Aldosterone induces interleukin-18 through endothelin-1, angiotensin II, Rho/Rho-kinase, and PPARs in cardiomyocytes

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INTERLEUKIN-18 (IL-18), A member of the IL-1 family, is a proinflammatory cytokine with multiple biological functions (36, 38). IL-18, originally identified as an interferon-γ-inducing factor (34), can induce TNF-α and IL-6 expression in murine macrophages (34). IL-18 is expressed by both immune and nonimmune cells and plays a critical role in the pathophysiology of various diseases, including myocardial ischemia, myocardial infarction, and myocarditis. In particular, IL-18 is thought to induce a proinflammatory response in the myocardium through different mechanisms, including increased expression of endothelial cell adhesion molecules (55) and production of proinflammatory cytokines, such as TNF-α, IL-1β, and IL-8. It also brings about the expression of inducible nitric oxide synthase (37, 41). These molecules are implicated in the modulation of myocardial contractile function (7, 26) and myocyte apoptosis (29). Myocardial hypertrophy is a major cause of myocardial dysfunction and cardiac remodeling. It has been shown that IL-18 induces cardiac myocyte hypertrophy through activation of phosphatidylinositol 3-kinase, Akt, and GATA-4 signaling pathway (6). Furthermore, atrial natriuretic peptide expression is upregulated in the myocardium of patients with congestive heart failure, leading to myocardial hypertrophy (49). In addition, the effect of IL-18 on left ventricular (LV) function was examined. Daily intraperitoneal injection of IL-18 increased LV end-diastolic pressure and reduced β-adrenergic responsiveness to isoproterenol (57). We and others have reported that there are increased levels of circulating IL-18 in patients with acute coronary syndromes and congestive heart failure (24, 25, 34). Furthermore, an epidemiological study suggested that IL-18 can predict cardiovascular death in patients with stable and unstable angina (24, 61). IL-18 signaling has been studied in depth in immune cells, where it activates nuclear factor-κB (NF-κB) and p38 mitogen-activated protein kinase. This signaling involves IL-1 receptor-associated kinases-1 and -4 and adaptor proteins like myeloid differentiation protein-88 and tumor necrosis factor receptor-associated factor-6 (45). In recent studies, it was shown that IL-18 could activate activator protein-1 and NF-κB-dependent inflammatory genes in vascular smooth muscle cells. However, there have so far been few studies that have precisely addressed how IL-18 expression is regulated under both physiological and pathophysiologic conditions.

Aldosterone is an important mediator of the renin-angiotensin-aldosterone system that is involved in a variety of pathophysiological processes associated with cardiovascular events. Aldosterone has been reported to induce vascular inflammation (44), endothelial dysfunction (12), cardiac fibrosis (43), and cardiac hypertrophy (33). These results indicate that aldosterone is an important risk factor for cardiovascular diseases. Endothelin-1 (ET-1) and angiotensin II (ANG II) also contribute to pathophysiologic conditions, including cardiac hypertrophy and remodeling (8, 21). Furthermore, aldosterone has been reported to upregulate ET-1 (14, 58) and angiotensin-converting enzyme activity and ANG II (18). These findings suggest that aldosterone is closely related to ET-1- and ANG II-induced cardiovascular disorders. However, the cellular mechanism whereby aldosterone contributes to cardiovascular disorders is still not well understood.
A small GTPase, RhoA, and Rho binding protein Rho-kinase participate in cytoskeletal organization, smooth muscle contraction, and gene expression. ANG II increases the TNF-α gene expression through RhoA and Rho-kinase in cardiomyocytes (23). Recently, RhoA was reported to be one of the targets of the pleiotropic effect of statins (53). In dilated cardiomyopathy patients, simvastatin improved functional class assessed by New York Heart Association and decreased the concentration of TNF-α in blood (35). ET-1, similar to ANG II, has been reported to induce myocyte hypertrophy through RhoA. Accumulating evidence has shown that Rho-kinase also plays an important role in such pathophysiological conditions, including hypertension (16), coronary vasospasm (30), inflammation (28), and atherosclerosis (27).

NF-κB is the one of the most important transcription factors related to the mechanism of inflammation. NF-κB has been known to be activated by proinflammatory cytokine, lipopolysaccharide, and reactive oxygen species (1). The activation of NF-κB has been reported to play an important role in the pathogenesis of cardiac remodeling and heart failure (40). The IL-18 gene sequence contains the NF-κB binding site (13). Furthermore, it has been reported that TNF-α induces IL-18 expression in cardiomyocytes via NF-κB activation (5). However, there have so far been few reports concerning the relationship and mechanism between NF-κB activation and aldosterone-, ET-1-, and ANG II-induced IL-18 expression in cardiomyocytes.

Peroxisome proliferator-activated receptors (PPARs) are nuclear receptors that regulate gene expression by binding with the retinoid X receptor to PPAR-responsive elements. PPARs have three independent isoforms: PPAR-α, -β/δ, and -γ. PPARs have been reported to regulate lipid metabolism (50) and possess pronounced anti-inflammatory activities (32). Furthermore, clinical trials have shown that fibrates (PPAR-α/γ agonist) have a beneficial effect on cardiovascular disease and stroke (2), whereas pioglitazone (PPAR-γ agonist) reduces the composite of all-cause mortality, nonfatal myocardial infarction, and stroke in patients with type 2 diabetes who have a high risk of macrovascular events (PROactive study) (10). Recent studies have shown PPARs inhibit cardiac hypertrophy, have an antifibrotic effect, and suppress cardiac remodeling by inhibition of NF-κB activation (4, 11, 19, 20). However, there have so far been few reports addressing the interaction of aldosterone and IL-18 and the effect of PPAR agonists on these molecules. This study was designed to clarify the mechanism of the effect of aldosterone on IL-18 expression and the role of the Rho/Rho-kinase pathway and PPAR agonists, pioglitazone and bezafibrate, in rat cardiomyocytes.

MATERIALS AND METHODS

Materials. Sprague-Dawley rats were purchased from Japan SLC (Shizuoka, Japan). All procedures involving animals conformed to the Guide for the Care and Use of Laboratory Animals published by the US National Institutes of Health (NIH Publication no. 85-23, revised 1996) and were approved by the Animal Research Committee of Hyogo College of Medicine. The standard culture media were DMEM and DMEM/nutrient mixture F-12 (DMEM/F-12, 1:1 vol/vol) from Gibco-BRL. Olmesartan (Daichi-Sankyo, Tokyo, Japan), an ANG II type I receptor blocker; simvastatin (Banyu, Tokyo, Japan), an 3-hydroxy-3-methylglutaryl coenzyme A (HMG-CoA) reductase inhibitor; pioglitazone (Takeda, Osaka, Japan), a PPAR-γ agonist; and bezafibrate (Kissei, Nagano, Japan), a PPAR-α/γ agonist were kindly donated by Daichi-Sankyo, Banyu, Takeda, and Kissei, respectively. All other materials and chemicals were obtained from commercial sources.

Cell culture. Primary cultures of neonatal rat cardiomyocytes were prepared as previously described (46, 54). The culture medium was DMEM/F-12 supplemented with 5% calf serum. 5-Bromodeoxyuridine (100 mM) was added during the first 24 h to prevent proliferation of nonmyocyte cells. The cells were seeded at 37°C in an atmosphere of 5% CO₂. Two days after removal from serum-containing medium, the cultures were used for further experiments. The cells were cultured with or without various concentrations of aldosterone for various times. For some experiments, cardiomyocytes were treated with mineralocorticoid receptor (MR) inhibitors, spironolactone and eplerenone; a protein synthesis inhibitor, cycloheximide; pioglitazone; bezafibrate; endothelin A receptor (ETAR) antagonist, BQ-123; endothelin B receptor (ETBR) antagonist, BQ-788; olmesartan; RhoA inhibitor, C₃ toxin; Rho-kinase inhibitor, fasudil; simvastatin; mevalonolactone; specific NF-κB inhibitor, pyrrolidin dihydrocarnamate (PDTC); SN50, which is characterized as a cell-permeable peptide that acts as a specific inhibitor of transcription of the NF-κB active complex into the nucleus; and a nonfunctional mutant of SN50, SN50M (39), simultaneously with aldosterone stimulation. For each experimental condition, six myocyte cultures from three separate isolations were included in the study. The cell viability after various stimulations or exposure to drugs at different times and concentrations exceeded 95% as assessed by trypan blue exclusion (data not shown).

Design of primers and probes. Oligonucleotide primers and TaqMan probes for rat IL-18 were designed based on the cDNA sequences reported in the GenBank database. The forward primer was 5'-AACCCGGCCCTGTTTCTCGA-3' and the reverse primer was 5'-TCAGTCTG-GTCGGGATTGCT-3'. The TaqMan probe was 5'-ACATGCTCT-GATTACGACCAGACC-3'. The primers and the TaqMan probe for rat β-actin were purchased from Applied Biosystems (Tokyo, Japan).

Isolation of total RNA and PCR. Total RNA was extracted from cardiomyocytes by using TRIzol (Invitrogen), according to the manufacturer's protocol. Real-time quantitative reverse transcription-polymerase chain reaction (RT-PCR) was performed to screen for the expression of IL-18 and β-actin. RT-PCR reactions were performed with the TaqMan One-Step RT-PCR Master Mix Reagents Kit with an ABI Prism 7900 HT Detection System (Applied Biosystems), according to the manufacturer's protocol. The same amount of reagents, primers, and probes was used for every reaction. To obtain a calibration curve, serial dilutions of stock standard RNA (total RNA extracted from rat cardiomyocytes: 100, 50, 25, 12.5, and 6.25 ng) were used. The threshold cycle (Ct) is defined as the fractional cycle number at which the fluorescence generated by cleavage of the probe passes a fixed threshold value above baseline. The target message in the unknown samples is quantified by measuring the Ct and by using a calibration curve to determine the starting target message quantity. Ct values ranging from 27 to 30 in the assays for β-actin, and from 33 to 36 for IL-18, were adopted. The reaction mixtures were subjected to the following amplification scheme: one cycle at 48°C for 30 min (reverse transcription) and one cycle at 95°C for 10 min (AmpliTaq Gold activation), followed by 40 cycles each consisting of denaturation at 95°C for 15 s and extension/annealing at 60°C for 1 min. The relative amount of each mRNA was normalized by comparison with the quantity of mRNA of a housekeeping gene, β-actin.

IL-18 protein synthesis. Cell lysates were prepared from cardiomyocytes suspended in phosphate-buffered saline, pH 7.4, by sonication (59). IL-18 was measured using a human IL-18 ELISA Kit (Medical and Biological Laboratories, Aichi, Japan), according to the manufacturer's protocol. The assay uses a sandwich ELISA method that employs two monoclonal antibodies against two different epitopes of human IL-18 in a 96-well ELISA format. The concentrations of ET-1 and ANG II secreted in response to aldosterone stimulation were measured by SRL (Tokyo, Japan).

Rho-kinase activity. Rho-kinase activity was measured using the Rho-Kinase Assay Kit (CycLex, Nagano, Japan), according to the
manufacturer’s protocol. This assay uses a peroxidase-coupled anti-phospho-myosin binding subunit (of myosin phosphatase) threonine 696 monoclonal antibody as a reporter molecule in a 96-well ELISA format.

**RESULTS**

Aldosterone induced IL-18 expression in a time- and dose-dependent manner via the MR by a genomic reaction. The ability of aldosterone to induce IL-18 mRNA expression was first examined in cultured rat neonatal cardiomyocytes. Aldosterone increased IL-18 mRNA expression in a time- and dose-dependent manner in rat neonatal cardiomyocytes (Fig. 1, A and B). A significant increase was found that peaked at 48 h

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**NF-κB activity.** The NF-κB activity was measured using the NF-κB/p65 ActivELISA Kit (IMGENEX, San Diego, CA), according to the manufacturer’s protocol. This kit uses a sandwich ELISA with an anti-p65 antibody.

**Statistical analysis.** Values are reported as means ± SE. The statistical analysis was performed using an ANOVA followed by the Bonferroni test (Statview version 5, Abacus Concepts). Differences were considered to be statistically significant when the probability value, $P$, was <0.05.
after stimulation by a concentration of 500 nM aldosterone (fivefold increase; Fig. 1A). Figure 1B shows a significant increase using 500 nM aldosterone at 48 h (fivefold increase). With these results, we determined IL-18 mRNA expression at 48 h after stimulation with 500 nM aldosterone using various antagonists.

To determine whether the aldosterone-induced IL-18 mRNA expression was mediated via the MR, the effects of MR antagonists were examined. Addition of an MR antagonist, spironolactone (1 μM) or eplerenone (10 μM), resulted in a significant reduction in aldosterone-induced IL-18 mRNA expression. Spironolactone and eplerenone alone did not significantly affect the basal levels of IL-18 mRNA expression (Fig. 1C). Therefore, aldosterone-induced IL-18 mRNA expression in cardiomyocytes is likely to be mediated via the MR.

Aldosterone increased IL-18 mRNA expression with a peak induction at 48 h after addition. This was a considerably long time for mRNA expression. Therefore, to determine whether aldosterone-induced IL-18 mRNA expression requires de novo protein synthesis, the effect of a protein synthesis inhibitor, cycloheximide, was assessed on mRNA expression. Aldosterone-induced IL-18 mRNA expression was completely inhibited by treatment with cycloheximide (100 μM). Cycloheximide alone did not significantly affect the basal levels of IL-18 mRNA expression (Fig. 1C). These results indicate that IL-18 expression requires de novo protein synthesis via an MR-mediated genomic reaction.

Aldosterone increases IL-18 mRNA and protein expression via the ETAR and ANG II receptor. Aldosterone has been reported to upregulate ET-1 and ANG II production (14, 18, 58). To determine whether aldosterone-induced IL-18 mRNA expression is mediated by ET-1 and ANG II production, the effects of ET-1 receptor and ANG II receptor (AT-IIIR) antagonists on IL-18 mRNA expression were assessed. The ETAR antagonist, BQ-123 (1 μM), and the AT-IIIR antagonist, olmesartan (10 μM), led to a significant reduction in aldosterone-induced IL-18 mRNA expression. However, the ETBR antagonist BQ-788 (1 μM) did not inhibit these effects. BQ-123, BQ-788, and olmesartan alone did not significantly affect the basal levels of IL-18 mRNA expression (Fig. 2A). Furthermore, ET-1 (10 nM) and ANG II (100 nM) induced IL-18 mRNA expression with peak inductions at 4 and 8 h after stimulation, respectively (Fig. 2, B and C). These results indicate that IL-18 is likely produced in response to aldosterone through a pathway involving ET-1 and ANG II production via ETAR and AT-IIIR. Aldosterone (500 nM) increased the ET-1 and ANG II production in the conditioned media, and concentration of ET-1 and ANG II peaked at 24 h after stimulation (Fig. 3, A and B). Without aldosterone stimulation, ET-1 and ANG II were not detected in the conditioned media. Moreover, ET-1 (10 nM) and ANG II (100 nM) increased the production of IL-18 protein measured at 12 h, and the concentration peaked at 24 h after stimulation (Fig. 3C). IL-18 protein expression is represented as the fold increases compared with the beginning of various stimulations (at 0 h). Subsequently, aldosterone (500 nM) increased IL-18 protein expression with a peak induction at 60 h after stimulation (Fig. 3D). These results indicate that aldosterone induces IL-18 expression through intermediates, ET-1 and ANG II, via ETAR and AT-IIIR.

The Rho/Rho-kinase pathway is involved in aldosterone-induced IL-18 expression. The Rho/Rho-kinase pathway is associated with ET-1- and ANG II-induced gene expression in cardiomyocytes. To determine whether the Rho/Rho-kinase pathway is involved in the aldosterone-induced IL-18 expression, the effects of RhoA and Rho-kinase inhibitors and statin were evaluated on IL-18 mRNA expression. Treatment with RhoA inhibitor C3 toxin (1 μM) and the Rho-kinase inhibitor fasudil (100 μM) led to a significant reduction in aldosterone-induced IL-18 mRNA expression. C3 toxin and fasudil alone did not significantly affect the basal levels of IL-18 mRNA expression.
expression (Fig. 4A). Furthermore, HMG-CoA reductase inhibitor simvastatin (1 μM), which is known to inhibit the isoprenylation of small G protein, including RhoA, also inhibited the aldosterone-induced IL-18 mRNA expression. Mevalonate (100 μM), in combination with simvastatin, reversed the inhibitory effects of simvastatin on IL-18 mRNA expression. Simvastatin and mevalonate alone did not significantly affect the basal levels of IL-18 mRNA expression (Fig. 4B). Rho-kinase activity, determined by phosphorylation of myosin-binding subunit of myosin phosphatase, which is known to be one of the target molecules of Rho-kinase, was observed with a peak induction at 24 h after stimulation by aldosterone (500 nM) (Fig. 5A). Rho-kinase activity is represented as the fold increases compared with the beginning of various stimulations (at 0 h). These effects were prevented by the AT-IIR antagonist olmesartan (10 μM) and ETAR antagonist BQ-123 (1 μM). However, the PPAR-γ agonist pioglitazone (10 μM) and PPAR-α/γ agonist bezafibrate (100 μM) did not inhibit these reactions (Fig. 5B). Furthermore, Rho-kinase activity was up-regulated also by ET-1 (10 nM) and ANG II (100 nM), with a peak induction at 2 h (Supplemental Fig. S1, A and C). (The online version of this article contains supplemental data.) BQ-123 and olmesartan inhibited the ET-1- and ANG II-induced Rho-kinase activity, respectively. However, pioglitazone and bezafibrate did not inhibit these effects (Supplemental Fig. S1, B and D). These results indicate that aldosterone induces IL-18 expression via the Rho/Rho-kinase pathway through ET-1 and ANG II production.

PPAR agonists pioglitazone and bezafibrate attenuated the aldosterone-induced IL-18 expression. PPAR agonists inhibit inflammatory responses and suppress the production of inflammatory cytokines in several tissues and cells (22). Cardiomyocytes were treated with aldosterone in the presence or absence of PPAR-γ agonist pioglitazone (10 μM) or PPAR-α/γ agonist bezafibrate (100 μM) (Fig. 6). Addition of pioglitazone or bezafibrate led to a significant reduction in the aldosterone-induced IL-18 mRNA expression. Pioglitazone and bezafibrate also inhibited the ET-1- and ANG II-induced IL-18 mRNA expression. Pioglitazone and bezafibrate alone did not significantly affect the basal levels of IL-18 mRNA expression (Supplemental Fig. S2, A and B). However, pioglitazone and bezafibrate did not inhibit the aldosterone-induced ET-1 and ANG II secretion (data not shown) and aldosterone-, ET-1-, and ANG II-induced Rho-kinase phosphorylation. Furthermore, pioglitazone and bezafibrate did not induce Rho-kinase phosphorylation by themselves (Fig. 5B, Supplemental Fig. S1, B and D). These results indicate that PPAR agonists attenuate
Aldosterone-induced IL-18 expression at a point downstream from Rho-kinase.

Aldosterone induced NF-κB activity, and pioglitazone and bezafibrate inhibited this action. NF-κB is a key transcription factor that regulates inflammatory processes. The activation of NF-κB has been reported to increase proinflammatory proteins. Lastly, we examined whether aldosterone induced NF-κB activity. Aldosterone (500 nM) upregulated NF-κB activity with a peak at 24 h after start of stimulation (Fig. 7A). NF-κB activity is represented as the fold increases compared with the beginning of various stimulations (at 0 h). PPAR agonists have been shown to inhibit NF-κB activity. The aldosterone-induced NF-κB activity was inhibited by pioglitazone (10 μM) and bezafibrate (100 μM) (Fig. 7B). ET-1 (10 nM) and ANG II (100 nM) also induced NF-κB activity with a peak at 2 h after start of stimulation, and pioglitazone and bezafibrate inhibited the ET-1- and ANG II-induced NF-κB activity (Supplemental Fig. S3). Specific NF-κB inhibitor PDTC (100 μM) and SN50 (10 μM) inhibited the aldosterone-, ET-1-, and ANG II-induced NF-κB activity, but not SN50M (10 μM), a nonfunctional mutant of SN50 (Fig. 7B, Supplemental Fig. S3, B and D). Likewise, PDTC and SN50 inhibited the aldosterone-induced IL-18 mRNA expression, but not SN50M (Fig. 7C). These results indicate that PPAR agonists attenuate the aldosterone-induced NF-κB activity.

**DISCUSSION**

In the present study, we demonstrated for the first time that aldosterone, a proinflammatory cytokine, induces IL-18 mRNA expression in cultured neonatal rat cardiomyocytes. This effect was blocked by an MR antagonist. Furthermore, the aldosterone-induced IL-18 mRNA expression was blocked by a protein synthesis inhibitor, suggesting that the aldosterone-induced IL-18 mRNA expression proceeds via an MR-mediated genomic reaction. The maximal induction of IL-18 was 48 h at the mRNA level and 60 h at the protein level after aldosterone stimulation. These results indicate the possibility that some molecules might act as an intermediate in aldosterone-induced IL-18 expression. ET-1 and ANG II have been reported to be produced in response to aldosterone stimulation (14, 18, 58). This study examined the effect of the ET-1 and AT-IIR on aldosterone-induced IL-18 expression. The ETAR antagonist BQ-123 and AT-IIR antagonist olmesartan inhibited the aldosterone-induced IL-18 mRNA expression. However, the ETBR antagonist BQ-788 did not inhibit the aldosterone-induced IL-18 mRNA expression. Furthermore, ET-1 and ANG II induced IL-18 mRNA expression maximally at 4 and 8 h, respectively. Moreover, the ET-1 and ANG II protein concent-

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**Fig. 6.** The effects of Pio and Bez on Aldo-induced IL-18 mRNA expression. Cultured cardiomyocytes from neonatal rats were stimulated by Aldo (500 nM) and incubated with or without Pio (10 μM) and Bez (100 μM) for 48 h. Addition of Pio or Bez led to a significant reduction in Aldo-induced IL-18 mRNA expression. The ratio of IL-18/β-actin was determined using the RT-PCR, as described in MATERIALS AND METHODS. The values are means ± SE (n = 6). *P < 0.05 vs. Aldo at 500 nM only.

**Fig. 7.** The effects of Pio, Bez, pyrrolidine dithiocarbamate (PDTC), SN50, and SN50M on Aldo-induced NF-κB activity and on Aldo-induced IL-18 mRNA expression. A: Aldo, at a concentration of 500 nM, activated NF-κB maximally at 24 h after stimulation. B: Aldo-induced NF-κB activity was inhibited by Pio (10 μM), Bez (100 μM), PDTC (100 μM), and SN50 (10 μM) but not by SN50M (10 μM). C: Aldo-induced IL-18 mRNA expression was significantly reduced by PDTC and SN50 but not by SN50M. NF-κB activity was determined by phosphorylation of p65, and the ratio of IL-18/β-actin was determined using the RT-PCR, as described in MATERIALS AND METHODS. The values are means ± SE (n = 6). *P < 0.05 vs. the control group. #P < 0.05 vs. Aldo at 500 nM only.
trations, which peaked at 24 h, were upregulated by aldosterone stimulation. These results suggest that, initially, aldosterone stimulates ET-1 and ANG II synthesis in cardiomyocytes, and then ET-1 and ANG II upregulate the IL-18 expression.

Rho-kinase and RhoA are involved in the ET-1- and ANG II-induced signal transduction pathway. Rho-kinase has been reported to be involved in the signal transduction pathway in ET-1- and ANG II-induced cardiovascular hypertrophy in vivo and in vitro (17, 30). Rho-kinase has also been reported to be involved in the pathogenesis of LV remodeling after a myocardial infarction and is associated with upregulation of proinflammatory cytokines (15). These reports suggest that Rho-kinase is involved in the ET-1- and ANG II-induced IL-18 expression. In fact, fasudil, a Rho-kinase inhibitor, significantly reduced the IL-18 mRNA expression. Furthermore, Rho-kinase was phosphorylated by ET-1 or ANG II stimulation in this study.

An HMG-CoA reductase inhibitor, a statin, prevents the development of cardiac hypertrophy in a cholesterol-independent manner. This mechanism is due, in part, to the inhibition of isoprenylation of small G proteins, including geranylgeranylation of Rho and Rac, and farnesylation of Ras (53). A recent study demonstrated that short-term statin therapy has a beneficial effect in patients with nonischemic, dilated cardiomyopathy. Statin decreases the markers of inflammation while improving neurohormonal imbalance and the cardiac function (35). Simvastatin, an HMG-CoA reductase inhibitor, decreases the myocardial TNF-α expression in heart transplant recipients (56). RhoA has been reported to be involved in the ET-1- and ANG II-induced gene expression. Furthermore, this molecule is involved in ET-1- and ANG II-induced cardiac hypertrophy (3). In this study, simvastatin led to a significant reduction in the IL-18 mRNA expression. Furthermore, the specific RhoA inhibitor C3 toxin inhibited the aldosterone-induced IL-18 mRNA expression. There is thus a possibility that simvastatin might inhibit the participation of the small G protein RhoA, in combination with Rho-kinase, which is involved in the signal transduction pathway of ET-1- and ANG II-induced IL-18 expression. The inhibition of the IL-18 expression by simvastatin might, therefore, be a novel mechanism for the pleiotropic effects of HMG-CoA reductase inhibitors.

PPAR-α activators inhibit the inflammatory responses in aortic smooth muscle cells (51), and PPAR-γ activators suppress the production of inflammatory cytokines in activated macrophages (22, 42). Furthermore, in cardiomyocytes, PPAR-γ inhibits ANG II-induced and mechanical stretch-induced cardiac hypertrophy, and PPAR-α and -γ inhibit lipopolysaccharide-induced TNF-α expression (52, 60). On the other hand, angiotensin type 1 receptor blockers induce PPAR-γ activity, and statins activate PPAR-α activity via the Rho signal cascade (31, 48). Consequently, the activation of PPARs is thought to have a beneficial effect on cardiovascular diseases. In this study, the effects of PPAR-γ agonist pioglitazone and PPAR-α/γ agonist bezafibrate on aldosterone-induced IL-18 expression were examined. Pioglitazone and bezafibrate significantly reduced the IL-18 mRNA expression. Furthermore, pioglitazone and bezafibrate significantly reduced ET-1- and ANG II-induced IL-18 mRNA expression. However, pioglitazone and bezafibrate did not inhibit the aldosterone-induced ET-1 and ANG II induction and aldosterone-, ET-1-, and ANG II-induced Rho-kinase phosphorylation. These results indicate that pioglitazone and bezafibrate inhibit IL-18 expression downstream of Rho-kinase. The mechanism by which PPARs inhibit the IL-18 expression is unknown. However, ligand-activated PPARs are known to positively regulate the gene expression through binding to a specific DNA sequence (PPAR response element) (47) or by inhibiting other types of gene expression, in part, through antagonism of the activities of other transcription factors, such as NF-κB (42). A recent study revealed that aldosterone activates NF-κB, and PPAR agonists inhibit NF-κB activation (9). In this study, the effects of PPAR agonists pioglitazone and bezafibrate on aldosterone-, ET-1-, and ANG II-induced NF-κB activity were also examined. Pioglitazone and bezafibrate attenuated the aldosterone-, ET-1-, and ANG II-induced NF-κB activity. From these results, aldosterone appears to induce the IL-18 expression at least through ET-1 and ANG II via the Rho/Rho-kinase and PPAR/NF-κB pathway (Fig. 8). Further studies are needed to fully elucidate this mechanism.

In conclusion, the induction of IL-18 in cardiomyocytes might cause a deterioration of cardiac function in an autocrine

![Fig. 8. Schematic diagram of the proposed signaling pathways involved in the Aldo-induced IL-18 in cardiomyocytes. Aldo is proposed to induce the IL-18 expression, at least through ET-1 and ANG II, via the Rho/Rho-kinase and peroxisome proliferators-activated receptor (PPAR)/NF-κB pathway. ETAR, endothelin A receptor; AT-IR, ANG II receptor.](http://ajpheart.physiology.org/DownloadedFrom/10.220.33.6/16700553.pdf)
and paracrine fashion. The inhibition of IL-18 expression by PPAR agonists might provide some beneficial cardiovascular effects. The MR antagonist, AT-II receptor antagonist, ETA antagonist, Rho/Rho kinase inhibitor, statin, and PPAR agonists may, therefore, play a critical role in the aldosterone-induced cardiovascular diseases, such as heart failure, ischemia-reperfusion, and myocardial infarction. These effects have already been reported in our laboratory’s clinical studies related to the serum concentrations of IL-18 (24, 25, 34).

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