Expression of the bradykinin B_1 receptor gene is up-regulated in vascular smooth muscle cells (VSMCs) in response to a variety of inflammatory stimuli. We isolated the 5'-flanking region of the human bradykinin B_1 receptor gene and examined its promoter activity by transient transfection analysis. This region (−2582 to +34) showed promoter activity inducible by lipopolysaccharide (LPS), tumor necrosis factor α (TNF-α), and interleukin-1β (IL-1β) in VSMCs. Further deletion analysis revealed that constructs containing 111 base pairs of 5'-flanking sequence were sufficient for transcriptional induction. Mutagenesis of a nuclear factor κB (NF-κB)-like site at −64 to −55 abolished most of the LPS, TNF-α, and IL-1β inducibility, whereas a mutation of a cyclic AMP response element at −50 to −43 markedly reduced the basal promoter activity, and a mutation of the activator protein 1 (AP-1) site at −78 to −72 had minimal effects. Nuclear extracts from LPS, TNF-α, and IL-1β-treated VSMCs, IL-1β-treated human hepatoma HepG_2, and human lung fibroblast IMR-90 cells showed strong inducible binding activity to the NF-κB-like site by gel shift assays. These results demonstrated that NF-κB-like nuclear factor was involved in the inducible expression of the human bradykinin B_1 receptor gene during inflammatory processes.

Kinins are biologically active peptides that are formed locally after tissue damage and inflammatory stimuli from precursor kininogens by limited proteolysis (1). Kinins exert a broad spectrum of physiological and pathological effects, including smooth muscle contraction, vasodilation, increased vascular permeability, and pain induction, by binding to their cell surface receptors (2). Two subtypes of kinin receptors, B_1 and B_2, have been characterized on a pharmacological basis (3). Whereas the B_2 receptor is responsive to the intact kinins, bradykinin (BK) and Lys-BK (kallidin), the B_1 receptor has a higher affinity for the carboxypeptidase metabolites of kinins, des-Arg<sup>9</sup>-BK and des-Arg<sup>10</sup>-kallidin (4). Expression cloning of B_1 and B_2 receptor cDNAs confirms the existence of these two subtypes and reveals that they both belong to the G protein-coupled superfamily of receptors with seven transmembrane domains (5–11). Activation of the kinin receptors leads to generation of inositol phosphate and transient increases in intracellular Ca<sup>2+</sup> levels via coupling to phospholipase C, involving, at least, the G<sub>i</sub>, G<sub>q/11</sub>, and G<sub>13</sub> proteins (12–15).

The bradykinin B_2 receptor is constitutively expressed in a variety of tissues and cultured cell lines and mediates most of the in vivo effects usually assigned to kinins (16). The bradykinin B_1 receptor, however, is not present to any significant extent in normal tissues. It is expressed in a limited number of cultured cell types, such as embryonic lung fibroblasts, vascular smooth muscle cells, and endothelial cells (17–19). B_1 receptor-mediated responses are up-regulated in a time and protein synthesis-dependent process (4). Recent studies in animal models of hyperalgesia and in B<sub>1</sub> receptor knockout mice with induced peritoneal inflammation suggest that the B<sub>1</sub> receptor may play an important role in the pathogenesis of chronic inflammatory diseases (20–22).

The genes encoding B<sub>1</sub> receptors have been cloned from the rat (2) and human (23, 24). The human B<sub>1</sub> receptor gene was located close to the B<sub>2</sub> receptor gene within the same chromosomal region at 14q32 (23). The B<sub>1</sub> receptor is induced following tissue injury, following prolonged in vitro incubation of tissues, or upon exposure in vivo or in vitro to proinflammatory mediators, such as lipopolysaccharide (LPS) (4). Whereas cytokines, including IL-1β, IL-2, and IL-8 but not TNF-α or IL-6, induced the B<sub>2</sub> receptor, continuous incubation with the glucocorticoid dexamethasone or the protein synthesis inhibitor cycloheximide suppressed the induction in vitro (25, 26). To understand the molecular mechanism underlying the regulated expression of the B<sub>1</sub> receptor gene, we isolated and characterized the promoter of the human B<sub>1</sub> receptor gene. In this study, we present evidence that nuclear factor κB (NF-κB) is involved in the dynamic regulation of human B<sub>1</sub> receptor gene expression.

EXPERIMENTAL PROCEDURES

Cell Culture—Rat vascular smooth muscle cells (VSMCs) were explanted from a media layer of the thoracic aorta of male Sprague-Dawley rats (200–250 g) according to the method of Biro et al. (27) and maintained in Dulbecco’s modified Eagle’s medium (Life Technologies, Inc.) supplemented with 10% fetal bovine serum (HyClone, Logan, UT), 100 units/ml penicillin, 100 μg/ml streptomycin sulfate, and 25 mM Hepes, pH 7.4. These cells exhibit a “hill and valley” growth pattern and are characterized by positive immunostaining with monoclonal antibodies against smooth muscle α-actin (28). Experiments using VSMCs were performed between passages 3 and 10. Human hepatoma HepG<sub>2</sub> cells were cultured in Dulbecco’s modified Eagle’s medium containing 10% fetal bovine serum and antibiotics. Human diploid lung fibroblasts, IMR-90 cells obtained from ATCC (Rockville, MD) were cultured as described.
described (7). Bovine arterial endothelial cells (BAECs) from ATCC were grown in RPMI 1640 medium (Life Technologies, Inc.) supplemented with 20% fetal bovine serum, 2 mM L-glutamine, and antibiotics. cDNA (Escherichia coli serotype 055:B5), sodium salicylate, pyrroli- dine dithiocarbamate, cycloheximide, and dexamethasone were purchased from Sigma. Recombinant human IL-1β was from R&D Systems (Minneapolis, MN).

Cloning of the 5′-Flanking Region of the Human B1 Receptor Gene—A human lambda genomic library (Stratagene, La Jolla, CA) was screened with an oligonucleotide probe, 5′-AGA AAA CTC CTC CAA AAG CAG CTC TCA-3′, specific for exon I of the human bradykinin B1 receptor gene (GenBank™ accession no. U30271) as described previously (11, 23). Positive clones were picked for further characterization. Lambda phage DNA was digested and subjected to Southern blotting to identify fragments containing exon I and possibly the 5′-flanking sequence of the human B1 receptor gene, which were in turn subcloned into pBlue-Script KS II vector (Stratagene) and sequenced on both strands using a dsDNA cycle sequencing system (Life Technologies, Inc.).

Construction of Reporter Plasmids—A 2.6-kb fragment spanning nucleotides −2582 to +34 relative to the transcription start site (29) was amplified by polymerase chain reaction (PCR) and cloned upstream of a firefly luciferase gene in the promoterless plasmid pGL2-Basic (Promega, Madison, WI). The resulting construct was named p-2582Luc. The same fragment as above was also inserted into pGL2-Basic in the reverse orientation, giving rise to p-2582Luc. A series of deletion constructs containing 5′-flanking fragments of the human B1 receptor gene was made by PCR. Constructs containing these 5′-progressively removed fragments were named p-1743Luc, p-825Luc, p-589Luc, p-251Luc, p-111Luc, and p-43Luc. Mutant constructs were as follows (lowercase indicates mutation): p-2582mLuc, with the AP-1 site (−78 to −72) mutated from AGACTCA to AGActc; p-2582m3Luc, with 2 bp in the NF-κB-like site (−64 to −55), mutated from GGCAATCCCC to GgCAATCCCC; and p-2582m2Luc, with the cAMP response element (CRE) (−50 to −43), mutated from TGGATCTA to TGATcAGA. Mutants were created using a PCR site-directed mutagenesis using p-2582Luc as the template (30). A double mutant construct, p-2582m2CNLuc, with both the CRE and NF-κB-like sites mutated as above, was prepared with the same method using p-2582m2Luc as the PCR template. Another construct, p1900Luc, with a 1.9-kb PCR-generated fragment upstream of the translation start codon ATG as described previously (23), was also produced. All constructs were sequenced to confirm the product fidelity.

Transfection and Luciferase Assays—Twenty-four hours before transfection, BAECs, HepG2 cells, or VSMCs were seeded at a concentration that would achieve 60–70% confluence in 12-well tissue culture plates. Liposome-mediated cotransfection using a mixture of 400 μl of Opti-MEM, 4.8 μl of Lipofectin (for BAECs) or Lipofectamine (for HepG2 and VSMCs), 0.6 μl of the reporter construct, and 0.2 μg of pCMV-bgal (Clontech Laboratories, Inc.) (as a control for measuring transfection efficiency) per well was carried out as recommended by the manufacturer (Life Technologies, Inc.). The transfection mixture was removed from growth medium 24 h after being washed with ice-cold phosphate-buffered saline (PBS), cells were scraped into 1.2 ml of cold PBS, pelleted, and resuspended in 500 μl of Buffer A (10 mM Hepes-KOH, pH 7.9, 1.5 mM MgCl2, 10 mM KCl, 0.5 mM DTT, 0.2 mM phenylmethylsulfonyl fluoride) and incubated for 10 min with shaking. The nuclear extract was centrifuged at 14,000 × g for 5 min at 4 °C, and the supernatant was aliquoted and stored at −80 °C. Protein concentrations were determined by the Bradford method (29).

Electrophoretic Mobility Shift Assay—A double-stranded oligonucleotide containing a wild-type NF-κB-like site (−73 to −50, 5′-CAC TTT TGC GGC ACC CAC CAC AAT-3′), was labeled with [γ-32P]ATP and T4 polynucleotide kinase (Life Technologies, Inc.) and used as nested specific probes for rat and human B1 receptor genes and β-actin, respectively.

Nuclear Extract Preparation—Confluent cells were washed once with serum-free medium and then treated with IL-1β (2 ng/ml), TNF-α (10 ng/ml), or LPS (10 μg/ml) or left untreated as controls. Nuclear proteins were extracted by a modified method of Dignam et al. (34). After being washed with ice-cold phosphate-buffered saline (PBS), cells were scraped into 1.2 ml of cold PBS, pelleted, and resuspended in 500 μl of Buffer A (20 mM Hepes-KOH, pH 7.9, 1.5 mM MgCl2, 10 mM KCl, 0.5 mM DTT, 0.2 mM phenylmethylsulfonyl fluoride) and incubated for 20 min on ice with shaking. The nuclear extract was centrifuged at 14,000 × g for 5 min at 4 °C, and the supernatant was aliquoted and stored at −80 °C. Protein concentrations were determined by the Bradford method (29).

RESULTS

Induction of Bradykinin B1 Receptor Gene Expression—It has been shown that stimulation of human lung fibroblast IMR-90 cells with IL-1β increased both the levels of the B1 receptor mRNA and the number of B1 receptors (7, 17, 29). We examined whether expression of the B1 receptor gene was induced in rat VSMCs and human hepatoma HepG2 cells following IL-1β stimulation. Because preliminary studies revealed that serum in the medium increased the B1 receptor message, we washed cells once with serum-free Dulbecco’s modified Eagle’s medium before incubation with mediators in serum-free medium to eliminate the stimulatory effects of serum. The B1 receptor mRNA levels were lowest in unstimulated VSMCs, but it was markedly increased by treatment with IL-1β for 3 h (Fig. 1). In contrast, the same dose of IL-1β failed to induce B1 receptor mRNA expression in HepG2 cells after prolonged incubation (data not shown). We next examined the levels of the B1 receptor mRNA in VSMCs incubated with various stimuli and/or CHX and DEX. Proinflammatory agents LPS
and TNF-α were also able to up-regulate the B1 receptor mRNA. The protein synthesis inhibitor CHX augmented the effects of IL-1β and LPS, whereas CHX alone induced an increase in B1 receptor message. The synthetic glucocorticoid DEX reduced B1 receptor mRNA levels in unstimulated cells and suppressed up-regulation by LPS. In contrast to the B1 receptor, the level of β-actin transcript remained the same irrespective of the presence of various mediators (Fig. 1).

**Potential Regulatory Elements in the 5′-Flanking Region of the Human B1 Receptor Gene**—We previously cloned a BamHI fragment of the human B1 receptor gene containing the sole coding exon (23). The fragment of about 1.9 kb, upstream of the translation start site ATG in the BamHI fragment, was found to have a significant basal promoter activity in HepG2 cells (23). In a preliminary study, however, we found that the fragment was not able to confer IL-1β or LPS inducibility on the reporter gene in VSMCs and HepG2 cells. As demonstrated above, B1 receptor gene expression is up-regulated in response to stimuli by LPS and IL-1β. Thus, the 1.9-kb fragment could not represent the promoter for the B1 receptor gene, at least in VSMCs under inflammatory stimuli. Later, we and other investigators found that the human B1 receptor mRNA in IMR-90 cells includes two noncoding exons, indicating that the 1.9-kb fragment is not the 5′-flanking region of the gene (24). In this study, we isolated the 5′-flanking region of the B1 receptor gene for elucidating the molecular mechanism of up-regulation of B1 receptor expression. Sequence determination on both strands was extended 1500 bp compared with the longest reported sequence (24). In addition to the consensus TATA box, several other potential transcription factor binding sites, including Sp1, AP-1, NF-κB, and CRE, were found in the 5′-flanking region by computer search (Fig. 2).

**Inducible Promoter Activity of the 5′-Flanking Region of the Human B1 Receptor Gene**—To assess whether the 5′-flanking region of the B1 receptor gene has inducible promoter activity, transient transfection was performed in VSMCs and HepG2 cells. In VSMCs, LPS (10 μg/ml), IL-1β (2 ng/ml), and TNF-α (10 ng/ml) increased 3–4-fold the luciferase activity of p-2582Luc, which contained the longest 5′-flanking region (Fig. 3, A and B). p1900Luc, with a basal promoter activity of about

![Figure 1](http://www.jbc.org/)

**FIG. 1.** Reverse transcription-PCR Southern blot analysis of rat bradykinin B1 receptor mRNA. VSMCs were washed once with serum-free medium and then incubated for 3 h with serum-free medium (control), 10 μg/ml LPS, 2 ng/ml IL-1β, 10 ng/ml TNF-α, 10 μg/ml CHX, 10 μg/ml CHX plus 10 μg/ml LPS, 10 μg/ml CHX plus 2 ng/ml IL-1β, 1 μM DEX, 1 μM DEX plus 10 μg/ml LPS, or 10% fetal bovine serum (FBS). CHX and DEX were applied to the cells 1 h before LPS and IL-1β were applied. Total RNA was prepared, 2 μg of total RNA was used in the reverse transcription reaction, and PCR was conducted for 30 cycles (B1 receptor) or 25 cycles (β-actin), followed by Southern blot analysis as described under "Experimental Procedures."

![Figure 2](http://www.jbc.org/)

**FIG. 2.** Sequence of the 5′-flanking region of the human bradykinin B1 receptor gene. The transcription initiation site is designated +1. The TATA box and several other potential regulatory elements are double-underlined and labeled.
1/7 of that of p-2582Luc, showed no inducibility of the luciferase activity in the presence of LPS, IL-1β, or TNF-α. The luciferase activity of p-2582Luc in HepG2 cells was about 1/5 that of VSMCs (with respect to promoterless pGL2-Basic) and was also inducible by IL-1β with about 1.8-fold induction (Fig. 3C).

On the contrary, p1900Luc had basal promoter activity more than 8-fold higher than p-2582Luc in HepG2 cells, although it was not responsive to IL-1β. The plasmid p2582Luc, containing the 5'-flanking sequence inserted in the reverse orientation, showed minimal luciferase activity and was not responsive to...
To determine whether the induction of promoter activity is sensitive to antioxidants and steroidal and nonsteroidal anti-inflammatory drugs, we incubated VSMCs transfected by p-2582Luc with pyrrolidine dithiocarbamate (PDTC), DEX, or sodium salicylate before stimulation with LPS. As shown in Fig. 4, DEX inhibited the basal activity of p-2582Luc and prevented LPS induction, whereas PDTC or sodium salicylate had no apparent effects on the basal activity but suppressed the LPS inducibility.

Cellular Specificity of the Human B1 Receptor Gene Promoter—A series of deletion constructs (Fig. 3A), including p1900Luc, were transfected into BAECs, HepG2 cells, and VSMCs to investigate the basal cellular specificity of the B1 receptor gene promoter. Due to variations in transfection efficiency, it is difficult to make an accurate comparison of the basal activities of the same construct in different cells. We therefore compared the basal activities of the series of deletion constructs within each cell line. In VSMCs and BAECs, which are known to express B1 receptors (19), the series of deletion constructs exhibited a similar profile of basal activities (Fig. 5): the 5'-flanking region had higher basal promoter activity than the 1.9-kb fragment, and the p-111Luc showed a level comparable (about 80%) to that observed with the p-2582Luc, implying that the upstream sequence of 2400 bp in p-2582Luc has little contribution to its basal activity. However, in HepG2 cells, the deletion constructs in the 5'-flanking region had a profile of relative basal activity different from those in VSMCs and BAECs (Fig. 5). Constructs containing truncated 5'-flanking sequences had significantly lower basal promoter activities than that of p1900Luc, and the p-111Luc showed a higher basal level than p-2582Luc, indicating negative regulatory elements residing between −2582 and −111.

Identification of LPS-Responsive Elements—To delineate LPS-responsive elements within the 5'-flanking region of the human B1 receptor gene, transient transfection of VSMCs was performed with the series of deletion constructs in the presence or absence of LPS. Fig. 6 shows that the induction of promoter activity upon stimulation with LPS was evident in all constructs except for p-47Luc. The level of induction by LPS in p-111Luc was similar to all other constructs containing additional upstream sequences up to 2582 bp. The promoter activity of p-47Luc was about 4.5% that of p-2582Luc and showed no induction with LPS treatment. The data do not indicate that upstream elements are LPS-responsive but rather that an LPS-responsive region located at −111 to −47 in the 5'-flanking portion mediates the LPS induction of the promoter activity.

NF-κB-like Site Mediating the Induction by LPS and IL-1β—Fig. 2 shows the presence of a CRE site, an NF-κB-like site and an AP-1 site in the LPS-responsive region. In addition, CHX superinduced B1 receptor mRNA, whereas PDTC, an NF-κB activation inhibitor, reduced the LPS inducibility, suggesting the involvement of NF-κB in the induction of B1 receptor gene expression. To test this hypothesis, we performed electro-
and TNF-α. VSMCs, and it was increased upon stimulation by LPS, IL-1 indicating the specificity of the NF-κB-like binding site (Stratagene) (data not shown), or oligonucleotides containing the C/EBP, OCT-1, (Fig. 7A) or oligonucleotides containing the C/EBP, OCT-1, AP-1 binding site (Stratagene) (data not shown), indicating the specificity of the NF-κB-like binding. With nuclear extracts from IMR-90 and HepG2 cells, the same specific binding complexes induced by IL-1β were also observed, indicating that the NF-κB-like site is also functional in IMR-90 and HepG2 cells (Fig. 7A). To further determine the functional importance of the NF-κB-like site, we mutagenized this site in construct p-2582mNLuc. The sequence was changed from 5'-GGC AAT CCC C-3' to 5'-CTC AAT CCC C-3', identical to the mutant oligonucleotide competitor used in the gel shift assay. When p-2582mNLuc was transfected into VSMCs, mutation of the NF-κB-like site almost abolished the induction of promoter activity by LPS, IL-1β, and TNF-α (Fig. 8). A similar result was obtained with HepG2 cells: the observed IL-1β inducibility of p-2582Luc was completely abrogated by the mutation (data not shown), indicating that the NF-κB-like site appears to be necessary for the induction by IL-1β. To support the key role of the NF-κB-like site in the induction of B1 receptor gene expression, we also mutagenized the CRE and AP-1 sites. The p-2582mALuc exhibited almost the same promoter activity as the wild type p-2582Luc. On the other hand, the CRE mutant p-2582mCLuc was still responsive, but with a diminished induction and at a lower basal level. As expected, the double mutant p-2582mCNLuc was not responsive to LPS and IL-1β and demonstrated a much lower basal activity (Fig. 8).

**DISCUSSION**

Kinin receptors were classified into two subtypes, B₁ and B₂ (3). Whereas the B₂ receptor is constitutively expressed, the B₁ receptor is highly induced following tissue injury or inflammatory stimuli (4). However, the molecular mechanism of this induction is not understood. In the present study, we characterized the promoter of the human bradykinin B₁ receptor gene. We first showed that expression of the bradykinin B₁ receptor gene is inducible in primary cultured rat vascular smooth muscle cells but not in human hepatoma HepG2 cells. We then demonstrated that the 5'-flanking region of the human B₁ receptor gene, extending from −2582 to +34, conferred

**Fig. 6. Identification of LPS-responsive elements in VSMCs.** VSMCs were transiently transfected as described under “Experimental Procedures” with deletion constructs in Fig. 3A. The cells were then treated for 3 h with control medium or 5 μg/ml of LPS, and the luciferase activity was measured. Relative luciferase activity was determined as in Fig. 5. The data are presented as means ± S.E. from two independent experiments, each performed in duplicate.

**Fig. 7. Induction of NF-κB-like binding activities by LPS, IL-1β, or TNF-α.** A, interaction of induced nuclear factors with the NF-κB-like site in the human bradykinin B₁ receptor gene promoter. Nuclear proteins were extracted from control VSMCs (lane 2), HepG2 cells (lane 6), IMR-90 cells (lane 8), 4-h LPS-treated VSMCs (lane 3), 3-h TNF-α-treated VSMCs (lane 5), 2.5-h IL-1β-treated VSMCs (lane 4), HepG2 cells (lane 7), and IMR-90 cells (lane 9). Lane 1, no extracts. The binding to the radiolabeled oligonucleotide containing the NF-κB-like site was determined by electrophoretic mobility shift assay as described under “Experimental Procedures.” The induced complexes are indicated by arrowheads. B, competition assay. Nuclear proteins from 4-h LPS-treated VSMCs (from A, lane 3) were incubated with the radiolabeled probe in the absence (lane 1) or presence of 100-fold molar excess of cold oligonucleotide (the same as that used for labeling) (lane 2), the NF-κB consensus sequence DNA derived from the promoter of IRF-1 transcription factor (lane 3), or the oligonucleotide mutated in the NF-κB-like site (lane 4). Analysis by electrophoretic mobility shift assay was performed as in A. S and NS represent specific and nonspecific complexes, respectively.
full responsiveness to LPS and IL-1β by transient transfection of the fragment-driven luciferase reporter gene. Functional analysis showed that a 111-bp sequence from the transcription initiation site, which contained the NF-κB-like binding site, was sufficient for the induction of transcription in VSMCs in response to LPS, IL-1β, and TNF-α. Exposure to LPS, IL-1β, and TNF-α increased the formation of NF-κB-like complexes with this element, as demonstrated by gel shift assays. In contrast, mutation of this NF-κB-like site abolished most of the LPS, IL-1α, and TNF-α inducibility of the B1 receptor promoter construct in the same cells. Taken together, these data for the first time suggest that the NF-κB-like nuclear factor is involved in the bradykinin B1 receptor gene induction process.

NF-κB is a pleiotropic transcription factor involved in the regulation of many genes implicated in the immune response and inflammatory processes (35). In resting cells, NF-κB is sequestered in the cytosol by association with inhibitory proteins of the IκB family. Stimulation by agents such as TNF-α, LPS, and IL-1β initiates a phosphorylation-dependent proteolytic degradation of IκB, allowing active NF-κB to translocate into the nucleus and inducing transcription by binding to defined promoter elements (35). The B1 receptor was suggested to play an important role in the actions of kinins in chronic inflammation (4). The involvement of NF-κB-like nuclear factor in the transcriptional induction of the B1 receptor gene is consistent with the role for NF-κB in the activation of genes mediating inflammation.

As noted, NF-κB is critical for the inducible expression of genes involved in inflammation, and we therefore tested the effects of pyrrolidine dithiocarbamate and sodium salicylate, inhibitors of NF-κB activation (34, 36). We did not observe apparent inhibitory effects of both agents on the basal transcriptional activity of the human B1 receptor gene promoter, but we did observe some inhibitory effects on induction by LPS. This lack of effect might be attributed to an NF-κB-like activity constitutively expressed or induced by serum in VSMCs (37, 38), because PDTC and sodium salicylate seemed to block activation of NF-κB only before stimulation, and they did not alter the NF-κB binding activity (34, 36).

Glucocorticoids have been used as anti-inflammatory drugs (39). It was reported that the synthetic glucocorticoid dexamethasone inhibited the stimulant effect of LPS on the responsiveness to des-Arg9-BK in isolated rabbit aortic strips (25, 26). We examined the effect of dexamethasone on the induction of the B1 receptor in VSMCs. Dexamethasone decreased the expression of the B1 receptor mRNA under basal conditions, which is in agreement with the observation that continuous exposure to dexamethasone inhibited the development of the contractile response to B1 agonists in rabbit aortic strips and human umbilical vein rings (25, 40) and suppressed the LPS inducibility (see Fig. 1). Our transient transfection experiments demonstrated that dexamethasone down-regulated the basal transcriptional activity of the human B1 receptor gene promoter and inhibited the induction by LPS in VSMCs. Thus, dexamethasone inhibited, at least in part, B1 receptor expression at the transcriptional level.

Both in vitro and in vivo studies have suggested that cytokines play an important role in the B1 receptor induction process (4, 25, 26). Our study has shown that the protein synthesis inhibitor cycloheximide enhanced B1 induction by LPS and IL-1β, indicating that the induction of the B1 receptor by LPS and IL-1β does not require new protein synthesis and that IL-1 is not essential for the LPS induction, although VSMCs produce IL-1 in response to endotoxin (41). The enhanced effect by cycloheximide is generally attributed to superinduced NF-κB activity due to blocking of the synthesis of the inhibitory protein IκB (42). In addition, our results showing that recombinant human TNF-α up-regulates B1 receptor gene expression and increases NF-κB-like binding activity in VSMCs are in contradiction with the finding that TNF-α had no effect on the inducibility (39).
spontaneous development of the response to kinins in vitro (26). In addition to potential species and experimental system differences, one hypothesis that might explain the discrepancy is that TNF-α may have inhibitory effects in the posttranscription process.

Early studies showed that the 1.9-kb fragment upstream of the translation start codon ATG in exon III (which covers intron II, exon II, and part of intron I) had a high basal promoter activity in HepG2 cells based on CAT assays (23). On the basis of this finding, we inferred that this fragment might be the promoter for the human B1 receptor gene. Based on their finding in transformed IMR-90 cells, Yang and Polgar (29) thought that intron II of the human B1 receptor gene might function as an alternative promoter. We included this 1.9-kb fragment in the current study, inserted upstream of the luciferase reporter instead of the CAT reporter that was used previously (23). This fragment exhibited a strong basal promoter activity in HepG2 cells, which was 6–8-fold higher than that of the 2582-bp 5′-flanking promoter. It is of interest to note that the basal promoter activity of this fragment in VSMCs is 6-fold lower than that of the 2582-bp 5′-flanking promoter. Because the 5′-flanking promoter was able to confer IL-1β inducibility in both VSMCs and HepG2 cells and the 1.9-kb fragment was not, it is not likely that the 1.9-kb fragment serves as the promoter in VSMCs and HepG2 cells under inflammatory conditions. Whether and under what conditions this 1.9-kb fragment or part of it may function as an alternative promoter still needs further investigation.

The NF-κB-like site is functional in VSMCs as well as in IMR-90 and HepG2 cells. IMR-90 cells are known to express the B1 receptor gene by LPS and IL-1β, but due to the low transfection efficiency, we did not use IMR-90 cells. Whether and under what conditions this 1.9-kb fragment serves as the promoter for the human B1 receptor gene. Based on their basis of this finding, we inferred that this fragment might function as an alternative promoter. We included this 1.9-kb fragment in the current study, inserted upstream of the luciferase reporter instead of the CAT reporter that was used previously (23). This fragment exhibited a strong basal promoter activity in HepG2 cells, which was 6–8-fold higher than that of the 2582-bp 5′-flanking promoter. It is of interest to note that the basal promoter activity of this fragment in VSMCs is 6-fold lower than that of the 2582-bp 5′-flanking promoter. Because the 5′-flanking promoter was able to confer IL-1β inducibility in both VSMCs and HepG2 cells and the 1.9-kb fragment was not, it is not likely that the 1.9-kb fragment serves as the promoter in VSMCs and HepG2 cells under inflammatory conditions. Whether and under what conditions this 1.9-kb fragment or part of it may function as an alternative promoter still needs further investigation.

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