Angiotensin II (ATII)-inducible Platelet-derived Growth Factor A-chain Gene Expression Is p42/44 Extracellular Signal-regulated Kinase-1/2 and Egr-1-dependent and Mediated via the ATII Type 1 but Not Type 2 Receptor

INDUCTION BY ATII ANTAGONIZED BY NITRIC OXIDE*

(Received for publication, January 25, 1999, and in revised form, May 11, 1999)

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Angiotensin II (ATII) and platelet-derived growth factor (PDGF) are two vasoconstrictors implicated in the maintenance of normal vascular homeostasis. PDGF A-chain levels increase in cultured vascular smooth muscle cells (SMCs) exposed to ATII. The molecular mechanisms underlying this induction are not known. We used transient transfection analysis to show that ATII can increase reporter gene activity driven by fragments of the PDGF-A promoter bearing recognition elements for the transcription factor, Egr-1. Nuclear run-off experiments indicate that ATII induces Egr-1 expression at the level of transcription. Gel shift and supershift studies show that Egr-1 protein accumulates in the nuclei of SMCs exposed to ATII and binds to the proximal region of the PDGF-A promoter in a specific, time-dependent manner. ATII induced extracellular-signal regulated kinase (p42/44 ERK) activity as did phorbol 12-myristate 13-acetate. The specific MEK1/2 inhibitor, PD98059, suppressed both PDGF-A and Egr-1 endogenous and promoter-dependent expression inducible by ATII. The ATII type 1 receptor (AT1) antagonist, Losartan, inhibited ATII-induction of p42/44 ERK, as well as Egr-1 and PDGF-A, whereas neither PD123319, an AT2 receptor antagonist, nor wortmannin, an inhibitor of phosphatidylinositol 3-kinase and c-Jun N-terminal kinase, had any effect. ATII induction of Egr-1 and PDGF-A was blocked by SIN-1, a NO donor. In addition, this pathway was blocked by overexpression of NO synthase. Collectively, these findings demonstrate that ATII activation of the PDGF-A promoter is mediated via the MEK/ERK/Egr-1 pathway and AT1 receptor and that this process is antagonized by NO.

Angiotensin II (ATII), a peptide hormone with potent vasoconstrictor activity, has long been implicated in the pathobiology of hypertension. In vascular smooth muscle cells (SMCs), ATII stimulates protein synthesis (1), cellular hypertrophy (2–5), migration (6), extracellular matrix synthesis (7, 8), and the activation of a large number of transcription factors. These include Jak/STAT (9), Ets-1 (10), SRF (11), Mox (11), c-Jun (12, 13), JunB (12), and c-Fos (14). ATII is produced in the vessel wall by the actions of renin, which converts angiotensinogen to ATII, which is then cleaved to ATII by angiotensin-converting enzyme. Two ATII receptor subtypes have been described, AT1 and AT2. Signal transduction through G-protein-coupled AT1 receptors involves phospholipase C, phospholipase A₂, phosphodiesterase D, adenylate cyclase, and the release of intracellular calcium (reviewed in Refs. 15 and 16). The AT1 receptor also regulates neointimal thickening after mechanical injury to the rat carotid artery wall and ATII infusion (17). AT2 receptor signaling is less well understood, but evidence suggests that this receptor is involved in growth inhibition (18), Bcl-2 dephosphorylation (19), and apoptosis (20, 21).

Platelet-derived growth factor (PDGF) consists of an A-chain and B-chain held together in homo- or heterodimeric configuration by disulfide bonds (reviewed in Refs. 22 and 23). It is a potent mitogen and chemotactant for mesenchymal cells in culture and a vasoconstrictor with activity comparable to that of ATII (24). Expression of the PDGF-A gene, which resides on human chromosome 7p21-p22 and spans approximately 24 kilobase pairs (25), is under the transcriptional control of the immediate-early gene product, Egr-1 (26), in cultured endothelial cells and SMCs exposed to phorbol 12-myristate 13-acetate (PMA) (27, 28). This involves the displacement of Sp1, which is required for basal expression of the gene, by Egr-1 from overlapping binding sites in the proximal promoter. Several groups have demonstrated that ATII can increase PDGF-A mRNA expression in SMCs (29–33). Indeed, Wong et al. (34) have shown that PDGF-A mRNA and protein expression after arterial balloon injury is blocked by perindopril inhibition of angiotensin-converting enzyme. Presently, however, the regulatory mechanisms underlying ATII induction of PDGF-A are not known.

* This work was supported in part by grants from the National Heart Foundation of Australia (to L. M. K.), National Health and Medical Research Council (to L. M. K. and C. N. C.), Merck Sharp & Dohme (to L. M. K.), and a New South Wales Department of Health Infrastructure Grant (to C. N. C.). The costs of publication of this article were defrayed in part by the payment of page charges. This article must therefore be hereby marked “advertisement” in accordance with 18 U.S.C. Section 1734 solely to indicate this fact.

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The abbreviations used are: ATII, angiotensin II; AT1, ATII type 1 receptor; AT2, ATII type 2 receptor; CAT, chloramphenicol acetyltransferase; p42/44 ERK, extracellular-signal-regulated kinase-1/2; NAME, N-nitro-L-arginine methyl ester; NO, nitric oxide; NOS, nitric oxide synthase; eNOS, endothelial NO synthase; PBS, phosphate-buffered saline; PDGF, platelet-derived growth factor; PMA, phorbol 12-myristate 13-acetate; SIN-1, 3-morpholinosydnonimine; SMCs, smooth muscle cells; Egr-1, early growth response factor-1; bp, base pairs; Sp, specificity protein; FBS, fetal bovine serum; MEK, MAP kinase/ERK; DTT, dithiothreitol; PMSF, phenylmethylsulfonyl fluoride; MAP, mitogen-activated protein; oligo, oligonucleotide; MOPS, 4-morpholinepropanesulfonic acid; DN, dominant-negative.
Nitric oxide (NO) is a potent vasodilator, along with prostacyclin, produced by vascular endothelium. NO regulates blood pressure and regional blood flow through its paracrine action on neighboring SMCs. NO activates guanylate cyclase and inhibits a large number of biological processes, such as SMC proliferation and migration, adhesion molecule expression, leukocyte adhesion, and platelet aggregation (reviewed in Refs. 35–37). NO is produced from L-arginine via the metabolic activity of constitutive endothelial NO synthase (eNOS). It is also produced in cultured SMCs exposed to extracellular stimuli (38). SMCs exposed to lipopolysaccharide, alone or in combination with interferon-γ, interleukin-1β, tumor necrosis factor-α, or forskolin increase expression of inducible NO synthase (39–42). Interestingly, interleukin-1β or forskolin increase expression driven by both Egr-1 and PDGF-A promoters as well as the endogenous genes, in a MEK-dependent manner. Finally, we demonstrate that ATII induction of Egr-1 and PDGF-A in SMCs is mediated through the AT1 but not AT2 receptor and that this pathway is modulated by NO.

In this paper, we show that ATII-induced PDGF-A expression is mediated by the activation and specific interaction of Egr-1 with the PDGF-A promoter. ATII induces Egr-1 expression at the level of transcription and stimulates reporter gene expression driven by both Egr-1 and PDGF-A promoters as well as the endogenous genes, in a MEK-dependent manner. Finally, we demonstrate that ATII induction of Egr-1 and PDGF-A in SMCs is mediated through the AT1 but not AT2 receptor and that this pathway is modulated by NO.

**EXPERIMENTAL PROCEDURES**

**Chemicals**—ATII, phorbol 12-myristate 13-acetate (PMA), (S)-(+)-1-[4-(dimethylamino)-3-methylphenyl]methyl]-5-[diphenylacetyl]-4,5,6, 7-tetrahydro-1H-imidazo[4,5-c]pyridine-6-carboxylic acid (PD123319) and N-nitro-L-arginine methyl ester (L-NAME) were purchased from Sigma. Wortmannin, 2-[2-amino-3-methoxyphenyl]-4H-1-benzopyran-4-one (PD98059), and 3-morpholinosydnonimine (SIN-1) were purchased from Calbiochem. Losartan was a generous gift of Merck, Sharp & Dohme, Sydney, Australia.

**Cell Culture**—Rat vascular SMCs were obtained from Cell Applications, Inc. (Rockville, MD), maintained in M199 plus 10% fetal bovine serum (FBS), 50 μM neomycin, and 50 μg/ml streptomycin at 37 °C and 5% CO₂. Cultures were passaged every 3–4 days in 75-cm² flasks and used in experiments between passages 3 and 7. Cells seeded for preparation of nuclear extracts were maintained as described until 80% confluent and then treated with ATII and nuclear extract. Cells that were prepared for nuclear run-off were treated the same as cells for nuclear extraction except that the cells were incubated in 0.2% FBS for 24 h.

**Preparation of Nuclear Extracts**—SMC monolayers exposed to agonist were washed twice with ice-cold PBS, pH 7.4, then scraped into 2 ml of cold PBS. The cells were pelleted by centrifugation at 250 × g for 10 min at 4 °C. The pellet was resuspended in cold PBS, and the suspension was transferred to Eppendorf tubes. Cells were pelleted by centrifugation for 20 s at 4 °C, then lysed by the addition of ice-cold hypotonic solution (Buffer A) consisting of 10 mM HEPES, pH 8.0, 1.5 mM MgCl₂, 10 mM KCl, 0.5 mM dithiothreitol (DTT), 200 mM sucrose, 0.5% Nonidet P-40, 0.5% phenylmethylsulfonyl fluoride (PMSF), 1 μg/ml leupeptin, 1 μg/ml aprotinin, and incubating the suspension on ice for 5 min. The suspension was centrifuged, and the nuclei were lysed in an ice-cold solution (Buffer C) consisting of 20 mM HEPES, pH 8.0, 100 mM KCl, 0.2 mM EDTA, 20% glycerol, 1 mM DTT, 0.5 mM PMSF, 1 μg/ml leupeptin, 1 μg/ml aprotinin. Cellular debris was pelleted by centrifugation, and the supernatant fraction containing DNA binding proteins was combined with an equal volume of Buffer D (20 mM HEPES, pH 8.0, 100 mM KCl, 0.2 mM EDTA, 20% glycerol, 1 mM DTT, 0.5 mM PMSF, 1 mg/ml leupeptin, 1 mg/ml aprotinin). Extracts were immediately frozen on dry ice and then transferred to −80 °C storage until use.

**Electrophoretic Mobility Shift Assay**—Binding reactions for gel shift assays were performed in 20 μl of 10 mM Tris-HCl, 50 mM MgCl₂, 1 mM EDTA, 1 mM DTT, 5% glycerol, 1 mM PMSF, 1 μM salmon sperm DNA, 5% sucrose, 1 μg poly(dI-dC), 32P-labeled oligonucleotide (150,000 cpm), and 2.5–3 μg of nuclear extract. The reaction was incubated for 35 min at 22 °C. In supershift studies, 2 μg of the appropriate affinity purified rabbit antipeptide antibody was incubated with the binding mix for 10 min at 22 °C before addition of the probe. In competition studies a 100-fold molar excess of unlabeled probe was added to the binding solution for 10 min at 22 °C before addition of the probe. Bound complexes were separated from free probe by loading the samples onto a 6% non-denaturing polyacrylamide gel and electrophoresing at 200 V for 2.5 h. Gels were dried and subjected to autoradiography.

**Northern Blot Analysis**—Northern analysis was performed using total RNA prepared by TRIzol extraction (Life Technologies) electrophoresed on 1% MOPS gels. Prehybridization and hybridization with 32P-labeled Egr-1 and PDGF-A cDNA was performed essentially as described previously (27).

**Western Blot Analysis**—Western blot analysis was performed essentially as described previously (47, 48) using mouse monoclonal antibodies recognizing the phosphorylated (p) form (Thr-183/Tyr-185) of JNK (1,500, Santa Cruz Biotechnology) or rabbit polyclonal antibodies recognizing PDGF-A (1,500, Genzyme) and chimeriluminescence detection (NEN Life Science Products).
ATII Induction of PDGF-A Mediated by Egr-1 and AT1 Receptors

RESULTS

ATII Activates PDGF-A Promoter-dependent Reporter Gene Expression—The molecular mechanisms underlying the capacity of ATII to stimulate PDGF-A mRNA expression in SMCs are unknown. SMCs were transfected with a CAT reporter plasmid, f28, driven by 71 bp of the PDGF-A promoter including the TATA box. Reporter activity in SMCs transfected with this construct increased severalfold following exposure to \(10^{-7}\) M ATII (Fig. 1). CAT activity in SMCs transfected with the proximal PDGF-A promoter sequence was incubated with nuclear extracts of SMCs exposed to ATII (10\(^{-7}\) M) for 1 h. Electrophoretic mobility shift analysis revealed the formation of three distinct nucleoprotein complexes (Fig. 2A). The electrophoretic mobility of these complexes was virtually identical to the pattern observed when PMA (100 ng/ml) was used in lieu of ATII, is not known. The gels were dried and autoradiographed overnight at –80 \(^\circ\)C. The arrow indicates the ATII-inducible complex. The data are representative of three independent experiments.

ATII Induces the Specific Interaction of Nuclear Proteins with the PDGF-A Promoter—A double-stranded, \(^{32}\)P-labeled oligonucleotide (\(^{32}\)P-Oligo A) bearing the bp -76/-47 PDGF-A promoter sequence was incubated with nuclear extracts of SMCs exposed to ATII (10\(^{-7}\) M) for 1 h. Electrophoretic mobility shift analysis revealed the formation of three distinct nucleoprotein complexes (Fig. 2A). The electrophoretic mobility of these complexes was virtually identical to the pattern observed when PMA (100 ng/ml) was used in lieu of ATII (Fig. 2A). However, only one of the three complexes was induced in response to agonist (Fig. 2A, arrow).

Time course studies revealed the transient nature with which this inducible nucleoprotein complex formed. After induction within 1 h, the intensity of the complex remained high for 4 h and then declined progressively to preinduced levels by 9 h (Fig. 2B, arrow). Whereas the relative intensities of the nucleoprotein complexes varied between nuclear extract preparations (compare Figs. 2, A and B), the inducible complex (arrow) consistently formed in response to ATII. Interestingly, induction of this complex within 1 h precedes the earliest detection of inducible PDGF-A mRNA in SMCs exposed to ATII, which typically occurs within approximately 4 h (29, 31, 32). Further experiments determined that the complex formed in a dose-dependent manner at ATII concentrations as low as 10\(^{-10}\) M (data not shown).

We next performed competition studies to demonstrate that interaction between the protein and DNA components of the inducible complex was specific. A 100-fold molar excess of un-labeled Oligo A abrogated the formation of all three complexes (Fig. 3A), whereas the same molar excess of an unrelated oligonucleotide (E74) had no effect (Fig. 3A). Supershift analysis defined the identity of these complexes. The inducible (center) complex was completely supershifted when nuclear extract was preincubated with antibodies directed to Egr-1 (Fig. 3B). The upper complex was abolished by antibodies recognizing Sp1 (Fig. 3B), whereas the lower complex was abrogated by Sp3 antibodies (Fig. 3B). Thus, ATII-inducible Egr-1 protein binds to the PDGF-A promoter in a specific and transient manner.

ATII Induces Egr-1 Expression in SMCs at the Transcriptional Level—The rapid activation of Egr-1 protein suggested that induction of this nuclear factor by ATII was mediated at least in part at the level of transcription. To explore this further, we performed nuclear run-off analysis with nuclei isolated from SMCs exposed to ATII for 1 h. ATII (10\(^{-7}\) M) stimulated new Egr-1 mRNA synthesis within 30 min (Fig. 4). Levels of \(\beta\)-actin transcripts did not change (Fig. 4). These
findings thus indicate that new Egr-1 transcription is stimulated in SMCs incubated with ATII.

ATII Induction of PDGF-A Mediated by Egr-1 and AT1 Receptors

NO Inhibits ATII-Inducible Egr-1 and PDGF-A Expression—Since ATII and PDGF-A are both potent vasoconstrictors (24, 49) and ATII can induce PDGF-A expression, we hypothesized that an endogenous vasodilator, such as NO, might interfere with the induction of PDGF-A. The positive regulatory role of Egr-1 in ATII signaling demonstrated herein prompted us to investigate whether NO could antagonize the activity of this transcription factor. The inability of NO donors to inhibit the induction of Egr-1 by ATII in rat cardiac fibroblasts (50) argued against this possibility. Nevertheless, we found that ATII-inducible Egr-1 promoter-expression was abolished by overexpression of eNOS (Fig. 6).

To determine whether NO could influence the expression of
endogenous Egr-1 and PDGF-A, we performed Northern blot analysis and assessed the effect of the NO donor, SIN-1, on Egr-1 and PDGF-A transcript levels in SMCs. In this system, the MEK1/2 inhibitor blocked the induction of PDGF-A (Fig. 7) and Egr-1 (Fig. 8), whereas wortmannin (1 μM) had no effect on either transcript (Figs. 7 and 8) was abolished by prior exposure to SIN-1 (Figs. 7 and 8). The NO donor also blocked ATII-inducible PDGF-A protein expression (Fig. 9). These findings, taken together, demonstrate the involvement of the MEK/ERK pathway in ATII-inducible endogenous PDGF-A and Egr-1 mRNA expression and the capacity of NO to antagonize this process.

ATII Induction of PDGF-A and Egr-1 in SMCs Is Mediated via the AT1 Receptor—

To determine which ATII receptor subtype mediates the induction of Egr-1 and PDGF-A by ATII, we used pharmacologic agents with the capacity to selectively block signaling from each subtype. Northern blot analysis revealed that ATII-inducible PDGF-A (Fig. 7) and Egr-1 (Fig. 8) expression was abrogated by prior exposure to 10 μM Losartan, a specific, competitive inhibitor of the AT1 subtype (reviewed in Ref. 51). In contrast, neither transcript was influenced by PD123319 (1 μM) (Figs. 7 and 8), a non-peptide inhibitor of AT2 (reviewed in Refs. 52 and 53).

ATII Induction of PDGF-A and Egr-1 in SMCs Is Mediated via the AT1 Receptor—To determine which ATII receptor subtype mediates the induction of Egr-1 and PDGF-A by ATII, we used pharmacologic agents with the capacity to selectively block signaling from each subtype. Northern blot analysis revealed that ATII-inducible PDGF-A (Fig. 7) and Egr-1 (Fig. 8) expression was abrogated by prior exposure to 10 μM Losartan, a specific, competitive inhibitor of the AT1 subtype (reviewed in Ref. 51). In contrast, neither transcript was influenced by PD123319 (1 μM) (Figs. 7 and 8), a non-peptide inhibitor of AT2 (reviewed in Refs. 52 and 53).

In an assay of p42/44 ERK phosphorylation, Losartan almost completely abrogated the activity of this kinase induced by ATII (Fig. 10), an effect comparable to inhibition observed following exposure to PD98059 (Fig. 10). In contrast, neither PD123319 nor wortmannin modulated ATII-induced p42/44

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**Fig. 5.** Egr-1- and PDGF-A-promoter-dependent gene expression is MEK-dependent. SMCs were transfected with 10 μg of −480-CAT (A) or 5 μg of pPDGFALuc9 (B) prior to growth arrest and exposure to 10^{-7} M ATII at 37 °C for 24 h. One h prior to the addition of ATII, the cells were treated with 30 μM PD98059 or 1 μM wortmannin. CAT activity was normalized to the concentration of protein in the lysate. Firefly luciferase activity was normalized to levels of Renilla luciferase. C, wortmannin inhibits ATII-inducible JNK phosphorylation. Growth-arrested SMCs were exposed to 1 μM wortmannin for 1 h prior to incubation with ATII (10^{-7} M) for 8 min. Lysates (10 μg) were assessed for JNK phosphorylation by Western blot analysis using antibodies (Santa Cruz Biotechnology) specifically recognizing phosphorylated (Thr-183/Tyr-185) JNK and chemiluminescence.

**Fig. 6.** Egr-1 promoter activity is blocked by dominant-negative (DN) p42/44 ERK and coexpression of eNOS. SMCs were transfected with 10 μg of −480-CAT together with 5 μg of pcDNA3, DN-ERK1/2-pcDNA3, or eNOS-pcDNA3, prior to growth arrest and exposure to 10^{-7} M ATII at 37 °C for 24 h. CAT activity was normalized to the concentration of protein in the lysate as described under “Experimental Procedures.”

**Fig. 7.** PDGF-A expression inducible by ATII is MEK-dependent and is blocked by NO and AT1 receptor antagonists. Growth-arrested SMCs were exposed to ATII (10^{-7} M) for 4 h after prior incubation (1 h) with 3 or 30 μM PD98059, 1 μM wortmannin, 1 μM PD123319, 1 μM Losartan, or 1 μM SIN-1 at 37 °C. Total RNA was isolated, and Northern blot analysis was performed with 32P-labeled PDGF-A cDNA.
ERK activity (Fig. 10). The L-arginine analogue and NOS inhibitor, L-NAME, which blocks NOS consumption of NADPH by interrupting electron flux has been used previously to investigate the role of NO in pathologic settings such as vascular hypertension (54), glomerular damage (55), and pre-eclampsia (56). L-NAME and ATII together increased p42/44 ERK activity beyond that observed in the presence of ATII alone (Fig. 10). L-NAME had no effect on ERK1/2 phosphorylation in the absence of ATII (Fig. 10). Superinduction of ATII-inducible ERK1/2 phosphorylation by the NOS inhibitor supports the present findings demonstrating the capacity of NO to inhibit ATII signaling (Figs. 6–8). Moreover, these data are consistent with previous observations in which L-NAME modulates NO-dependent signaling (57–59). Taken together, these data indicate that ATII induction of PDGF-A is Egr-1-, ERK1/2-, MEK1/2-, and AT1 receptor-dependent and that this process is antagonized by NO.

**DISCUSSION**

In this paper, we demonstrate that ATII-inducible PDGF-A expression in vascular SMCs is regulated through the AT1, but not AT2 receptor, and requires the activation of p42/44 ERK and promoter interaction by Egr-1. Transient transfection analysis determined that ATII increased reporter gene expression driven by segments of the PDGF-A promoter bearing recognition elements for Egr-1. Gel shift and supershift studies demonstrated that Egr-1 protein accumulates in the nuclei of SMCs exposed to ATII and binds to the proximal region of the PDGF-A promoter in a specific, time-dependent manner. ATII activated p42/44 ERK phosphorylation with magnitude comparable to PMA. PD98059, but not wortmannin, blocked ATII-inducible PDGF-A- and Egr-1-promoter-dependent expression and inhibited endogenous expression of both genes. Losartan inhibited ATII-inducible p42/44 ERK activity, Egr-1, and PDGF-A expression. ATII signaling was blocked by SIN-1 and overexpression of eNOS and, conversely, was augmented by L-NAME.

This study demonstrates for the first time that ATII induction of PDGF-A is mediated by MEK/ERK activation and transactivation by Egr-1 in vascular SMCs. The MEK/ERK pathway also mediates phosphorylation of certain other transcriptional activators and even the AT1 receptor itself. For example, MEK inhibitors block ATII-induced serine phosphorylation of Stat3 in rat fibroblasts (60), as well as phosphorylation and nuclear translocation of AT1 receptors in rat brain neurons (61, 62). Although ATII also activates JNK, possibly via p21-activated kinase in rat vascular SMCs (63), wortmannin, which inhibits JNK activity (64), had no attenuating effect on ATII induction of Egr-1 or PDGF-A. Specific events mediating ATII induction of Egr-1/PDGF-A upstream of MEK are not precisely known. Recent studies in vascular SMCs (65) and cardiac myocytes...
(66) indicate, however, that the non-receptor kinase, c-Src, is required for ATII activation of p42/44 ERK. c-Src is involved in the activation of p21, an upstream activator of MEK, in SMCs exposed to ATII (67). p42/44 ERK activation by ATII also involves protein kinase C-ζ (68) which also lies upstream of MEK (69–71).

The availability of benzimidazole-based pharmacologic inhibitors that selectively block AT1 or AT2 receptor activity prompted us to delineate the receptor subtype that mediates ATII induction of Egr-1 and PDGF-A. Inhibitors such as these have been used by other groups to shed light on pathways utilized by ATII in vivo. For example, ATII-induced vascular wall hypertrophy in rats is blocked by both Losartan and PD123319 (72), while Losartan restored normal arterial pressure, PD123319 had no effect (72). Our demonstration that ATII induction of p42/44 ERK activity, Egr-1, and PDGF-A expression is mediated through the AT1 receptor is supported by observations in vivo. For example, Kim et al. (73) reported that Egr-1 induction and neointima formation after arterial injury is blocked by candesartan cilexetil, an antagonist of the AT1 receptor. This group later showed that p42/44 ERK and JNK activation after injury could be suppressed by the AT1 receptor antagonist, E4177 (74).

Recent investigations in a number of laboratories have revealed that NO plays a crucial antiproliferative role in vascular cells. Rat vascular SMC replication in culture is inhibited by overexpression of eNOS (75). When eNOS-SMC transfectants were seeded onto the luminal surface of balloon-injured rat carotid arteries, neointima formation was blocked and vascular diameter increased (75). NO donors also inhibit SMC proliferation in rat arteries following balloon angioplasty (76). Moreover, adenoviral gene transfer of eNOS in injured rat arteries inhibits neointima formation (77). Balloon injury of pig coronary arteries results in decreased constitutive NOS activity (78). This is, at least in part, due to NO inhibition of medial SMC proliferation (77). Recent studies reveal that NO inhibition of SMC growth is regulated by its ability to down-regulate Cdk2 and cyclin A gene transcription (79). The capacity of NO to suppress the expression of PDGF-A (in the present study), which is chemoattractant for SMC (80), is consistent with the ability of NO to inhibit SMC migration through the AT1 receptor (6). The precise mechanism with which NO modulates Egr-1 and PDGF-A expression is presently unclear. Yu et al. (81) reported that NO donors block SMC proliferation by preventing Ras-dependent activation of Raf-1.

Several other transcription factors are targets of selective inhibition by NO. For example, NO donors inhibit the activation of NF-κB in vascular endothelial cells exposed to tumor necrosis factor-α by stabilization of IκB-α, the cytoplasmic inhibitor of NF-κB. In contrast, AP-1 (82), GATA (82), and IRF-1 (83) are unaffected by NO. Cytokine-inducible vascular cell adhesion molecule-1 (83, 84), intercellular adhesion molecule-1 (83), and M-CSF (85) expression is inhibited by NO via its capacity to suppress NF-κB. Our demonstration that NO blocks ATII-inducible, Egr-1-dependent PDGF-A expression is consistent with the capacity of NO to inhibit activation of transcription factors and their dependent pathophysiologically relevant genes. NO inhibition of vasoconstrictor expression supports the antiatherogenic and antihypertensive properties of this endogenous mediator of homeostasis.

Acknowledgments—We thank Fernando S. Santiago for excellent technical assistance and Drs. Kathleen M. Sakamoto (UCLA School of Medicine), James K. Liao (Department of Medicine, Brigham and Women’s Hospital and Harvard Medical School), and Melanie H. Cobb (Southwestern Medical Center, University of Texas) for plasmids — 480-CAT, eNOS-pcDNA3, and dominant-negative ERRK1/2 (pCEP4/LK18R, pCEP4/LK521R), respectively.

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