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Reactive Oxygen Species-Mediated Signaling Pathways in Angiotensin II-Induced MCP-1 Expression of Proximal Tubular Cells

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ABSTRACT

Angiotensin II (AngII) has pleiotropic effects, the most well known of which is the generation of reactive oxygen species (ROS) and chemokines in inflammatory lesions. Monocyte chemoattractant protein-1 (MCP-1) is considered a major chemokine in the pathogenesis of kidney diseases. We examined signaling pathways of AngII-induced MCP-1 expression and the role of ROS in the murine proximal tubular cells (mProx) using various inhibitors. Furthermore, we compared the signaling pathways between mProx and mesangial cells (MC). AngII-induced MCP-1 protein expression in mProx at 6 h was largely blocked by ROS (*N*-acetylcysteine; 82 ± 14%), Ras (*N*-acetyl-*S*-*trans,trans*-farnesyl-L-cysteine; 82 ± 13%), and nuclear factor-κB (NF-κB) (partheno-lide; 89 ± 7.9%) inhibitors. Both AT_1 receptor (AT1R) (Olmesartan; 41 ± 12%) and the AT2R (PD123319; 24 ± 11%) antagonists partially blocked the MCP-1 expression. Furthermore, mitogen-activated protein kinase (MAPK) pathways were also implicated in this protein expression, but it is less dependent on ROS/Ras pathways. In MC, protein kinase (calphostin C; 84 ± 2.8%) and NF-κB (89 ± 1.4%) inhibitors attenuated acute AngII-induced MCP-1 expression stronger than ROS/Ras inhibitors (1.0 ± 0.9/29 ± 9.5%). MAPK pathways, especially p38 MAPK, were involved in MC more than in mProx. AT1R (69 ± 8.6%) and AT2R (57 ± 21%) antagonists also were blocked. We suggested that, although NF-κB activation has a critical role, signaling pathways are different between mProx and MC. ROS-mediated signaling in mProx may have more contribution to AngII-induced inflammatory responses than to those in MC. *Antioxid. Redox Signal.* 7, 1261–1268.

INTRODUCTION

MONOCYTE CHEMOATTRACTANT PROTEIN-1 (MCP-1) is an important chemokine for macrophages and T lymphocytes, which are crucial players in renal diseases. MCP-1 is expressed by a variety of renal cells, including mesangial cells (MC) and tubular epithelial cells. In renal resident cells, its expression is up-regulated by various mediators, including cytokines and growth factors. Therefore, this particular chemokine may play a major role in the pathogenesis of renal injury, especially in the progression of tubulointerstitial damage (38, 41). Indeed, blockade of MCP-1 by antibodies or antisense oligonucleotides reduces tubulointerstitial damage in a variety of renal diseases (41).

Angiotensin II (AngII), a major peptide of the renin–angiotensin system (RAS), is increased locally in many tissues under a variety of pathophysiological conditions. Various tubulointerstitial insults, including ischemia and proteinuria, induce local RAS activation in tubulointerstitial or infiltrating cells (36, 37). On the other hand, previous studies have indicated that reactive oxygen species (ROS) are involved in the pathogenesis of glomerular and tubulointerstitial diseases

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(34). ROS also act as a second messenger of intracellular transduction through growth factor receptors in many forms of transcriptional regulations (32). AngII is known to induce intracellular ROS in many types of cells (8). Moreover, AngII-induced ROS are important for intracellular signalings, such as extracellular signal-regulated kinases 1/2 (ERK1/2), in renal cells (14). Therefore, special attention is paid to the ROS-related pathological conditions with locally elevated AngII in various diseases (13, 32, 33).

Cytokine-induced MCP-1 expression is regulated through a redox-sensitive mechanism. ERK activation is involved in the MCP-1 expression via small GTPase, Ras, protein kinase C (PKC), and phosphatidylinositol 3-kinase (PI3K) in certain cells (15). However, the signaling pathways of AngII-induced MCP-1 production through ROS remain unclear in renal resident cells. Severity of tubulointerstitial damage is a major prognosis factor in kidney diseases. On the other hand, oxidative stress plays a role in the tubulointerstitial damage. To approach the pathogenesis of AngII-mediated tubulointerstitial damage, we examined the signaling pathways of AngII-induced MCP-1 in mouse proximal tubular cells (mProx), as well as the contribution of ROS to this situation. Furthermore, we also used MC to compare the role of ROS between both renal resident cells.

MATERIALS AND METHODS

Reagents

AngII, *N*-acetyl-*S*-*trans,trans*-farnesyl-L-cysteine (AFC), *N*-acetylcysteine (NAC), PD123319, diphenyleneiodonium chloride (DPI), SB203580, parthenolide, and catalase were obtained from Sigma Chemical Co. (St. Louis, MO, U.S.A.). PD098059 and calphostin C were from Wako (Tokyo, Japan). Olmesartan (RNH-6270)was provided by Sankyo Co. (Tokyo, Japan).

Cells

The mProx (derivative; patent WO9927363, Japan, U.S., European Union) were kindly provided by CMIC Co. Ltd. (16, 38). This cell line was obtained from C57BL/6 adult mouse kidney (CLEA Japan, Tokyo, Japan) and cultured in Dulbecco's modified Eagle's medium with 10% fetal calf serum, 250 μg/ml penicillin, and 250 μg/ml streptomycin placed on a 10-cm dish at 37°C in 5% CO_2. Confluent cells were starved in the serum-free medium for 24 h. Thereafter, the cells were pretreated with several inhibitors and stimulated with AngII (19). Primary cultured mouse MC were prepared from outgrowths of glomeruli harvested from mice as described previously (12, 17). In brief, C57BL/6 mice (CLEA Japan) were killed by decapitation, and their kidneys were removed. After removal from the renal capsule, the kidneys were separated by repeated 30–50% Percoll (Amersham Biosciences, Uppsala, Sweden) gradient centrifugation. We used MC at the third passage in RPMI 1640 medium buffered with 25 m*M* HEPES at pH 7.4 supplemented with 20% fetal calf serum, 100 U/ml penicillin, 100 μg/ml streptomycin, 2 m*M* L-glutamine, 5 μg/ml transferrin, and 0.06 U/ml insulin (complete medium) in a 5% CO_2 environment at 37°C.

Semiquantitative reverse transcription–polymerase chain reaction (RT-PCR)

Total RNA was extracted from each sample using TRIZOL reagent (Invitrogen, Carlsbad, CA, U.S.A.) according to the manufacturer's protocol. One microgram of total RNA was transcribed from cDNA in a reaction mixture containing 1 × buffer, 10 m*M* dithiothreitol, dNTP (0.5 m*M* each), 0.5 μg of oligo(dT) primer, RNase H, and Superscript II. Each sample was assayed for MCP-1 and glyceraldehyde-3-phosphate dehydrogenese (GAPDH). cDNA was placed in separate tubes using primers for PCR. PCR was performed by incubating 1 μg of the sample cDNA, and was conducted under the following conditions: 30 cycles of denaturation at 94°C for 1 min, annealing of the primers at 55°C for 1 min, and primer extension at 72°C for 2 min using a thermal cycler. Primers were as follows: 5′-TGTCTGGACCCCATTCCTTC-3′ (forward primer), 5′-ACCAGCAAGATGATCCCAAT-3′ (reverse primer) for mouse MCP-1 of 140 bp; and 5′-GGAGAGAACCTGGTCCTCAG-3′ (forward primer), 5′-ACCCAGA AGACTGT GGATGG-3′ (reverse primer) for GAPDH of 300 bp. The amplification of PCR was performed for up to 30 cycles.

Enzyme-linked immunosorbent assay (ELISA)

MCP-1 concentrations in the culture supernatants were measured using an ELISA kit (Sigma). Culture supernatants (100 μl) were added to 96-well ELISA plates coated with anti-mouse MCP-1 monoclonal antibody for 24 h at 4°C and then washed three times with phosphate-buffered saline (PBS) and 0.05% Tween. Nonspecific sites were blocked by incubation in 5% bovine serum albumin and PBS for 1 h at room temperature. The plates were washed five times. We then added cultured medium from each sample and incubated the samples for 2 h at room temperature. The plates were washed five times and incubated with an anti-mouse MCP-1 monoclonal antibody for 1 h at room temperature. Quantification was performed by determination of colorimetric conversion at 450 nm of tetramethylbenzipine.

Assay of intracellular ROS

mProx were pretreated with each reagent as follows: olmesartan for 2 h; parthenolide for 90 min; catalase for 1 h, and NAC (the free radical scavenger), AFC (Ras inhibitor), PD098059 (ERK1/2 inhibitor), and calphostin C (PKC inhibitor) for 30 min. After pretreatment, the mProx were stimulated with AngII for 3 h. Confluent cells were incubated for 45 min in 5-(and 6)-chloromethyl-2′,7′-dichlorodihydrofluorescein diacetate (CM-H_2DCFDA) (Molecular Probes Inc., Eugene, OR, U.S.A.) and washed with Ringer solution. CM-H_2DCFDA is a nonpolar compound that readily diffuses into cells, where it is hydrolyzed to the nonfluorescent polar derivative 2′,7′-dichlorofluorescin (DCFH) and thereby trapped within the cells. In the presence of a proper oxidant, DCFH is oxidized to the highly fluorescent 2′,7′-dichlorofluorescein (DCF) (9, 21, 22, 26).

Western blot analysis

Western blotting was performed as described previously (17, 34). In brief, the cells were washed with ice-cold PBS, and

then lysed with lysis buffer (50 m*M* HEPES at pH 7.4, 1% NP40 in PBS, 0.1% sodium dodecyl sulfate, 50 m*M* NaF, 50 m*M* NaCl, 1 m*M* Na_3VO_4, 30 m*M* $Na_4P_2O_7$, 50 U/ml aprotinin, and 10 mg/ml leupeptin). Samples were directly resolved on 10% polyacrylamide gels and electrophoretically transferred to nitrocellulose membrane (Novex, San Diego, CA, U.S.A.) for 1 h at 150 mA. The filters were incubated with blocking buffer overnight. Then the filters were incubated with anti-phospho-p44/p42 mitogen-activated protein kinase (MAPK) (Thr^{202}/Tyr^{204}) monoclonal antibody (1:2,000) (Cell Signaling, Beverly, MA, U.S.A.). Blots were visualized by the enhanced chemiluminescence reaction (Amersham Life Science).

RESULTS

Ang II induces MCP-1 expression through AT_1 and AT_2 receptors (AT1R and AT2R) in cultured mProx

First, we examined basal kinetics of AngII-induced MCP-1 expression in mProx. We confirmed that AngII induces MCP-1 expression in a dose- (Fig. 1A) and time- (Fig. 1B) dependent manner. Olmesartan (1 μ*M*), AT1R blocker, inhibited AngII-induced MCP-1 protein expression (% inhibition: 3 h, 61 ± 6.6%; 6h, 41 ± 12.2%) (Fig. 1B, Table 1). Although less intensively, the AT2R antagonist, PD123319 (10 μ*M*), also inhibited AngII-induced MCP-1 expression (% inhibition: 3 h, 51 ± 2.1%; 6 h, 24 ± 11%) (Fig. 1B, Table1). The same inhibitory effect was found in mRNA expression (Tables 1 and 2).

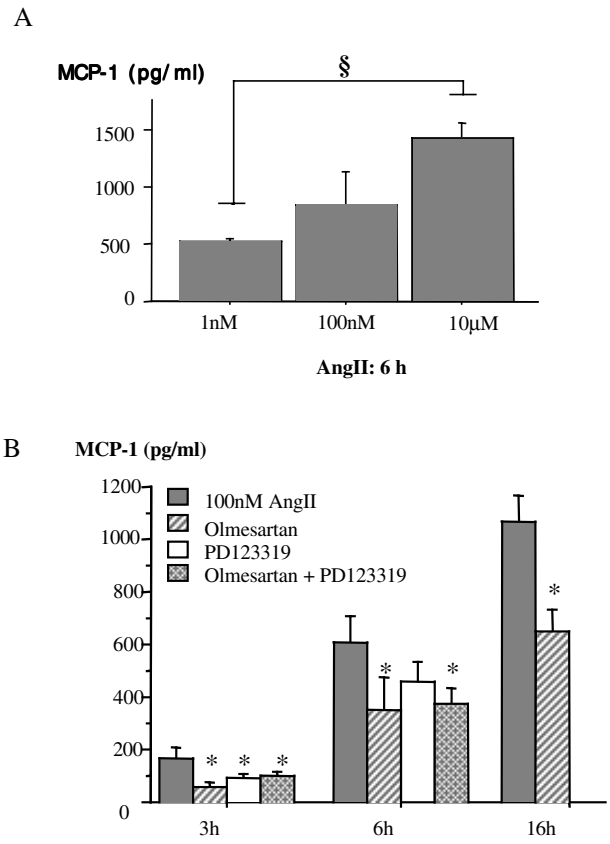

FIG. 1. (**A**) AngII induces MCP-1 production in mProx in a dose-dependent manner. Values represent means ± SD of three independent experiments. §$p < 0.05$. (**B**) AngII (100 n*M*) induces MCP-1 production in mProx in a time-dependent manner. Moreover, Olmesartan (1 μ*M*), PD123319 (10 μ*M*), and the combination partially blocked AngII-induced MCP-1 production in mProx. MCP-1 levels were determined by ELISA as described in Materials and Methods. Values represent means ± SD of three independent experiments. *$p < 0.05$ versus AngII.

TABLE 1. PERCENT INHIBITION OF ANGII-INDUCED MCP-1 EXPRESSION AT 6 H IN MPROX

Inhibitors	*Protein*	*mRNA*
Olmesartan (AT1R antagonist) (1 μ*M*)	41 ± 12	64 ± 7.7†
Olmesartan (10 n*M*)	39 ± 8.4	29 ± 16
PD123319 (AT2R antagonist)	24 ± 11	27 ± 2.1
Olmesartan (1 μ*M*) + PD123319	39 ± 9.9	ND
NAC (free radical scavenger)	82 ± 14*	75 ± 6.3†
DPI (NADH/NADPH oxidase inhibitor)	54 ± 13*	ND
AFC (Ras inhibitor)	82 ± 13*	79 ± 2.8†
Calphostin C (PKC inhibitor)	16 ± 2.6	25 ± 14
PD098059 (ERK1/2 inhibitor)	57 ± 10*	54 ± 3.2†
SB203580 (p38 MAPK inhibitor)	32 ± 12	8.9 ± 1.5
Parthenolide (NF-κB inhibitor)	89 ± 7.9*	94 ± 5.7†

*†$p < 0.05$ versus AngII.

AngII induces intracellular ROS production in mProx

Next, we evaluated AngII-induced ROS production in mProx. The cells were treated with AngII (100 n*M*) for 1–3 h. Figure 2A shows that intracellular ROS was increased in a time-dependent manner by AngII treatment. We also studied the mediators involved in AngII-induced ROS production using various inhibitors (Fig. 2B). NAC (5 mg/ml), a scavenger of free radicals (44), greatly reduced intracellular ROS production, whereas AFC (12.5 μg/ml), a Ras inhibitor, did not show this effect (42). Moreover, catalase (1,500 U/ml) treatment also strongly attenuated this production, indicating that hydrogen peroxide is the main source of ROS. We examined whether PD098059 (25 μ*M*) (ERK1/2 inhibitor), calphostin C (100 n*M*) (PKC inhibitor), or parthenolide (10 μg/ml) [nuclear factor-κB (NF-κB) inhibitor] inhibited AngII-induced ROS production (Fig. 2B). However, these inhibitors failed to block intracellular ROS production, suggesting that ROS production is upstream of the ERK, PKC, and PI3K pathways and NF-κB activation.

TABLE 2. PERCENT INHIBITION OF ANGII-INDUCED MCP-1 EXPRESSION AT 6 H IN MC

Inhibitors	*Protein*	*mRNA*
Olmesartan (AT1R antagonist) (1 μ*M*)	69 ± 8.6*	56 ± 8.5†
PD123319 (AT2R antagonist)	57 ± 21	39 ± 13
Olmesartan (1 μ*M*) + PD123319	71 ± 5.6*	ND
NAC (free radical scavenger)	1.0 ± 0.9	2.2 ± 1.8
AFC (Ras inhibitor)	29 ± 9.5	8.6 ± 7.0
Calphostin C (PKC inhibitor)	84 ± 2.8*	46 ± 2.8†
PD098059 (ERK1/2 inhibitor)	54 ± 3.5	69 ± 4.2†
SB203580 (p38 MAPK inhibitor)	57 ± 8.5	49 ± 16†
Parthenolide (NF-κB inhibitor)	89 ± 1.4*	58 ± 1.4†

*†$p < 0.05$ versus AngII.

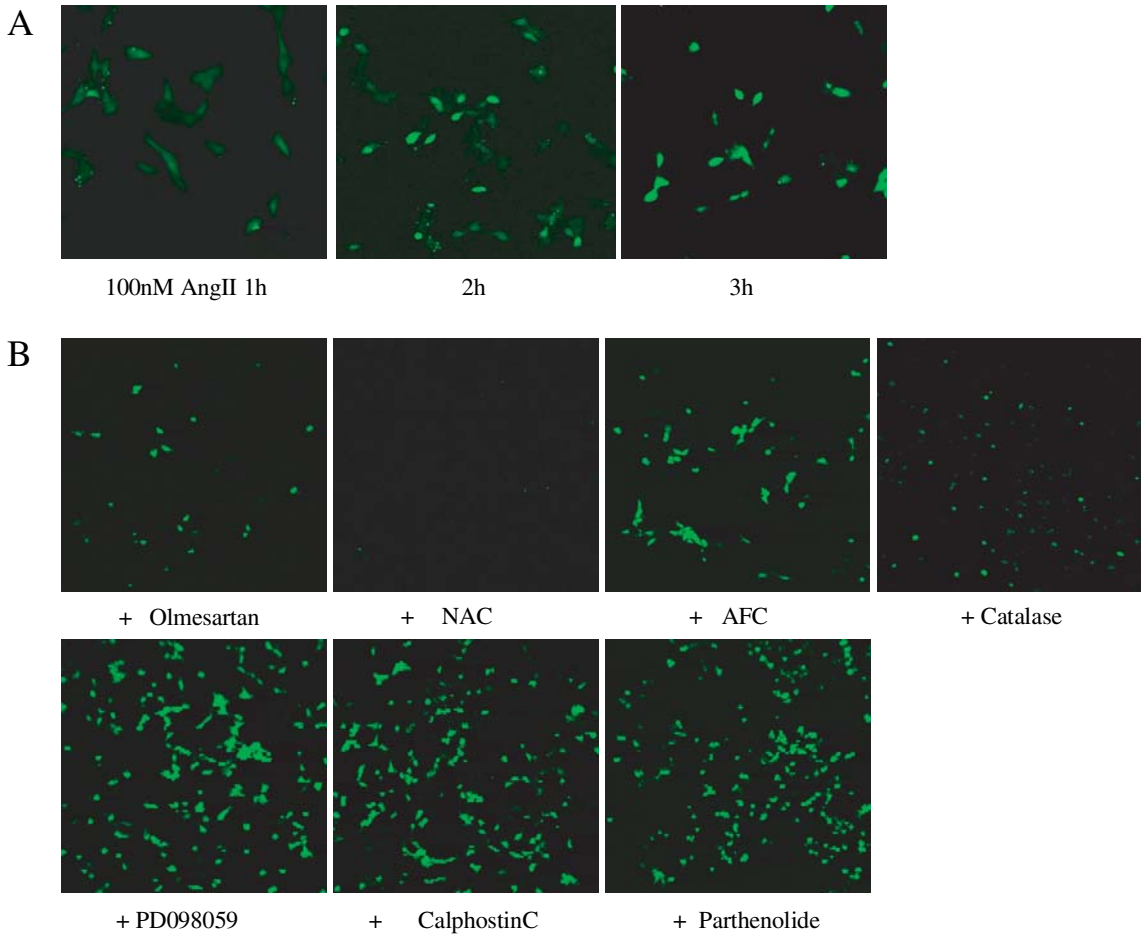

FIG. 2. (**A**) AngII induces ROS production in mProx. AngII induces ROS production in a time-dependent manner. (**B**) mProx were pretreated with various inhibitors before AngII stimulation for 3 h. Olmesartan (1 μ*M*) pretreatment was performed for 2 h, NAC, AFC, PD098059, and calphostin C for 30 min, catalase for 1 h, and parthenolide for 90 min. Panels show a representative experiment of $n = 5$ done.

AngII induces MCP-1 expression mainly through ROS, Ras, and NF-κB in mProx

To examine the ROS-mediated signaling cascade in the MCP-1 production, we focused on the early phase (3–6 h) after AngII stimulation. Accordingly, NAC pretreatment inhibited the AngII-induced MCP-1 expression in mProx, peaking at 3–6 h (data not shown). Table 1 shows that NAC, AFC, and parthenolide attenuated MCP-1 expression markedly at 6 h (80–90%), indicating that ROS, Ras, and NF-κB are major factors in this cascade. On the other hand, NADH/NADPH inhibitor (DPI; 4 μ*M*) caused attenuation of ~50%. The ERK1/2 inhibitor (PD098059) was more effective than the MAPK p38 inhibitor (SB203580) (10 μ*M*). The AngII-induced mRNA expression of MCP-1 was also regulated in the same manner at protein levels (Table 1).

AngII-induced ROS production and Ras activation are not major pathways for ERK1/2 activation in mProx

To determine whether ROS-dependent ERK activation is involved in AngII-induced MCP-1 expression in mProx, we examined the phosphorylation levels of ERK1/2 at 6 h using anti-phospho-p42/p44 MAPK antibody. NAC and AFC attenuated ERK1/2 phosphorylation at 6 h only by 26 ± 13% and 25 ± 6.0%, respectively. Therefore, these findings suggest that ROS production, and subsequent Ras activation, have small contribution to AngII-induced ERK1/2 phosphorylation (Table 3).

TABLE 3. PERCENT INHIBITION OF ERK1/2 PHOSPHORYLATION AT 6 H IN MPROX

Inhibitors	*% inhibition*
Olmesartan (AT1R antagonist) (1 μ*M*)	45 ± 2.0
PD123319 (AT2R antagonist)	28 ± 9.8
Olmesartan (1 μ*M*) + PD123319	42 ± 5.8
NAC (free radical scavenger)	26 ± 13
DPI (NADH/NADPH oxidase inhibitor)	37 ± 11
AFC (Ras inhibitor)	25 ± 6.0
Calphostin C (PKC inhibitor)	55 ± 22*
PD098059 (ERK1/2 inhibitor)	72 ± 17*
SB203580 (p38 MAPK inhibitor)	30 ± 13

*$p < 0.05$ versus AngII.

Table 4. Percent Inhibition of ERK1/2 Phosphorylation at 6 h in MC

Inhibitors	*% inhibition*
Olmesartan (AT1R antagonist) (1 μ*M*)	29 ± 13
PD123319 (AT2R antagonist)	27 ± 2.8
Olmesartan (1 μ*M*) + PD123319	47 ± 3.5
NAC (free radical scavenger)	11 ± 13
DPI (NADH/NADPH oxidase inhibitor)	8 ± 0.9
AFC (Ras inhibitor)	62 ± 29
Calphostin C (PKC inhibitor)	55 ± 25
PD098059 (ERK1/2 inhibitor)	78 ± 28
SB203580 (p38 MAPK inhibitor)	38 ± 8.6

On the other hand, Olmesartan, DPI, and calphostin C attenuated ERK1/2 phosphorylation by 45 ± 2.0%, 37 ± 11%, and 55 ± 22%, respectively, suggesting that AT1R-mediated NADH/NADPH oxidase and PKC activation may be mainly involved in ERK1/2 phosphorylation.

ROS is not a central mediator of AngII-induced MCP-1 expression in MC

To compare the signaling pathways of AngII-induced MCP-1 expression between MC and mProx, MC were also pretreated with these inhibitors. Although NAC and AFC failed to show sufficient inhibition (1.0 ± 0.9% and 29 ± 9.5%), PD098059 (54 ± 3.5%) and calphostin C (84 ± 2.8%) strongly inhibited the MCP-1 expression in MC (Table 2). In this regard, calphostin C (55 ± 25%), but not DPI (8 ± 0.9%), largely attenuated the AngII-induced ERK1/2 phosphorylation (Table 4). Parthenolide inhibited the MCP-1 expression in MC (89 ± 1.4%) and mProx (89 ± 7.9%) to the same extent (Tables 1 and 2). Accordingly, our results indicate that AngII-induced MCP-1 expression in MC may be mainly through PKC, ERK1/2, and NF-κB activation, but not ROS-dependent pathways as seen in mProx.

DISCUSSION

ROS, as a second messenger, regulate many intracellular signaling pathways and transcriptional factors (32). In addition, ROS is known to be involved in AngII-related pathophysiology (13, 36, 40). AngII is also a potent stimulator of MCP-1 expression in various cell types, including vascular smooth muscle cells (VSMC) (3, 10, 30), MC (29), and proximal tubular cells (36). Previous studies have revealed that ROS and MCP-1 are importantly involved in the development of tubulointerstitial damage, which is ameliorated by RAS blockade. Therefore, we examined the role of ROS in AngII-induced MCP-1 expression in mProx in comparison with that in MC. By using some inhibitors, our data indicate that AngII-induced intracellular ROS, and subsequent Ras and NF-κB activation, are the major pathway for the MCP-1 expression in mProx. By contrast, the AngII-induced MCP-1 expression in MC is less dependent on ROS production, and more dependent on PKC and MAPK pathways.

The beneficial effects of AT1R blockade may be due not only to the prevention of AT1R-mediated responses, such as fibrosis or proliferation (1, 6, 22), but also to the binding of free AngII to the AT2R, which results in some other beneficial effects, such as vasodilatation and antiproliferative responses (1). However, a recent report has indicated that AT2R may play an important role in tubulointerstitial leukocyte infiltration (6), cell differentiation, and apoptosis via ROS generation in proximal tubular cells (1, 31). In addition, persistent proteinuria causes local RAS activation followed by apoptosis of tubular cells via AT2R in a protein-overload model (1, 39). Therefore, AT2R-mediated signaling pathways, concomitant with ROS generation and the downstream signalings, should be carefully addressed in the tubulointerstitial inflammatory processes and remodeling. Indeed, our present data show that both AT1R and AT2R blockade inhibited AngII-induced MCP-1 expression in mProx.

Several signaling pathways, such as Ras, PKC, PI3K, ERK, and NF-κB, are known to be involved in MCP-1 expression (15, 34). The present study shows that inhibitors of the Ras, PKC, PI3K, and ERK pathways failed to attenuate AngII-induced intracellular ROS production in mProx, suggesting that those signalings were not positioned upstream of ROS. However, not only ROS but also Ras inhibitors strongly attenuated AngII-induced MCP-1 expression in mProx to the same extent. The small GTPase, Ras, plays a critical role in the regulation of numerous cellular functions, including cell proliferation and differentiation (27). Lysophosphatidylcholine is known to induce ROS production in some cells (43). On the other hand, lysophosphatidylcholine induces raf-1 activation transmitted by Ras and ERK1/2 activation, which is blocked by antioxidants (43). These findings indicate that intracellular ROS may be a potent activator of Ras (43). Therefore, our data suggest that the cascade of AngII-induced MCP-1 expression in mProx is mainly through the AngII-induced intracellular ROS production and, presumably, subsequent Ras activation.

MAPKs are encoded by the ERK genes. ERK1/2 has two isoforms: p44 (ERK1) and p42 (ERK2) (3, 4). ERK1/2 is activated by phosphorylation on threonine and tyrosine residues by MAPK kinase (MEK). ERK1/2 is also activated by stimulation of either AngII or platelet-derived growth factor, which in turn may affect cell proliferation and chemokine generation in many types of cells (3, 18, 32, 34, 39). ROS generation is also known to activate the ERK pathway (7, 43). Moreover, ERK activation by Ras seems to be involved in MCP-1 expression in human embryonic kidney 293 cells (15). In our study, although Ras inhibitor strongly inhibited AngII-induced MCP-1 expression in mProx, this inhibitor did not cause enough reduction of the ERK1/2 phosphorylation levels, suggesting that ERK pathways after Ras activation may have a small role in AngII-induced MCP-1 expression in mProx. On the other hand, PKC (calphostin C), p38 MAPK (SB203580), and ERK1/2 (PD098059) inhibitors largely inhibited the AngII-induced MCP-1 expression in MC, but not the free radical scavenger NAC. In agreement with our data, several reports have demonstrated that PKC and phosphorylated ERK1/2 are involved in MCP-1 expression in MC (9). Accordingly, our present findings suggest that AngII-induced MCP-1 expression in MC may be mainly through PKC-activated ERK1/2 phosphorylation, but not through ROS.

MCP-1 expression is regulated by several transcriptional factors, such as NF-κB, activator protein-1, and Sp-1 (32,

34). NF-κB is a well characterized transcription factor that is influenced by the cellular redox states (30). AngII-induced ROS activates NF-κB in cardiac fibroblasts (32), VSMC (2, 30, 31), tubular cells (25), and MC (28). The present study demonstrated that NF-κB inhibitor (parthenolide) attenuated AngII-induced MCP-1 protein expression in both mProx and MC (89 ± 2.7% and 89 ± 1.4%), indicating that, in spite of the difference in signaling pathways, NF-κB activation is crucial in both cells. This idea is supported by previous findings that in a number of experimental renal diseases both RAS blockade and NF-κB inhibition show beneficial effects (6, 23, 24, 37).

Inappropriate renal RAS activation by many factors, including proteinuria (20, 35, 37), ischemia (37), and antibody deposition (11, 36), may contribute to the pathogenesis of glomerular and tubulointerstitial diseases (5, 14). Overproduction of chemokines by AngII might be one of the key mechanisms in the pathogenesis of renal injury. The present study demonstrates that AngII strongly induces MCP-1 production in both renal cell types, but through different signaling cascades. Of interest, intracellular ROS production may be a critical process for the AngII-induced inflammatory responses in proximal tubular cells. Progression of tubulointerstitial damage finally determines the prognosis of renal diseases. Therefore, antioxidant therapy, in addition to RAS blockade, could be required and an interesting approach for the prevention of end-stage renal failure.

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ABBREVIATIONS

AFC, *N*-acetyl-*S*-*trans,trans*-farnesyl-L-cysteine; AngII, angiotensin II; AT1R and AT2R, AT_1 and AT_2 receptors, respectively; CM-H_2DCFDA, 5-(and 6)-chloromethyl-2′,7′-dichlorodihydrofluorescein diacetate; DCFH, 2′,7′-dichlorofluorescin; DPI, diphenyleneiodonium chloride; ELISA, enzyme-linked immunosorbent assay; ERK, extracellular signal-regulated kinase; GAPDH, glyceraldehyde-3-phosphate dehydrogenase; MAPK, mitogen-activated protein kinase; MC, mesangial cells; MCP-1, monocyte chemoattractant protein-1; mProx, mouse proximal tubular cells; NAC, *N*-acetylcysteine; NF-κB, nuclear factor-κB; PBS, phosphate-buffered saline; PI3K, phosphatidylinositol 3-kinase; PKC, protein kinase C; RAS, renin–angiotensin system; ROS, reactive oxygen species; RT-PCR, reverse transcription–polymerase chain reaction; VSMC, vascular smooth muscle cells.

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