Kinin B₁ receptor upregulation by angiotensin II and endothelin-1 in rat vascular smooth muscle cells: receptors and mechanisms

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Morand-Contant M, Anand-Srivastava MB, Couture R. Kinin B₁ receptor upregulation by angiotensin II and endothelin-1 in rat vascular smooth muscle cells: receptors and mechanisms. Am J Physiol Heart Circ Physiol 299: H1625–H1632, 2010. First published September 10, 2010; doi:10.1152/ajpheart.00735.2009.—Oxidative stress upregulates the kinin B₁ receptor (B₁R) in diabetes and hypertension. Since angiotensin II (ANG II) and endothelin-1 (ET-1) are increased in these disorders, this study aims at determining the role of these two prooxidative peptides in B₁R expression in rat vascular smooth muscle cells (VSMC). In the A10 cell line and aortic VSMC, ANG II enhanced B₁R protein expression in a concentration- and time-dependent manner (maximal at 1 μM and 6 h). In A10 cells, ANG II (1 μM) also increased B₁R mRNA expression at 3 h and the activation of induced B₁R with the agonist [Sar-D-Phe⁸]-des-Arg⁹-BK (10 nM, 5 min) significantly enhanced mitogen-activated protein kinase (MAPK) phosphorylation. The enhancing effect of ANG II on B₁R protein expression in A10 cells was normalized by the AT₁ receptor antagonist losartan but not by the AT₂ receptor (PD123319) receptor antagonist. Furthermore, it was inhibited by inhibitors of phosphatidylinositol 3-kinase (wortmannin) and NF-κB (MG132) but not of MAPK (PD098059). Whereas the ETₐ receptor antagonist (BQ788) had no effect, the ETₐ receptor antagonist (BQ123) blocked the effect of ANG II at 6–8 h but not at an early time point. BQ123 and BQ788 also blocked the increasing effect of ET-1 on B₁R protein expression. Antioxidants (N-acetyl-l-cysteine and diphenylpolyiodonium) abolished ANG II- and ET-1-increased B₁R protein expression. In conclusion, B₁R induction is linked to oxidative stress and activation of phosphatidylinositol 3-kinase and NF-κB. The newly synthesized B₁R is functional and can activate MAPK signaling in VSMC. The effect of ANG II is mediated by the AT₁ receptor and the subsequent activation of ET-1 release.

Oxidative stress; bradykinin; hypertension

Kinins are polypeptides formed locally after tissue damage and inflammation (24). They exert various physiological and pathological effects, including smooth muscle contraction, vasodilatation through the release of nitric oxide (NO) and prostaglandins by endothelial cells, increased vascular permeability and edema, induction of pain, and mitogenesis by the mitogen-activated protein kinase (MAPK) pathway (6, 10, 11, 22, 24). Kinins signal through the activation of two G protein-coupled receptors (GPCR): B₁ (B₁R) and B₂ (B₂R) (34). B₂R is constitutively expressed in a variety of tissues and cell lines and relays the majority of the vascular actions of bradykinin (BK) and Lys-BK (24). However, B₁R is preferentially activated by the C-terminal kininase I active metabolites des-Arg⁹-BK and Lys-des-Arg⁹-BK (27). Whereas the B₂R is virtually absent or weakly expressed in healthy tissues, its expression is enhanced during tissue injury or by exposure to proinflammatory cytokines, growth factors, or oxidative stress (7, 27). This induction can occur in a variety of cell types, including vascular smooth muscle cells (VSMC), endothelial cells, cardiomyocytes, fibroblasts, and neurons (7, 22, 28).

Prooxidative peptides such as angiotensin II (ANG II) and endothelin-1 (ET-1) may contribute to B₁R overexpression in cardiovascular diseases. The effects of ANG II are mediated by two GPCR, AT₁ and AT₂ (39). The AT₁ receptor relays the majority of physiological actions of ANG II and is expressed in normal adult tissues, while the AT₂ receptor is highly expressed in fetal tissues, decreases rapidly after birth, and occurs in a small amount in some adult tissues (38, 40, 44). ANG II stimulates the synthesis (15) and the release of ET-1 (23). ET-1, a peptide of 21 amino acids, is mainly produced by endothelial cells (32), yet other cell types such as cardiomyocytes and VSMC may also produce this peptide (12). The effects of ET-1 are similar to those produced by ANG II such as vasoconstriction, cell proliferation, and production of free radicals (23). The effects of ET-1 are mediated by two GPCR namely ETₐ and ETₐ (32). In blood vessels, ETₐ receptor is mainly expressed in VSMC and relays vasoconstriction, whereas the ETₐ receptor, mainly expressed in endothelial cells, causes vasodilatation by producing NO (35). The ETₐ receptor may also relay the contraction of blood vessels when expressed in VSMC (35).

Although some studies (9, 22) have reported the enhancing effect of ANG II on kinin B₁R expression, none have attempted to demonstrate a putative interaction between ANG II and ET-1 on B₁R induction. Thus this study was designed to assess the direct effects of ANG II and ET-1 and their interplay on the induction of kinin B₁R in VSMC. The following putative mechanisms of B₁R induction by ANG II and ET-1 were studied: MAPK via extracellular signal-regulated kinases 1 and 2 (ERK1/2), phosphatidylinositol 3-kinase (PI3-kinase), nuclear factor-κB (NF-κB), and oxidative stress. The functionality of the B₁R was also studied by assessing the effect of a selective B₁R agonist on MAPK signaling as previously shown in VSMC for B₁R induced by interleukin-1β (IL-1β; Ref. 6). Two types of rat VSMC were used: VSMC freshly derived from the thoracic aorta of the Sprague-Dawley rat and the VSMC A10 cell line derived from embryonic rat thoracic aorta. A10 cells have the advantage to be commercially available and to be cultured for a longer period of time without changing phenotype (21).

MATERIALS AND METHODS

Animal preparation. Male Sprague-Dawley rats (200–225 g; 6–8 wk old) purchased from Charles River (Saint-Constant, Québec, Canada) were maintained on a standard rat chow diet and tap water available ad libitum with a 12:12-h light-dark cycle in a quiet...
were cultured in DMEM containing normal glucose (5.5 mM), 10% FBS, and 1% antibiotic-antimycotic (containing penicillin, streptomycin, and amphotericin B) at 37°C in 95% room air-5% CO₂ as described previously (14). The cells were passaged upon reaching confluence with 0.5% trypsin and used between passages 3 and 20 for A10 and passages 2 to 8 for aortic VSMC. The confluent VSMC after 24 h of incubation in DMEM with 1% FBS was exposed to different periods of time and concentrations of ANG II (0–24 h; 1 nM to 10 μM) or ET-1 (0–6 h). The functionality of the induced B₁R was assayed by measuring the effect on MAPK of a selective and stable B₁R agonist, [Sar-D-Phe⁸]-des-Arg⁹-BK (10 nM, 5 min), with or without stimulation with ANG II (1 μM, 6 h). For receptor antagonists studies, VSMC were incubated overnight in the absence or presence of antagonists of AT₁ (losartan, 10 μM), AT₂ (PD123319, 10 μM), ETA (BQ123, 1 μM), or ETA (BQ788, 1 μM) receptors and stimulated by ANG II (1 μM, 6 h) or ET-1 (100 nM, 2 h). The implication of ERK1/2 and PI3-kinase in the effect of ANG II (1 μM, 6 h) was investigated by using specific inhibitors, PD98059 (10 or 50 μM) and wortmannin (100 nM), respectively. To confirm the implication of NF-κB, a pretreatment of 30 min with an inhibitor of NF-κB (MG132, 10 μM) on VSMC was performed with or without stimulation with ANG II (1 μM, 6 h) or IL-1β (10 ng/ml, 6 h) used as a positive control. Finally, the effects of antioxidants on B₁R expression were measured on VSMC by adding N-acetylated-i-cysteine (NAC, 2 mM) or diphenylethylenodiomide (DPI, 10 μM) overnight before stimulation with ANG II (1 μM, 6 h) or ET-1 (100 nM, 2 h). Most of these treatments (concentration and time) were based on work already done in the past (8, 13, 25).

**Cell extracts.** After incubation, VSMC were washed twice with ice-cold PBS and lysed in 200 μl buffer containing 25 mM Tris HCl (pH 7.5), 25 mM NaCl, 1 mM sodium orthovanadate, 10 mM sodium fluoride, 10 mM sodium pyrophosphate, 2 mM EDTA, 1 mM sodium orthovanadate, 10 μg/ml aprotinin, 1% Triton X-100, 0.1% SDS, and 0.5 μg/ml leupeptin on the ice. The cell lysates were centrifuged at 12,000 rpm for 5 min at 4°C, and the supernatants were used for Western blot analysis. Cell viability was checked with the trypan blue exclusion technique and indicated that >90 ~ 95% cells were viable, like described previously (4).

**Western blot analysis.** Western blot analysis was performed as described previously (2). After SDS-PAGE, the separated proteins were electrophoretically transferred to a nitrocellulose membrane (Bio-Rad) with a Trans-Blot SD semi-dry apparatus (Bio-Rad) at 15 V for 45 min. After transfer, the membranes were incubated in PBS with 0.5% Tween-20 (PBS-Tween) containing 5% skim milk at room temperature for 2 h. The blots were cut in two pieces (~55 kDa) and then incubated with specific antibodies. The lower part was incubated with B₁R-specific antibody (M-19, 1:750), while the upper part was incubated with rat dynein-specific antibody (74–1, 1:5,000), both in PBS-Tween containing 5% skim milk at 4°C overnight. In another series of experiments, the blots were incubated with phosphorylated (p)-ERK1/2 (E-4, 1:2,000) or ERK1/2 (C-14, 1:2,000) in PBS-Tween at 4°C overnight to study the signaling pathway involved in B₁R activation. Blots were washed three times with PBS-Tween. The antigen-antibody complexes were detected by incubating the blots with bovine anti-goat IgG conjugated with horseradish peroxidase (HRP; 1:5,000) for B₁R detection, goat anti-mouse IgG conjugated with HRP (1:5,000) for dynein, and p-ERK1/2 or donkey anti-rabbit IgG conjugated with HRP (1:5,000) for ERK1/2 detection. This step was carried out in PBS-Tween containing 5% skim milk for 1 h at room temperature. The blots were then washed three times with PBS-Tween before the reaction with enhanced chemiluminescence, Western blotting detection reagents (Amersham). The B₁R antibody and the dynein antibody detected a single band at 35 and 74 kDa, respectively, while p-ERK1/2 and ERK1/2 antibody detected a doublet at 42 and 44 kDa. A quantitative analysis of the protein was performed by densitometric scanning of the autoradiographs employing the enhanced laser densitometer LKB Ultrascan XL and quantified using the gel-scan XL evaluation software (version 2.1) from Pharmacia (Baie d’Urfé, Québec, Canada).

**Quantitative real-time PCR.** After incubation, total cellular RNA from rat A10 VSMC was extracted with RNeasy mini kit, and a cDNA copy was synthesized using the QuantiTect reverse transcription kit, according to the manufacturer’s instructions (Qiagen, Mississauga, Canada). Quantitative real-time PCR was performed in SYBR Green Master mix (Qiagen) with 300 nM of each primer and signal detected using the DNA Engine Opticon 2 PCR apparatus (MJ Research, Walthman, MA). For standardization and quantification, rat 18S was amplified. The following primer pairs were designed by Vector NTI software and used: 5’-GGA CGC G CT AAC CAT AGC GGA ATT-3’ (forward, 367–391) and 5’-CCA GTT GAA ACG GTT CCC GTT GAT-3’ (reverse, 478–454) for amplification of rat B₁R (GenBank Accession No. NM_030851); and 5’-TCA ACT TTT GAT GTT AGC GCG CTT G-3’ (forward, 363–386) and 5’-TCC TGT GAT GTG GTA GCC GTT TCT-3’ (reverse, 470–447) for amplification of rat 18S (GenBank Accession No. X01117). PCR conditions were as follows: 95°C for 15 min, followed by 46 cycles at 94°C for 15 s, 60°C for 30 s, and 72°C for 30 s. The cycle threshold (Ct) value represents the cycle number at which a fluorescent signal rises statistically above background. The relative quantification of gene expression was analyzed by the 2^{−ΔΔCt} method (26). Real-time PCR was made on duplicates for each sample, and a blank control (no template) was included in all the experiments for negative controls.

**Drugs and reagents.** ANG II, ET-1, losartan, PD123319, BQ123, BQ788, wortmannin, PD098059, DPI, and NAC were purchased from Sigma-Aldrich Canada. [Sar-D-Phe⁸]-des-Arg⁹-BK was synthesized at the Research Institute of Biotechnology, National Research Council of Canada (Montreal, QC, Canada). The B₁R antibody (M-19, catalog no. sc-15048), dynein antibody (74–1, catalog no. sc-13524), p-ERK1/2 (E-4, catalog no. sc-7383), ERK1/2 (C-14, catalog no. sc-154), and all secondary antibodies were purchased from Santa Cruz Biotechnology (Santa Cruz, CA). The M-19 is an affinity purified goat polyclonal antibody raised against a peptide mapping near to the C-terminus of B₁R of mouse and rat origin. Its specificity was verified by showing a single band at the putative molecular weight of rat B₁R (35 kDa), which was displaced by the peptide sequence used to develop the antibody (data not shown). Other drugs were MG132 (Cayman Chemical, Ann Arbor, MI) and IL-1β (Cell Sciences, Canton, MA).

**Statistical analysis.** Data are means ± SE of n samples. B₁R protein and mRNA levels were measured relative to dynein and 18S levels, respectively. The p-ERK1/2 protein expression was normalized to ERK1/2 levels. Statistical analysis of data was performed with GraphPad Prism software. Results were analyzed by a one-way ANOVA in conjunction with a Bonferroni test where applicable or by a one-way ANOVA followed by Dunnett’s test for multiple comparisons with one control group. A difference between groups was considered statistically significant at P < 0.05.

**RESULTS**

**ANG II upregulates B₁R protein level.** To investigate the modulation of B₁R by ANG II, we examined the effect of different concentrations of ANG II on the expression of B₁R in A10 VSMC as well as in aortic cells. As shown in Fig. 1A, ANG II increased the expression of B₁R protein levels in a
concentration-dependent manner, with maximal stimulation at 1 μM in A10 cells. Control cells also displayed a basal expression of B₁R. Primary cultured aortic VSMC stimulated with ANG II (Fig. 1B) also showed a peak of B₁R expression at 1 μM, although 10 nM produced a highly significant response contrary to A10 cells.

We also examined optimal time for increased expression of B₁R by 1 μM ANG II. This concentration of ANG II was selected because it evoked the maximal induction of B₁R. As shown in A10 cells, B₁R protein levels were increased at 3 h and a peak was obtained at 6 h (Fig. 1C). The induction was not completely back to basal level at 16 and 24 h post-ANG II stimulation. Appropriate control performed at each end point after ANG II stimulation confirmed that the basal level of B₁R did not change over time (data not shown). In aortic VSMC, ANG II (1 μM) showed a similar profile of B₁R induction with a peak at 6 h and significant increases at 1 and 3 h (Fig. 1D). These results show that A10 cells are quite representative of aortic VSMC and, therefore, were used in the following experiments aimed at characterizing the ANG II response on B₁R expression.

ANG II upregulates B₁R mRNA level. To determine if ANG II (1 μM) increased B₁R expression at the mRNA level, quantitative RT-PCR was performed on A10 cells. Data show that B₁R mRNA levels increased significantly at 3 h post-ANG II stimulation (14.8 ± 6.9-fold increase compared with control baseline; *P < 0.05; n = 6) and returned to control levels 24 h after treatment with ANG II (0.8 ± 0.2-fold change vs. baseline values set at 1.0). Values obtained at 1 and 6 h were not significantly altered (data not shown).

Effect of B₁R agonist on ERK1/2 phosphorylation. To determine whether B₁R induced by ANG II was functional, the effect of the selective and stable B₁R agonist [Sar₉-D-Phe₈]-des-Arg⁹-BK was determined on ERK1/2. First, significant phosphorylation of ERK1/2 occurred in A10 cells stimulated for 6 h with ANG II (1 μM; Fig. 2). The addition of [Sar₉-D-Phe₈]-des-Arg⁹-BK (10 nM) during the last 5 min further enhanced the ERK1/2 phosphorylation by 30%. Conversely, the B₁R agonist had no significant effect on basal p-ERK1/2 in control
cells (Fig. 2). These results suggest that B1R is linked to a functional signaling pathway.

\textit{AT1} is implicated in the upregulation of B1R by ANG II. To determine which ANG II receptor subtype was implicated in the induction of B1R, two selective antagonists were used: losartan for the AT1 receptor and PD123319 for the AT2 receptor (39). Results in Fig. 3 show that inhibition of the AT2 receptor did not significantly alter the increased expression of B1R in response to ANG II. In contrast, ANG II-induced increased expression of B1R protein was significantly blocked by losartan. The AT1 and AT2 receptor antagonists did not have any effect on basal expression of B1R. These results suggest that ANG II modulates the expression of B1R via activation of the AT1 receptor.

\textit{PI3-kinase activated by ANG II contributes to the upregulation of B1R.} To investigate the role of ERK1/2 and PI3-kinase in the upregulation of B1R by ANG II, specific inhibitors were used: PD098059, which prevents the activation of ERK1/2 by MEK (1), and wortmannin, which interacts with the catalytic subunit of PI3-kinase (p110α; Ref. 42). Results indicate that wortmannin prevented the upregulation of B1R induced by ANG II, while PD098059 (10 μM) had no significant effect (Fig. 4). Even at a fivefold higher concentration (50 μM), PD098059 did not prevent the upregulation of B1R induced by ANG II [147 ± 5% for ANG II (n = 8), 138 ± 10% for 10 μM of PD098059 combined with ANG II (n = 4), and 144 ± 13% for 50 μM of PD098059 combined with ANG II (n = 4)]. The two inhibitors had no significant effect on basal expression of B1R. These results suggest that ANG II increased the expression of B1R protein via the PI3-kinase pathway.

\textit{NF-κB activated by ANG II and IL-1β contributes to the upregulation of B1R.} NF-κB is a transcription factor involved in the increased expression of B1R (24). To determine the implication of NF-κB in the response to ANG II, quiescent VSMC (A10 cells) were pretreated with an inhibitor of NF-κB (MG132; Ref. 20). IL-1β was used as a positive control for the upregulation of B1R (27). As shown in Fig. 5, MG132 significantly reduced the upregulation of B1R induced by ANG II and IL-1β. This inhibitor had no effect on B1R basal expression. These results suggest that ANG II induces B1R via the activation of NF-κB.
Implication of ETA receptor in the upregulation of B1R expression by ANG II. Since ANG II can increase the levels of ET-1, it was of interest to investigate the implication of ET-1 in ANG II-induced enhanced expression of B1R. To test this, we used selective antagonists of the ETA and ETB receptors, BQ123 (16) and BQ788 (17), respectively. As shown in Fig. 6, inhibition of the ETB receptor did not significantly alter the increased expression of B1R in response to ANG II. However, inhibition of the ETA receptor abolished the ANG II-induced increased expression of B1R. The antagonists for ETA and ETB alone did not have a significant effect on B1R basal expression. These results suggest that the increased B1R expression by ANG II is mediated via the activation of ETA receptor. As illustrated in Fig. 7, BQ123 had a little effect on ANG II-induced upregulation of B1R in the first hours but caused a complete inhibition after 6 and 8 h of stimulation with ANG II. This suggests that the ETA receptor intervenes in the secondary but not in the early phase of stimulation with ANG II.

B1R-induced upregulation by exogenous ET-1 treatment. To investigate if exogenous ET-1 can induce B1R expression, the
effect of ET-1 on the expression of B₁R was examined in A10 and aortic VSMC with various period of time. Results shown in Fig. 8 indicate that ET-1 (100 nM) increased the expression of B₁R in a time-dependent manner; a maximal response occurred at 2 h and remained elevated up to 4 h in both cell types to decline thereafter.

Implication of both ET-1 receptors in B₁R upregulation by ET-1. To determine which ET-1 receptor subtype was implicated in B₁R induction under treatment with ET-1, selective antagonists for each receptor were used: BQ123 for the ETA receptor and BQ788 for the ETB receptor. Results in Fig. 9 show that both antagonists blocked the increased expression of B₁R in response to ET-1. The antagonists for ETA and ETB did not have a significant effect on B₁R basal expression.

Oxidative stress produced by ANG II and ET-1 contributes to the upregulation of B₁R. To investigate the role of oxidative stress in the upregulation of B₁R, two antioxidants were used on A10 cells: DPI, an inhibitor of flavoenzymes such as NAD(P)H oxidase (30), and NAC, a scavenger of reactive oxygen species (ROS; Ref. 43). As shown in Fig. 10, either antioxidant prevented the upregulation of B₁R induced by the two peptides. The antioxidants had no direct effect on the basal expression of B₁R. These results suggest that ANG II and ET-1 induced B₁R via oxidative stress.

DISCUSSION

The present study shows that ANG II and ET-1 increased the expression of kinin B₁R by a mechanism involving oxidative stress, PI3-kinase, and NF-κB in VSMC. Our study provides the first demonstration that B₁R is increased by ANG II in a time- and concentration-dependent manner at the protein level. The data suggest that the upregulation of B₁R is due to increased protein synthesis and highlight an interaction between ANG II and ET-1. Most importantly, the novo synthesized B₁R is functional, as evidenced by the increased ERK1/2 phosphorylation evoked by the B₁R agonist [Sar-d-Phe⁸]-des-Arg⁹-BK in VSMC pretreated with ANG II. This is reminiscent of the activation of the MAPK pathway with des-Arg⁹-BK in VSMC pretreated with IL-1β (6). Our pharmacological data
also suggest that the enhanced expression of B1R by ANG II in VSMC is mediated by AT1 but not AT2 receptors as reported earlier on B1R mRNA in VSMC (22). The majority of vascular actions of ANG II are mediated by the AT1 receptor, which is predominantly expressed in VSMC (40). The AT2 receptor is almost undetectable in this cell type, while it is present in the adventitia (40, 44). Our data are congruent with the upregulation of B1R by endogenous ANG II via the AT1 receptor in the endothelium of small cardiac arteries and cardiomyocytes of two-kidney, one-clip hypertensive rats (9).

It is known that ANG II may stimulate the production (synthesis and release) of ET-1 in VSMC (12, 15). The data with the ETA antagonist suggest that endogenous ET-1 mediates the long-term effect (6–8 h) of ANG II on B1R induction. This is supported by the increased ET-1 level in the cultured medium of rat aortic VSMC that occurs 6 h after stimulation with 1 μM ANG II (23). Hence, ET-1 seems to be a key mediator in the sustained increased expression of B1R in VSMC stimulated by ANG II. This statement is further supported by the fact that exogenous ET-1 can time dependently induce B1R in the two types of VSMC. Exogenous ET-1 seems to operate through both ETA and ETB to enhance expression of B1R because both ET-1 receptor antagonists, BQ123 and BQ788, could individually inhibit ET-1-induced B1R. This is in keeping with the presence of both ETA and ETB receptors in VSMC (35, 37).

Stimulation of VSMC by ANG II or ET-1 causes increases of intracellular calcium and activation of PKC, MAPK, and ROS production (37, 40). These second messengers have been identified in vascular complications associated with ANG II and ET-1, notably cell proliferation, hypertrophy, and hyperplasia (35, 40, 41). Thus a possible mechanism by which these vasopeptides may enhance the expression of B1R is via the generation of ROS. This is supported by the results showing that ANG II- and ET-1-induced increased expression of B1R was attenuated to control levels by DPI and NAC. The contribution of oxidative stress in the overexpression of B1R was previously documented in a rat model of insulin resistance produced by chronic glucose feeding (18). Since DPI prevents the assembly of NAD(P)H oxidase subunits and NAC neutralizes free radicals, this study demonstrates that the assembly of the NAD(P)H oxidase and its activity (production of ROS) may be involved in the induction of B1R stimulated by ANG II and ET-1. Indeed, it is known that ANG II via ROS can activate NF-κB and stimulate degradation of its cytosolic inhibitor, IκB (33). Chronic infusion of ANG II induces B1R expression in rat aorta and spinal cord that was associated with the higher production of superoxide anion production by NAD(P)H oxidase and NF-κB activation (5, 31). Thus ANG II and ET-1 may promote the induction of B1R through the production of ROS and the subsequent activation of NF-κB in the A10 model of VSMC.

BK and ANG II share common signaling pathways leading to changes in vascular tone and architecture (27, 40). One of these mechanisms is MAPK pathways, and the best characterized is the ERK1/2 pathway. It was shown that ERK1/2 is important for NF-κB activation by IL-1β (a stimulus that can upregulate B1R) in a persistent stimulation but not in a rapid and transient stimulation (19). It appears, however, that ERK1/2 may not be implicated in ANG II-induced enhanced expression of B1R. PD098059, a MEK inhibitor, was unable to affect the enhanced expression of B1R in VSMC. On the other hand, wortmannin, an inhibitor of PI3-kinase, prevented the upregulation of B1R induced by ANG II, supporting the role of PI3-kinase in ANG II-induced increased expression of B1R. In this regard, ANG II, through the AT1 receptor, has been shown to activate PI3-kinase in a dose-dependent manner in vivo and in vitro in VSMC (36). ANG II has been reported to increase protein kinase B (Akt) and the transcription factors NF-κB in VSMC (29). NF-κB is the transcription factor that allows the increased expression of B1R (24). We also showed a role of NF-κB in ANG II-induced expression of B1R, because MG132, an inhibitor of NF-κB, blocked the ANG II-induced expression of B1R in VSMC. Taken together, it can be suggested that ANG II-induced expression of B1R is attributed to PI3-kinase and the transcriptional factor NF-κB but not to ERK1/2.

In addition to the harmful pronociceptive and proinflammatory consequences of B1R induction, evidence suggests that this receptor exhibits nephro- and cardioprotective effects, partly due to NO release, and could contribute to the benefit of ANG I-converting enzyme inhibitors in models of diabetes and cardiovascular diseases (reviewed in Ref. 7). Thus whether endogenous kinins can participate through B1R in the deleterious effects of ANG II and ET-1 in vascular disorders associated with diabetes and hypertension deserves further investigation.

In summary, ANG II and ET-1 increased the expression of B1R in aortic and A10 VSMC. The induction of B1R by ANG II is mediated by the AT1 receptor and reveals two components: a direct one in the early phase that is followed by the release of ET-1 that activates the ETB receptor in the second phase. A primary role of oxidative stress, PI3-kinase, and NF-κB is also highlighted in the induction of B1R by ANG II in VSMC. Further studies showing the phosphorylation of PI3K and MEK and also NF-κB activity by ANG II prior to and after antioxidant treatments would be desirable to confirm the hypothesis put forward that ANG II and ET-1 induced the protein B1R expression through ROS production and subsequent activation of NF-κB involving the PI3-kinase pathway.


