics, San Diego, CA) were cultured in smooth muscle basal medium supplemented with 5% fetal calf serum, gentamycin sulfate (10 mg/ml), and amphotericin-B (10 μg/ml) (Clonetics) in 95% air/5% CO_2 at 37° in a humidified incubator. The medium was replaced every 3 days, and on reaching confluency, cells were subcultured by detaching the monolayer with 0.05% trypsin and 1 mM EDTA. For treatments of the cells with IL-1β, cells at confluency in culture dish were made quiescent by incubation for 24 hr with serum-free medium and incubated for varying periods of time, as indicated, in serum-free medium containing IL-1β (10 units/ml) (Pepro Technology, Princeton, RH). All experiments were carried out with subcultured cells between the fifth and sixth passages.

[^{3}H]BK binding. Binding experiments were performed on membranes prepared from cultured human BSMCs as described previously (Trifilieff *et al.*, 1992). Briefly, cells were detached and centrifuged for 5 min at 850 × *g* at 4° in medium, resuspended, and homogenized in 25 mM triethylaminoethanesulfonic acid, pH 6.8, containing 1 mM 1,10-phenanthroline (buffer A). The homogenate was centrifuged at 50,000 × *g* for 30 min at 4°. The resulting pellet was resuspended in the appropriate volume (40 μg of protein/400 μl) of assay buffer (buffer A plus 140 μg/ml bacitracin, 10 μM captopril, 1 mM dithiothreitol, and 0.1% bovine serum albumin). For saturation experiments, the membrane preparations were incubated for 90 min at 25° with [^{3}H]BK (50 pM to 5 nM) (Du Pont-New England Nuclear, Boston, MA) in a final volume of 500 μl. In competition experiments, membranes were incubated in the same conditions with 0.5 nM radioligand and varying concentrations of either unlabeled BK (Sigma Chemical, St. Louis, MO) or BK analogues D-Arg^0-[Hyp^3,Thi^5,D-Tic^7,Oic^8]BK (Hoe 140) (gift from Dr. Wirth, Hoechst AG, Frankfurt, Germany) or D-Arg^0[Hyp^3,HypE-(*trans*-thio-phenyl)^7,Oic^8]BK (NPC 17761) (gift from Dr. Burch, Nova, Baltimore, MD). Experimental data from competition and saturation studies were analyzed as described previously (Gies *et al.*, 1989) using the LIGAND program (Elsevier-Biosoft, Cambridge, UK).

BK B_2 cDNA probe synthesis. Reverse transcription with Moloney murine leukemia virus-reverse transcription RNase H$^-$ (Stratagene, La Jolla, CA) was performed using total RNA extracted from human BSMCs (see below). PCR was run for 35 cycles of amplification with three steps of 1 min each: denaturation at 96°, annealing at 63°, and elongation at 72° with *Taq* DNA polymerase (Promega, Madison, WI) and with the specific primers 5′-CTTGGTGATCTGGGGGTGTACGCT-3′ and 5′-CGGTGCTAGTCCTGGTTGTGCTGC-3′, whose sequences correspond to nucleotides 600–623 and 872–895 of the human BK B_2 receptor gene (Hess *et al.*, 1992). PCR products were analyzed on a 1% agarose gel. After extraction, the 296-nucleotide PCR product was subcloned in pGEM-T plasmid (Promega). PCR product sequence analysis revealed a 100% homology with the human B_2 gene without any significant homology with other known genes. Sequence comparison with GenBank were done using the NCBI Blast program.

Northern blot. Total RNA was isolated by the guanidinium isothiocyanate method as described by Chomczynski and Sacchi (1987). Total RNA was separated in a 1% agarose/1 M formaldehyde gel and transferred to nylon membrane (Stratagene) in 20× SSC. Prehybridization was performed for 4 hr at 42° in a prehybridization solution containing 50% formamide, 0.01% SDS, 2× Denhardt's solution, and 5× SSC. Hybridization was carried out with α-^{32}P-random primer-labeled B_2 receptor and GAPDH cDNAs at 10^6 cpm/ml overnight at 42° in a solution containing 2.5% dextran sulfate, 10% salmon sperm (10 mg/ml) (Stratagene), 0.01% SDS, 4× Denhardt's solution, 50% formamide, and 5× SSC. Filters were washed twice with 2× SSC and 0.01% SDS at 42° for 15 min and 1× SSC and 1% SDS at 50° for 30 min before autoradiography exposure at −70° with Kodak X-AR 5 film.

Transcript stability analysis. At confluence, cells were treated or not with IL-1β (10 units/ml) for 3 hr and then treated with actinomycin-D (5 μg/ml) (Sigma Chemical). After varying times of incubation, total RNA was isolated from individual dishes, and the decay of mRNA was determined by Northern blotting. Half-life values were obtained from plots describing densitometrically determined absorbance units corrected by standardization with GAPDH versus time.

Nuclear run-on assay. Nuclei were prepared, as described previously (Greenberg *et al.*, 1984), from cultured cells treated or not with IL-1β (10 units/ml) for 3 hr by lysing in 10 mM Tris·HCl, pH 7.4, 10 mM NaCl, 3 mM $MgCl_2$, 0.5% Nonidet P-40, 2.75 mM dithiothreitol, and 20 units/ml recombinant ribonuclease (RNase) inhibitor on ice (Stratagene). The measurement of *in vitro* gene transcription was performed as described by Haddad *et al.* (1996). Briefly, isolated nuclei were resuspended in the same buffer without Nonidet P-40 and counted. Each reaction (final volume, 400 μl) was carried out in the presence of 5×10^7 isolated nuclei, 40 mM Tris·HCl, pH 8.3, 150 mM NH_4Cl, 7.5 mM $MgCl_2$, 0.625 mM ATP, 0.313 mM GTP, 0.313 mM CTP, 0.3 mCi of [α-^{32}P]UTP (800 Ci/mmol) (DuPont-New England Nuclear, Boston, MA), and 120 units/ml RNase inhibitor. Transcription reactions were allowed to proceed for 30 min at 27° before termination by the addition of 40 units recombinant RNase inhibitor and 75 units of RQ-1 DNase (Stratagene). After DNase treatment, the radiolabeled RNA that was formed was purified by phenol-chloroform extraction and precipitated three times with ethanol in the presence of 1.33 M ammonium acetate. An equal number of counts from each sample was added to slot blot, with three slots on the same membrane in which 10 μg of either pGEM-T plasmid (as control) or plasmid-containing inserts of human BK B_2 receptor cDNA or GAPDH cDNA had been immobilized. After hybridization for 72 hr at 42°, the filters were washed at a final stringency of 0.1× SSC and 0.1% SDS at 55°, including a 30-min digestion with 1 μg/ml RNase A and 10 units/ml RNase T_1 (Boehringer-Mannheim, Mannheim, Germany) at 37° to digest any single-stranded RNA not hybridized to DNA. After autoradiography, the film was scanned, and spots were quantified by calculation of the ratio between BK B_2 receptor cDNA signal and GAPDH cDNA signal.

PGE_2 determination. After 24 hr in serum-free medium, confluent cells in the 24 wells were incubated with or without IL-1β (10 units/ml) in serum-free medium for varying period of times, and the amounts of PGE_2 were quantified in the supernatants as described by Pradelles *et al.* (1985) by enzyme immunoassay (Stalergene, Paris, France)

cAMP determination. After 24 hr in serum-free medium, confluent cells in 24 wells were incubated with or without either IL-1β (10 units/ml) for various period times in for the kinetics experiments.

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Alternatively, cells were incubated with or without either IL-1β (10 units/ml) for 45 min, indomethacin (10 μM) (Sigma Chemical) for 45 min, or PGE_2 (100 ng/ml) (Sigma Chemical) for 45 min in the presence of the phosphodiesterase inhibitor 3-isobutyl-1-methylxanthine (1 mM) (Sigma Chemical). cAMP levels were measured from each batch of treated and untreated cells. Cells were harvested by the addition of boiling water (1 ml) directly to each well. Cells then were boiled for an additional 2 min before centrifugation at 12,000 × *g* at 4° for 10 min. The supernatant (1 ml) was collected, diluted 10-fold, and stored at −20°. Aliquots (500 μl) of diluted cell extracts were acetylated, and cAMP content was measured by radioimmunoassay (Amersham, Arlington Heights, IL), as described by Brooker *et al.* (1979). Briefly, a 100-μl acetylated sample was added to 100 μl of adenosine-3′,5′-cyclic phosphoric acid-2-*O*-succinimyl-3-[^{125}I]iodotyrosine methyl ester (2000 Ci/mmol) in 0.2% bovine serum albumin and 100 μl of anti-cAMP antibody in 0.2% bovine serum albumin. Samples were incubated overnight at 4°. Free and antibody-bound cAMP were separated by the addition of a binding protein and centrifuged. The amount of [^{125}I]cAMP was determined with a radiospectrometer.

Accumulation of IPs. The effect of BK on the hydrolysis of IPs was assayed by monitoring the accumulation of ^{3}H-labeled IPs, as described by Berridge *et al.* (1983). Briefly, cells at confluency were incubated in IP- and serum-free medium with 5 μCi/ml *myo*-[^{3}H]inositol (Du Pont-New England Nuclear) at 37° for 48 hr. After treatment with IL-1β (10 units/ml) for 6 hr or with vehicle, human BSMCs were washed three times with Hanks' balanced salt solution and scraped into Hanks' balanced salt solution (GIBCO BRL, Paisley, UK) containing 10 mM LiCl (30,000 cells/assay; Sigma Chemical). After a 30-min incubation at 37°, BK was added to the cells at the indicated concentration, and incubation was continued for 30 min. Reactions were terminated by the addition of 10% ice-cold trichloroacetic acid followed by centrifugation at 1000 × *g* for 10 min. The trichloroacetic acid-soluble supernatants were extracted four times with diethylether, neutralized to pH 7 with 100 mM sodium tetraborate, and applied to a column of AG 1-X8 (formate form, 200–400 mesh; BioRad). The resin was washed successively twice with 6 ml of water and twice with 6 ml of 60 mM ammonium formate/5 mM sodium tetraborate to eliminate free *myo*-[^{3}H]inositol and glycerophosphoinositol. Total IPs were eluted twice with 6 ml of 1 mM ammonium formate/0.1 M formic acid. The amount of [^{3}H]IPs was determined in a liquid scintillation counter.

Statistical analysis. Results were expressed as mean ± standard error. Paired Student's *t* test was used for statistical analysis of the results. Values of $p < 0.05$ were considered significant.

Results

Human BSMCs express B_2 receptors. [^{3}H]BK binding on cells membranes was saturable (Fig. 1A). Scatchard analysis indicates the presence of a single class of binding sites with a dissociation constant (K_d) of 0.36 ± 0.01 nM and a maximum number of binding sites (B_{max}) of 89.29 ± 5.49 fmol/mg of proteins (Fig. 1A, *inset*). Competition experiments using [^{3}H]BK (0.5 nM) revealed that des-Arg^9[Leu^8]BK, a specific B_1 antagonist, was unable to displace [^{3}H]BK binding, whereas unlabeled BK and selective B_2 antagonists (Hoe 140, NPC 17761) dose-dependently inhibited the binding of [^{3}H]BK. The order of potency of these compounds was Hoe 140 = NPC 17761 > BK ≫ Des-Arg^9[Leu^8]BK, and with the respective K_i (mean ± standard error, five experiments) values, 0.11 ± 0.03, 0.16 ± 0.01, and 1.20 ± 0.15 nM, ineffective (Fig. 1B).

IL-1β up-regulates B_2 receptors. In confluent cells exposed to IL-1β, the maximal number of B_2 binding sites (B_{max}) increased time-dependently (Fig. 2). Using Scatchard analysis, we determined a 24.6 ± 5.6% increase in B_{max} after 3 hr and a 71.5 ± 16.1% maximal increase after 6 hr. After 24 hr, the density of BK receptors returned to 27 ± 9.24% above the basal level. This treatment did not affect the binding affinity of [^{3}H]BK. Scatchard analysis of the saturation curves from IL-1β untreated and treated (10 units/ml for 6 hr) cell membranes gave K_d values of 0.36 ± 0.01 and 0.36 ± 0.03 nM and B_{max} values of 89.29 ± 5.49 and 152.9 ± 11.15 fmol/mg of proteins, respectively (mean ± standard error, four experiments) (Fig. 2, *inset*). Unlabeled BK and selective B_2 antagonists dose-dependently inhibited the binding of [^{3}H]BK (0.5 nM) to IL-1β-treated cell membranes with the same K_i values than in competition binding assays using IL-1β-untreated cell membranes (data not shown).

IL-1β increases BK-stimulated IP formation. In the presence of 10 mM LiCl, BK dose-dependently (10^{-10} to 10^{-4} M) stimulated [^{3}H]IP accumulation in cells (Fig. 3). A 2-fold increase in IP production was observed when human BSMCs were pretreated with IL-1β (10 units/ml) for 6 hr at each dose of BK (Fig. 3). IL-1β treatment alone caused no significant change in basal IP formation.

IL-1β increases B_2 receptor mRNA levels. Because IL-1β has a critical role in gene expression, we studied the

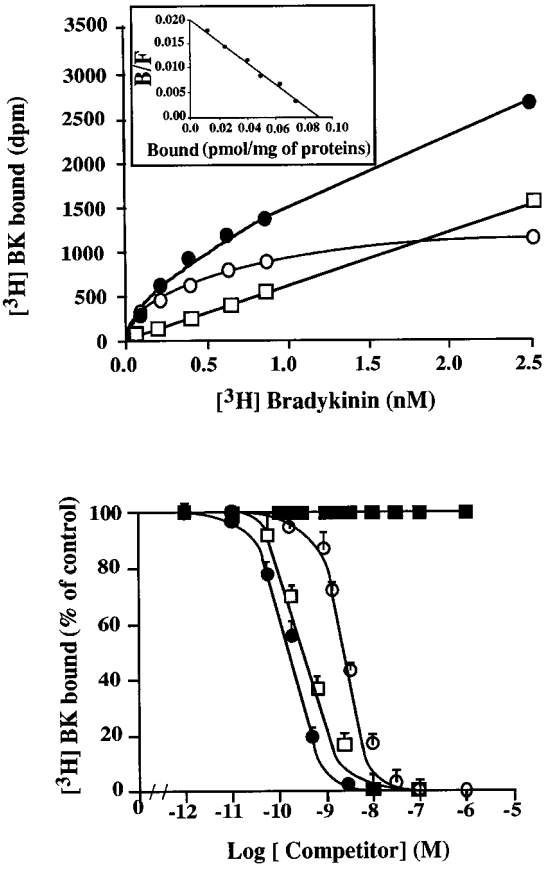

Fig. 1. Characterization of BK receptors on human BSMCs. A, Scatchard analysis of saturation experiments with [^{3}H]BK on human BSMC membrane preparations. *Inset*, binding of [^{3}H]BK to cells membrane preparations. K_d (0.39 nM) and B_{max} (90.0 fmol/mg of proteins) were obtained by Scatchard analysis. ●, Total binding. □, Nonspecific binding. ○, Specific binding. This experiment is representative of five experiments performed in triplicate. B, Inhibition of [^{3}H]BK binding by BK analogues Hoe 140 (●), NPC 17761 (□), BK (○), and des-Arg^9-[Leu^8]BK (■). Values are the mean ± standard error of five experiments, each performed in triplicate.

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effect of IL-1β treatment on B_2 mRNA levels. RNA was harvested from cells treated with IL-1β for 0, 1, 2, 3, 6, and 12 hr and analyzed by Northern blotting. We first synthesized a specific 296-bp cDNA probe for B_2 mRNA hybridization (see Materials and Methods), and we accounted for differences in loading and transfer of the RNA by hybridization of the blot with a GAPDH cDNA probe. These probes allowed to show that IL-1β time-dependently increased B_2 mRNA levels with a 5-fold maximum after 3 hr (Fig. 4).

Regulation of gene transcription by IL-1β. To determine the precise mechanisms by which IL-1β increased steady state B_2 mRNA levels, we first measured the B_2 mRNA half-life in the presence of actinomycin-D (5 μg/ml). IL-1β increased the half-life of B_2 mRNA from 2.9 to 3.6 hr (Fig. 5). This slight enhancement of mRNA stability may not account for the large increase of mRNA, suggesting the occurrence of a transcriptional regulation. Nuclear run-on experiments demonstrated that treatment for 3 hr with IL-1β (10 units/ml) increased the rate of B_2 gene transcription by 5-fold, calculated from the ratio of transcription of B_2 receptor gene to that of the *GAPDH* gene (Fig. 6). Thus, IL-1β-

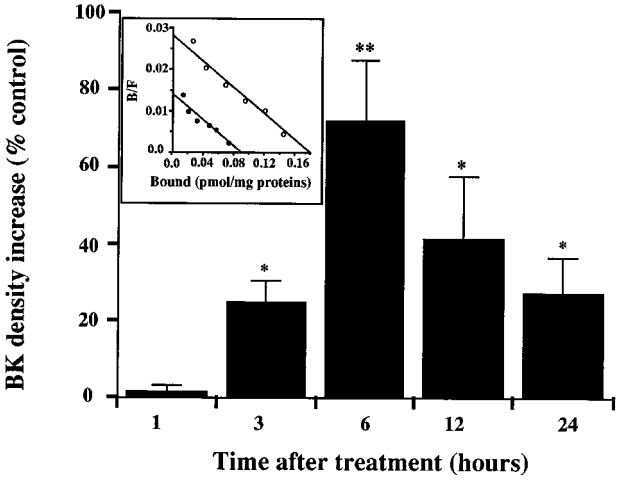

Fig. 2. Effect of IL-1β treatment on the expression of B_2 receptors by human BSMCs. Confluent cells were treated or not with IL-1β (10 units/ml) for various time periods and then assayed for saturation binding with [^{3}H]BK. Values represent the mean ± standard error of four experiments, each performed in triplicate. *, $p < 0.05$; **, $p < 0.01$, Significantly different compared with control. *Inset*, Scatchard plots of [^{3}H]BK binding to IL-1β (10 units/ml)-treated (○) for 6 hr and untreated cells (●). K_d (0.36 and 0.36 nM) and B_{max} (91.25 and 180.0 fmol/mg of proteins) for control and treated cells, respectively, were obtained by Scatchard analysis. This experiment is representative of four experiments performed in triplicate.

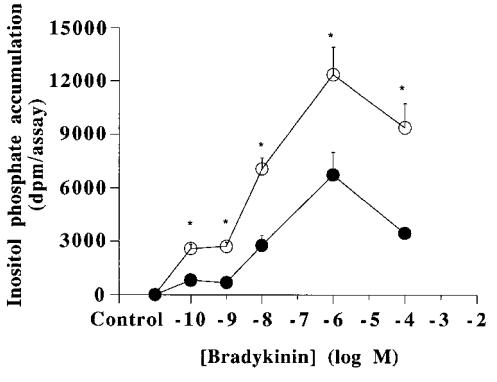

Fig. 3. Effect of IL-1β on BK-induced IP formation in BSMCs. Untreated (●) or IL-1β (10 units/ml for 6 hr)-treated cells (○) were stimulated with increasing concentration of BK as described in the text. Values represent the mean ± standard error of four experiments each, performed in triplicate. *, $p < 0.05$, Significantly different compared with untreated cells.

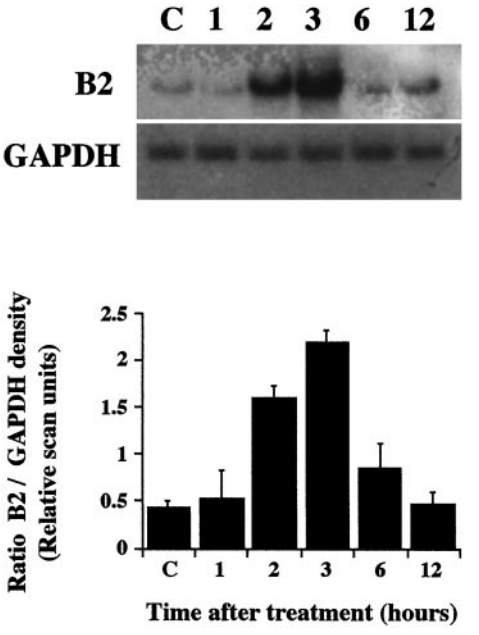

Fig. 4. Kinetic effect of IL-1β treatment on the steady state level of B_2 receptor mRNA in human bronchial smooth muscle cells. *Top*, confluent cells were untreated (*control*) or treated with IL-1β (10 units/ml) for 1–12 hr. Total RNA was extracted and subjected to Northern blot analysis using 15 μg of RNA/lane. RNA was transferred to nylon membranes and hybridized with a ^{32}P-labeled B_2 receptor cDNA probe as described in the text. For standardization, the same blot was probed again with a cDNA fragment of the *GAPDH* gene. Blot shown is representative of three independent experiments. *Bottom*, absorbance of the B_2 receptor mRNA signals standardized by GAPDH absorbance. Values represent the mean ± standard error of three experiments.

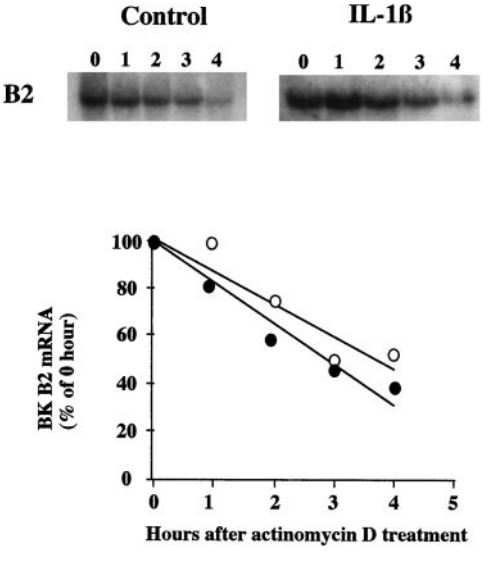

Fig. 5. Effect of IL-1β on BK B_2 mRNA half-life. *Top*, confluent cells were untreated (●) or treated with IL-1β (10 units/ml) (○) for 3 hr followed by administration of actinomycin-D (5 μg/ml). RNA was extracted at the indicated times after actinomycin-D treatment. Northern blot analysis was performed with 10 and 30 μg of total RNA for treated and untreated cells, respectively, as described in the legend to Fig. 3. Bottom, corrected (GAPDH standardized), absorbances were plotted as a percentage of 0-hr value against time. Data are representative of three independent experiments.

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induced increase in B_2 mRNA level was mainly due to an increase in the transcriptional rate of this gene with a slight increase of the mRNA stability.

IL-1β increases PGE_2 release and cAMP formation. When cells were stimulated with 10 units/ml IL-1β, PGE_2 was generated, reaching a rapid peak within 30–60 min compared with control cells (Fig. 7A). In the same experimental conditions, IL-1β elicited a time-dependent increase in cAMP formation (Fig. 7B). This increase appeared rapidly at 40 ± 5 min. No significant cAMP generation was detected before the maximal peak appeared.

IL-1β increases cAMP and mRNA through a prostanoid-dependent pathway. Indomethacin (10 μM) treatment totally prevented the increase in cAMP induced by IL-1β treatment for 45 min or 2 hr. The addition of exogenous PGE_2 (100 ng/ml) (Fig. 8A) totally restored the increase in cAMP. To determine the precise mechanisms by which IL-1β increased B_2 mRNA, we studied by Northern blotting the level of B_2 mRNA after treatment with indomethacin (10 μM) and after the addition of exogenous PGE_2 (100 ng/ml) (Fig. 8B). Treatment with indomethacin (10 μM) for 3 hr was without effect on the basal level of B_2 mRNA. At the same concentration, indomethacin reduced, from 5- to 2-fold the increase in B_2 mRNA levels induced by IL-1β (10 units/ml) treatment. The addition of exogenous PGE_2 (100 ng/ml) restored the increase in B_2 mRNA induced by IL-1β that was prevented by indomethacin. Treatment with PGE_2 alone (100 ng/ml) induced a 3-fold increase in B_2 mRNA level (Fig. 8B).

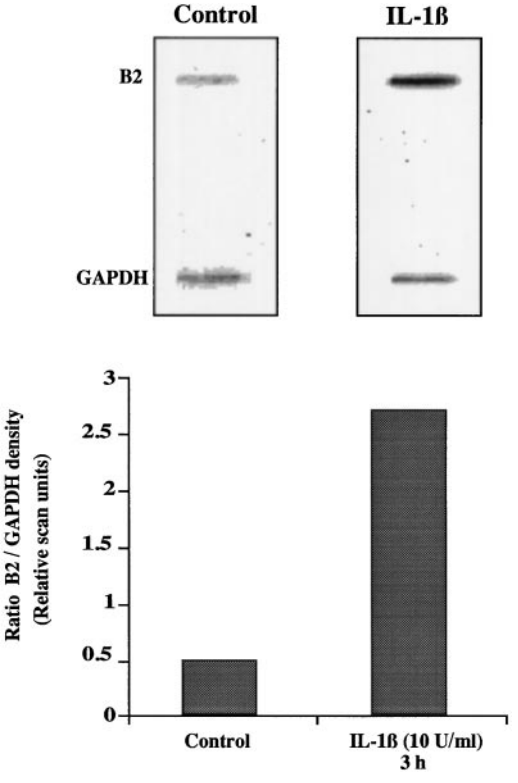

Fig. 6. Effect of IL-1β on the transcription rate of BK B_2 receptor gene in human BSMCs. *Top*, confluent cells were untreated (*control*) or treated with IL-1β (10 units/ml) for 3 hr. Nuclei and RNA probes were processed as described in the text. *Bottom*, optical densities of the B_2 receptor mRNA signals standardized by GAPDH absorbance. Results are representative of two independent experiments.

Discussion

Several studies report the effects of IL-1β on the expression of different genes through activation of the transcription factor NF-κB [Kessler *et al.*, 1992; Baldwin, 1996 (for review)]. Bathon *et al.* (1992) found that the incubation of human synovial fibroblasts in culture with IL-1β increased the number of BK receptors, but the molecular mechanism by which this up-regulation occurred was not investigated. In contrast, no effect of IL-1β on BK B_2 expression was noted on 3T3 fibroblasts (Burch and Tiffany, 1989). Airway hyperresponsiveness to BK has been described after intratracheal administration of recombinant human IL-1β in rats (Tsukagoshi *et al.*, 1995), suggesting an effect of this cytokine on the expression of BK receptor in airway smooth muscle.

Here, we report the first evidence of B_2 receptor expression by human BSMCs in culture and its up-regulation by IL-1β. [^{3}H]BK binds to a single class of saturable and high affinity sites, with densities and affinities in agreement with those found in other human cell types, such as synovial cells (Bathon *et al.*, 1992). The inhibition constants obtained for different BK analogues were similar to those found in the guinea pig trachea (Da Silva *et al.*, 1995) and indicate the presence of the B_2 receptor subtype. IL-1β treatment caused a time-dependent increase in B_2 receptor density, with a

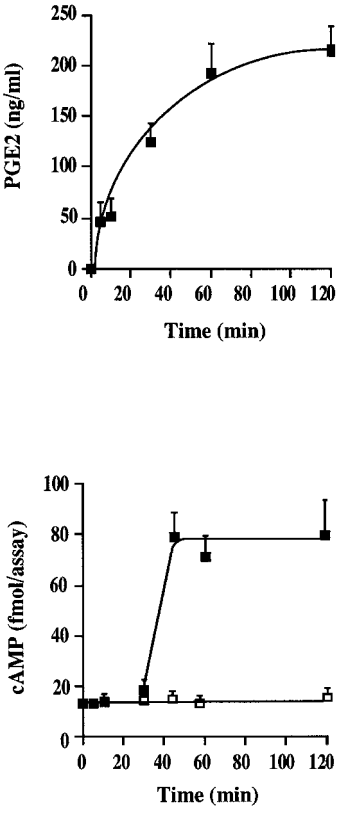

Fig. 7. A, Kinetic effect of IL-1β (10 units/ml) treatment on PGE_2 release in human BSMCs. PGE_2 release in the supernatant of treated and untreated cells was measured for indicated time by enzyme immunoassay. Values represent the mean ± standard error of four experiments each performed in triplicate of PGE_2 values in treated cells corrected by substraction of PGE_2 values in control cells. B, Kinetic effect of IL-1β treatment on cAMP formation in human BSMCs. cAMP in treated (■) and untreated (□) cells was measured by radioimmunoassay after extraction. Values represent the mean ± standard error of four experiments, each performed in triplicate.

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maximal effect after 6 hr of treatment, without affecting the receptor affinity. Results of kinetics of studies fit with the onset of airway hyperresponsiveness to BK after IL-1β treatment reported by Tsugagoshi *et al.* (1995). These authors failed to found up-regulation of B_2 receptors, most probably due to their used of total lung membrane preparations for binding experiments, whereas we uses pure smooth muscle cells preparations. The ability of IL-1β to enhance kinin binding to human BSMCs reinforces the hypothesis that local inflammation may determine cellular responsiveness. This is also supported by studies (Herxheimer and Stresemann, 1961) in which airways of asthmatic patients were more responsive to BK than their noninflamed counterparts. Moreover, we showed that the increase in B_2 receptors was correlated to an enhancement of IP formation, indicating a functional consequence of the up-regulation of B_2 receptors. In canine tracheal smooth muscle cells, forskolin treatment induced an increase in the density of BK receptors, which enhanced BK-induced IP accumulation and the rise in intracellular calcium concentration (Yang *et al.*, 1994). However, we cannot exclude an increase in the efficacy of G protein coupling because it has been reported that IL-1β increases BK-induced GTPase activity without any increase in receptor density (Burch *et al.*, 1988).

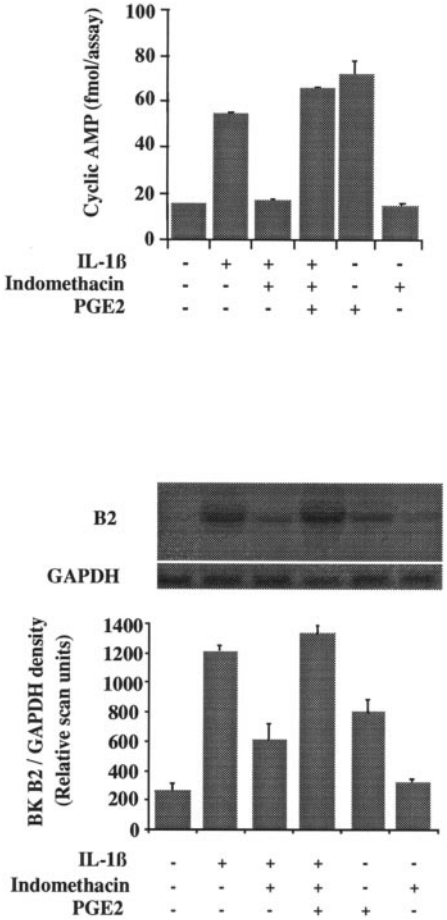

Fig. 8. A, Effect of IL-1β (10 units/ml), indomethacin (10 μM), and/or PGE_2 (100 ng/ml) treatment on cAMP formation. Cells were treated or not with IL-1β (10 units/ml) for 45 min in presence or absence of indomethacin and/or with PGE_2. cAMP was measured by radioimmunoassay after extraction. Values shown are mean ± standard error of three experiments, each performed in duplicate. B, Effect of IL-1β (10 units/ml) and/or indomethacin (10 μM) and/or PGE_2 (100 ng/ml) on the level of B_2 receptor mRNA. *Top*, cells were treated or not with IL-1β (10 units/ml) for 3 hr in presence or absence of indomethacin and/or PGE_2. Total RNA was extracted, and Northern blot analysis was performed using 15 μg of RNA/lane. Blot shown is representative of three experiments. *Bottom*, absorbances of the B_2 receptor mRNA signals standardized by GAPDH absorbance. Values represent the mean ± standard error of three experiments.

It has been largely reported in the literature that IL-1β is an efficient stimulating factor for prostanoids formation, such as PGE_2 (Angel *et al.*, 1994), but all reports investigated the late phase of apparition of PGE_2, involving protein synthesis such as cyclooxygenase II or phospholipase A_2 (Croxtall *et al.*, 1996). In the current study, IL-1β induced a rapid increase of PGE_2 release (compared with control cells), followed by an increase in cAMP formation, and both were totally prevented by indomethacin treatment. Moreover, both PGE_2 and cAMP increase preceded the increase in B_2 mRNA levels, which also was prevented by indomethacin treatment. Taken together, these data demonstrate the involvement of a prostanoid-dependent pathway in B_2 gene expression. Furthermore, the addition of exogenous PGE_2 restored totally the IL-1β-induced cAMP and B_2 mRNA increases that were prevented by indomethacin. This agrees with the observation that PGE_2 is a potent activator of adenylate cyclase (Brunton *et al.*, 1976; Nishigaki *et al.*, 1996). Therefore, we propose that IL-1β up-regulates B_2 gene expression via PGE_2, which acts as an autocrine factor for adenylate cyclase activation. However, we cannot exclude the participation of other prostanoids, such as PGI_2.

The addition of PGE_2 totally restored the increase in B_2 mRNA level after inhibition by indomethacin. Because PGE_2 increased cAMP level and mRNA in our cells, cAMP might be involved in BK B_2 gene expression induced by IL-1β. A cAMP-responsive element has been identified in the 5′-flanking region of the B_2 receptor gene (Ma *et al.*, 1994; Kammerer *et al.*, 1995). Interestingly, indomethacin at the concentration used to prevent totally the IL-1β-induced cAMP increase did not completely inhibit IL-1β-induced B_2 mRNA increase. Moreover, PGE_2 at the concentration required to induce the same level of cAMP as that generated by IL-1β treatment was less efficient to induce B_2 mRNA expression than IL-1β. We conclude that IL-1β increases B_2 gene expression predominantly through a prostanoid- and cAMP-dependent pathway, which might be followed by protein kinase A activation of the transcription factor cAMP response element-binding protein. Another activation process, insensitive to indomethacin, also may occur as a minor part of B_2 gene overexpression. Such a pathway might be related to mRNA stability or to the activation of protein kinase A through a cAMP-independent pathway (Gwosdow *et al.*, 1994).

In conclusion, IL-1β induces B_2 receptor overexpression mainly through a prostanoid pathway that activates cAMP formation, leading to gene transcription. The current study provides the first information regarding the molecular mechanism regulating BK B_2 gene expression in human BSMCs.

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