Histone Deacetylase Inhibition Attenuates Cardiac Hypertrophy and Fibrosis through Acetylation of Mineralocorticoid Receptor in Spontaneously Hypertensive Rats

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ABSTRACT

Inhibition of histone deacetylases (HDACs) by valproic acid (VPA) attenuates inflammatory, hypertrophic, and fibrotic responses in the hearts of spontaneously hypertensive rats (SHRs); however, the molecular mechanism is still unclear. We hypothesized that HDAC inhibition (HDACi) attenuates cardiac hypertrophy and fibrosis through acetylation of mineralocorticoid receptor (MR) in SHRs. Seven-week-old SHRs and Wistar-Kyoto rats were treated with an HDAC class I inhibitor (0.71% w/v in drinking water; VPA) for 11 weeks. Sections of heart were visualized after trichrome stain as well as H&E stain. Histone modifications, such as acetylation (H3Ac [acetylated histone 3]) and fourth lysine trimethylation (H3K4me3) of histone 3, and recruitment of MR and RNA polymerase II (Pol II) into promoters of target genes were measured by quantitative real-time polymerase chain reaction after chromatin immunoprecipitation assay. MR acetylation was determined by Western blot with anti-acetyl-lysine antibody after immunoprecipitation with anti-MR antibody. Treatment with VPA attenuated cardiac hypertrophy and fibrosis. Although treatment with VPA increased H3Ac and H3K4me3 on promoter regions of MR target genes, expression of MR target genes as well as recruitment of MR and Pol II on promoters of target genes were decreased. Although HDACi did not affect MR expression, it increased MR acetylation. These results indicate that HDACi attenuates cardiac hypertrophy and fibrosis through acetylation of MR in spontaneously hypertensive rats.

Introduction

Cardiac hypertrophy and fibrosis are compensatory mechanisms to volume or pressure overload and are frequently associated with chronic diseases such as hypertension (Gupta et al., 2005). Hypertension is a condition associated with increased expression of proinflammatory cytokines that activate hypertrophic mediators, which results in cardiac hypertrophy (Tokuda et al., 2004; Kudo et al., 2009). Cardiac fibrosis is also initiated by the actions of proinflammatory cytokines and is mediated by fibroblast activation (Ratcliffe et al., 2000; Kanzaki et al., 2001).

The mineralocorticoid receptor (MR) is a member of steroid hormone receptors and is known for its role in the development of cardiac diseases in response to ligand binding (Pitt, 2004; Messaoudi et al., 2013). MR regulates cardiac function through induction of inflammation and extracellular matrix, as well as expression of tenasin-X (TNX), plasminogen activator inhibitor-1 (PAI-1), orosomucoid-1 (ORM-1), and collagen IV (Fejes-Toth and Naray-Fejes-Toth, 2007). Expression of TNX, PAI-1, and extracellular matrix proteins, including collagen IV, is regulated by MR ligands and is abundant in the heart (Schellings et al., 2004; Chun and Pratt, 2005). TNX is an essential regulator of collagen deposition by fibroblasts and is upregulated during fibrosis in response to tissue injury. PAI-1 is an inhibitor of tissue-type plasminogen activator in plasma, which consequently suppresses the activity of plasmin. Increased PAI-1 expression is associated with greater risk of cardiovascular disease (Brown et al., 2002).

AABBREVIATIONS: ACE, angiotensin-converting enzyme; Aldo, aldosterone; AR, androgen receptor; ChIP, chromatin immunoprecipitation; DOCA, deoxycorticosterone acetate; H3Ac, acetylated histone 3; HDAC, histone deacetylase; HDACi, histone deacetylase inhibitor; H3K4me3, fourth lysine trimethylation of histone 3; HW, heart weight; MR, mineralocorticoid receptor; PCR, polymerase chain reaction; Pol II, RNA polymerase II; qRT-PCR, quantitative real-time polymerase chain reaction; SHR, spontaneously hypertensive rats; TL, tibia length; TNX, tenasin-X; VPA, valproic acid; WKY, Wistar-Kyoto rats.
MR ligand increased the expression of ORM-1, which is induced by proinflammatory cytokines and glucocorticoid (Vannice et al., 1984). Patients with diabetes and metabolic syndrome have high plasma ORM-1 levels that may be responsible for the increased incidence of cardiovascular problems in this syndrome (Engstrom et al., 2003). Therefore, MR is a potential target of hypertrophy and fibrosis.

The transcriptional activity of MR is regulated mainly by ligands such as aldosterone (Aldo). MR resides in the cytosol and translocates into the nucleus after ligand binding. The binding complex of MR with its ligand binds to hormone response elements in the promoter of target genes recruits coregulatory proteins and results in gene transcription (Yang and Young, 2009). However, post-transcriptional modifications, such as acetylation, phosphorylation, ubiquitylation, and sumoylation, play critical roles in regulating its transcriptional activity (Faus and Haendler, 2006). Phosphorylation of MR enhances its binding affinity for DNA response elements (Massaad et al., 1999), whereas acetylation of MR inhibits recruitment of MR and RNA polymerase II (Pol II) on promoter of MR target genes and prevents development of hypertension (Lee et al., 2013).

Histone deacetylases (HDACs) regulate pathologic cardiac conditions such as fibrosis (Kee et al., 2006) and hypertrophy (Antos et al., 2003; Kook et al., 2003). Several groups have shown that HDAC inhibitors can prevent cardiac hypertrophy in various animal models. For example, HDAC inhibitors increase the expression of antihypertrophic transcription factors such as Kruppel-like factor 2 in cultured cardiomyocytes, which prevents cardiac hypertrophy in culture (Kee and Kook, 2009; Liao et al., 2010). When transgenic mice that overexpress Hop are treated with HDAC inhibitor, increased cardiac mass is significantly reduced (Kook et al., 2003). HDAC inhibitors block cardiac fibrosis through multiple mechanisms. Induction of collagen synthesis mediated by transforming growth factor-beta is prevented by HDAC inhibitors in cultured rat ventricular fibroblasts (Kong et al., 2006). HDAC inhibitor is capable of suppressing differentiation of fibroblasts, which involves cardiac fibrosis (Guo et al., 2009). Other groups also have reported that HDAC inhibitor can prevent cardiac hypertrophy and fibrosis in animal models of hypertension, such as spontaneously hypertensive rats (SHRs) (Cardinale et al., 2010) and deoxycorticosterone acetate (DOCA)–salt hypertensive rats (Iyer et al., 2010). The mechanism for preventing cardiac hypertrophy and fibrosis in SHRs by HDAC inhibitor is still unclear, however. We hypothesized that HDACi attenuates cardiac hypertrophy and fibrosis through MR acetylation in SHRs.

### Materials and Methods

**Animals.** The investigation was conducted in accordance with the National Institutes of Health Guide for the Care and Use of Laboratory Animals and was approved by the Institutional Review Board of Kyungpook National University, and every effort was made to minimize both the number of animals used and their suffering. Seven-week-old male Wistar-Kyto rats (WKYs) and SHRs were purchased from SLC Co. (Shizuoka, Japan). Valproic acid (VPA) was purchased from Sigma-Aldrich (St. Louis, MO). Rats were administered VPA (0.71% w/v) via their drinking water for 11 weeks. Rats were anesthetized with sodium pentobarbital (50 mg/kg i.p.). Wet heart and lung weights were measured and normalized against tibia length. Tissues were frozen in liquid nitrogen and stored at −80°C until further study.

**Histology.** For H&E and trichrome stains, heart tissues were fixed in 4% formalin for overnight, dehydrated, and embedded in paraffin. The paraffin-embedded samples were sectioned at 3-μm thickness. The slides were examined using light microscopy.

**Quantitative Real-Time Polymerase Chain Reaction and Microarray.** Tissues (about 100 mg) were homogenized in liquid nitrogen using a glass homogenizer. RNA was extracted by using Trizol reagent (Invitrogen, Carlsbad, CA) according to the manufacturer's recommendations. Total RNA (2 μg) was reverse-transcribed into cDNA by using RevertAidTM first-strand cDNA synthesis (Fermentas, Glen Burnie, MD) in a 20-μl reaction volume according to the manufacturer's instructions. Quantitative real-time polymerase chain reaction (qRT-PCR) was performed using ABI Prism 7000 sequence detection system (Applied Biosystems, Foster City, CA). Ten microliters of SYBR Green PCR master mix (TaKaRa Bio., Otsu, Shiga, Japan), 4 μl of cDNA, and 200 nM primer set were used for amplification in 20-μl reaction volume. The primer sets used in the RT-PCR are shown in Table 1.

All samples were amplified in triplicate in a 96-well plates; cycling conditions were the following: 2 minutes at 50°C, 10 minutes at 95°C, and 40 cycles at 95°C for 15 seconds followed by 1 minute at 60°C. The relative mRNA expression level was determined by calculating the values of Δ cycle threshold (ΔCt) by normalizing the average Ct value

### Table 1

<table>
<thead>
<tr>
<th>Gene (NCBI Accession No.)</th>
<th>Primer Sequence (5’ to 3’)</th>
<th>Tm</th>
</tr>
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<tr>
<td>qRT-PCR (for rat)</td>
<td>Nppa (NM_012612)</td>
<td>F: ATCTGATGTTCAAGTTCAAGACC R: CTCTGAGAGGGTTGACTTC</td>
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<tr>
<td></td>
<td>Nppb (NM_031545)</td>
<td>F: ACAATCCAGATGCAAAGCT R: GGGGCTTGGTCCTTTGAGA</td>
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<tr>
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<td>TNX (NC_005119.2)</td>
<td>F: TATGGGAGCACAGATGGAATCA R: TCAGTGTCGGAAGCTCATAC</td>
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<tr>
<td></td>
<td>Collagen-IV (NM_001135009)</td>
<td>F: GCCCTCAAGCGATAGTCTACC R: TATAAATGGACTGCTCGGAAT</td>
</tr>
<tr>
<td></td>
<td>ORM-1 (NM_053288)</td>
<td>F: GTGTGACGGAGCAATGAAA R: CATGGCCTCATCTGGCATAC</td>
</tr>
<tr>
<td></td>
<td>PAI-1 (NM_012620.1)</td>
<td>F: AGGGGCAAGCATGAGACAA R: TACCGGCGAGGAAATTAA</td>
</tr>
<tr>
<td></td>
<td>GAPDH (NM_017008)</td>
<td>F: GTTGACCTCATGCGCTACCAT R: TGTAGGGAGAGTGCCTGATG</td>
</tr>
<tr>
<td>ChIP assay (for rat)</td>
<td>ORM-1</td>
<td>F: GGAGGTTGCTCACAATCCAG R: TCCCCAGGCGGTGTGTATAG</td>
</tr>
<tr>
<td></td>
<td>PAI-1</td>
<td>F: CCTCCTGATGCTGCTCCCAAC R: CTTCCCTCCCTCCTCCGAC</td>
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compared with its endogenous control (Gapdh) and then calculating 2-\(\Delta\Delta Ct\) values. For microarray, RNA was extracted from left heart. Microarray analysis was performed using Affymetrix Rat ST1.0 (Affymetrix Inc., Santa Clara, CA). All primer sets used in qRT-PCR are shown in Table 1.

**Chromatin Immunoprecipitation Assay.** Chromatin immunoprecipitation (ChIP) analysis was performed according to the manufacturer’s instructions with minor modification using EZ ChIP kit (Upstate Biotechnology, Lake Placid, NY). In brief, tissues were fixed with 1% formaldehyde and washed with ice-cold phosphate-buffered saline. After homogenization, tissues were incubated in SDS lysis solution for 10 minutes on ice. The lysates were sonicated with 15 cycles of 100 amplitudes of sonication for 10 seconds followed by cooling on ice for 50 seconds. The lysates were precleared with protein G agarose beads for 2 hours. Then antibodies were added, and they were incubated at 4°C overnight. Antibodies to MR and trimethyl-H3-K4 were obtained from Abcam (Cambridge, UK). Anti–polymerase II and anti-H3Ac antibodies were obtained from Upstate Biotechnology. Soluble chromatin captured by specific antibodies was harvested by protein G agarose beads, which were washed serially with a low-salt solution, high-salt solution, lithium chloride solution, and Tris-EDTA solution twice. The antibody-chromatin complexes were eluted from the beads with a solution containing 1% SDS and 0.1 M NaHCO3. To reverse the crosslinking between DNA and chromatin, elutes were incubated at 65°C for 5 hours after the addition of NaCl to a final concentration of 0.2 M. The proteins were eliminated by digestion with protease K at 45°C for 2 hours, and the DNA was purified with a spin column. A specific promoter DNA was quantified by real-time polymerase chain reaction (PCR). All samples were amplified in triplicate in 96-well plates, and the following were the cycling conditions: 2 minutes at 50°C, 10 minutes at 95°C, and 40 cycles at 95°C for 15 seconds followed by 1 minute at 60°C. All primer sets used in ChIP assay are shown in Table 1.

**Immunoprecipitation and Western Blot.** The frozen tissues were homogenized in RIPA buffer containing protease inhibitors. The cell lysates were precleared with protein G agarose at 4°C for 2 hours. The supernatants were incubated overnight with 1 μg of MR antibody (Abcam, UK) or ac-K antibody (Abcam) at 4°C. The immunocomplexes were washed three times with lysis buffer (20 mM Tris, 150 mM NaCl, 1% NP-40, 0.5% SDS, 0.1% SDS, and 1× proteinase inhibitor cocktail) and subjected to Western blot analysis. For Western blot analysis, protein-matched samples (Bradford assay) were electrophoresed (SDS-PAGE) and then transferred to nitrocellulose membranes. The nitrocellulose membranes were blocked with 5% skim milk in TBS (25 mM Tris base and 150 mM NaCl) for 2 hours at room temperature and then incubated with 1 mg/ml of MR or ac-K antibody at 4°C for overnight. Secondary antibody (1:5000 diluted) was incubated at room temperature for 1 hour and then washed three times for 10 minutes each in TBST. The target proteins were detected with enhanced chemiluminescence plus detection reagents (Amersham, Pittsburgh,

Fig. 1. Effect of VPA treatment on heart weight. Cardiac hypertrophy was analyzed based on HW and TL ratios in WKYs (n = 6) and SHRs (n = 6). VPA was administered with 0.71% drinking water to 7-week-old WKYs and SHRs for 11 weeks. (A and B) HW/TL and LHW/TL ratios were increased in SHRs as compared with WKYs. Administration of VPA results in restoration of HW/TL and LHW/TL ratios. (C and D) Lung weight and right heart weight were similar in the WKY and SHR groups. VPA administration did not affect RHW/TL and LW/TL ratios. Data show the mean ± S.E. of six independent experiments. **P < 0.01 versus WKYs; *P < 0.05; ##P < 0.01 versus SHRs.
Statistics. Results are expressed as means ± S.E. Kruskal-Wallis test or one-way analysis of variance; post hoc Tukey’s comparison test was used for analysis of data. Differences were considered significant at P < 0.05. The Student t test was applied for analysis of significant differences between the two groups. The procedures were performed using SPSS software (release 19.0; SPSS Inc., Chicago, IL).

Results

HDACi Attenuated Left Heart Weight. Ratios of heart weight (HW/tibia length (TL) were used to show phenotypic changes attributable to hypertrophy-induced increased heart mass since it is not affected by change of body weight. To determine whether SHRs showed cardiac hypertrophy, we analyzed the HW/TL and left-side HW/TL. SHRs showed increased HW/TL and left HW/TL ratio compared with that of WKYs, which was attenuated by VPA treatment (Fig. 1, A and B). Weights of lung and right side of hearts were similar between WKY and SHRs. VPA treatment did not affect lung weight/TL and right HW/TL in SHRs (Fig. 1, C and D). Thus, SHRs showed cardiac hypertrophy compared with WKYs, which could be attenuated by VPA treatment.

HDACi Attenuated Cardiac Hypertrophy and Fibrosis. To confirm cardiac hypertrophy microscopically in SHRs, we performed H&E stain. SHRs had hypertrophy when compared with WKYs, which was restored by VPA treatment (Fig. 2A). Collagen deposition is shown as blue in trichrome stain. Trichrome stain revealed that SHRs had increased cardiac fibrosis compared with WKYs. Cardiac fibrosis of SHRs was attenuated by VPA treatment (Fig. 2B). Expression of atrial natriuretic peptide A (Nppa) and B (Nppb), markers of cardiac hypertrophy, were detected by real-time PCR. Nppa and Nppb mRNA expression were increased in SHRs, consistent with the hypertrophy seen by histology. Nppa and Nppb mRNA expression were decreased with VPA treatment in SHRs (Fig. 2, C and D).

HDACi Attenuated MR Target Gene Expression. We analyzed expression of four major MR target genes: TNX, collagen IV, ORM-1, and PAI-1 in the left side of hearts. Expression of MR target gene was investigated by qRT-PCR.

Fig. 2. Effect of VPA treatment on cardiac hypertrophy and fibrosis. Analyses of histology of WKY (n = 6) and SHR (n = 6) hearts were performed using H&E and trichrome stains. (A) SHR rats had increased levels of hypertrophy when compared with WKY rats as shown by H&E staining; these levels were attenuated by VPA treatment. (B) SHRs had increased cardiac fibrosis seen in blue compared with WKYs. VPA treatment results in attenuated cardiac fibrosis in SHR. Scale bar 50 μm. (C and D) Expression of atrial natriuretic peptide (ANP) and brain natriuretic peptide (BNP), cardiac hypertrophy markers, were detected by qRT-PCR. SHRs had increased expression of ANP and BNP mRNA, which were decreased with VPA treatment. Data show the mean ± S.E. of six independent experiments. *P < 0.05 and **P < 0.01 versus WKYs; #P < 0.05 and ##P < 0.01 versus SHRs.
MR target gene expression levels were higher in hearts of SHRs than in those of WKY rats. Treatment of VPA resulted in attenuated expression of TNX (Fig. 3A), collagen IV (Fig. 3B), ORM-1 (Fig. 3C), and PAI-1 (Fig. 3D).

HDACi Changed Histone Code Modifications in ORM-1 and PAI-1 Promoters. We investigated enrichment of H3Ac (acetylated histone 3) and H3K4me3 (fourth lysine trimethylation of histone 3) in ORM-1 and PAI-1 promoters by using ChIP assay. Results of conventional PCR and qRT-PCR show that enrichment of H3Ac and H3K4me3 in ORM-1 and PAI-1 promoters was comparable in WKYs and SHRs. On VPA treatment, enrichment of H3Ac and H3K4me3 in the ORM-1 (Fig. 4, A and B, respectively) and PAI-1 (Fig. 4, C and D, respectively) promoters significantly increased in the hearts of WKYs and SHRs.

HDACi Attenuated Recruitment of MR and Pol II on Promoters of Target Genes. Enrichment of MR and Pol II on promoters of ORM-1 and PAI-1 was analyzed by ChIP assay. Results of conventional PCR showed that enrichment of MR and Pol II on the ORM-1 promoter was higher in SHRs than in WKYs. Treatment of VPA resulted in decreased enrichment of MR and Pol II on the ORM-1 promoter (Fig. 5A). Recruitment of MR and Pol II on the ORM-1 promoter was confirmed by qRT-PCR (Fig. 5B). Results of conventional PCR (Fig. 5C) and qRT-PCR (Fig. 5D) showed that enrichment of MR and Pol II on the PAI-1 promoter increased in SHRs compared with WKYs and was attenuated by VPA treatment.

HDACi Increased MR Acetylation. MR acetylation was investigated by Western blot with anti–acetyl-lysine antibody after immunoprecipitation with anti-MR antibody. Expression of MR was not different in WKYs and SHRs. Treatment of VPA did not affect protein levels of MR; however, MR acetylation was significantly increased by VPA treatment (Fig. 6, A and B). Also, we performed Western blot using anti-MR antibody after immunoprecipitation using anti–ac-K antibody. Results of reverse immunoprecipitation showed that MR acetylation was increased by VPA treatment (Fig. 6, C and D).

Discussion
In the present study, we demonstrated that HDACi attenuates cardiac hypertrophy and fibrosis through MR acetylation in SHRs. Our results show that HDACi increases MR acetylation, which decreases the recruitment of MR and Pol II on target gene promoters; reduces the expression of MR target genes such as TNX, collagen IV, ORM-1, and PAI-1; and attenuates cardiac hypertrophy and fibrosis.
Fig. 4. Effect of VPA on histone code modifications in ORM-1 and PAI-1 promoters. Histone code modification was analyzed by ChIP assay. Homogenized tissues were sonicated and precipitated with specific antibodies with which DNA was extracted. Representative gels of conventional PCR (input: 27 cycles; others: 33 cycles) and RT-PCR showed that treatment of VPA resulted in increased enrichment of H3Ac and H3K4me3 on ORM-1 (A and B) and PAI-1 (C and D) promoter. Data show the mean ± S.E. of six independent experiments. *P < 0.05 and **P < 0.01 versus WKYs; #P < 0.05 and ##P < 0.01 versus SHRs.
HDACs and histone acetylases play critical roles in remodeling chromatin structures (Delcuve et al., 2012). The status of acetylated histone and nonhistone proteins such as transcription factors is controlled by the opposing actions of histone acetylases and HDACs (Yang and Seto, 2007; Patel et al., 2011). HDACs are emerging as targets for the treatment of several diseases, including cardiac hypertrophy and heart failure (Kee et al., 2006; Cho et al., 2010; Cao et al., 2011). In this study, we showed that VPA, an HDAC inhibitor, attenuated cardiac hypertrophy and fibrosis in SHRs. HDACi not only decreases heart weight, but it also reduces expression of Nppa and Nppb (Figs. 1 and 2). Although HDACs are known to induce chromatin condensation and transcriptional repression (Kuo and Allis, 1998), HDAC increases the transcriptional activity of MR in the kidneys of DOCA-salt–induced hypertensive rat. In accordance with the results, treatment of HDACi resulted in increased acetylation of MR in the hearts of SHRs (Fig. 6) and adult human cardiomyocyte cells (Supplemental Fig. 3). Acetylation of lysine in the hinge region of MR resulted in transcriptional repression of MR (Lee et al., 2013).

Several studies have reported that HDAC inhibition reduces the expression of collagen, tumor necrosis factor, and nuclear factor κB in spontaneously hypertensive rats (Cardinale et al., 2010) and DOCA-salt–induced hypertensive rats (Iyer et al., 2010). Our results also showed that the rate of downregulated genes was highest in extracellular matrix and inflammation-related genes (Supplemental Fig. 1). In particular, expression of MR target genes—such as TNX, collagen IV, ORM-1, and PAI-1—were decreased by VPA treatment (Fig. 3). VPA would likely upregulate MR corepressors, which downregulate expression of MR target genes; however, VPA treatment did not affect the expressions of MR corepressor genes (Supplemental Fig. 2). Acetylation of steroid hormone receptors is a post-transcriptional modification that plays an important role in regulating their activity (Wang et al., 2011). The androgen receptor (AR) is acetylated by Tip60, PCAF, and P300 in its hinge region (Fu et al., 2000). Mutations in AR acetylation sites...
dramatically impair AR function, stimulating the expression of AR target genes that regulate prostate cancer cell growth (Gaughan et al., 2002). When estrogen receptor α is acetylated by P300 in lysine residues 266 and 268, it stimulates DNA binding activity (Kim et al., 2006). On the other hand, acetylation of lysine residues 302 and 303 prevents its binding to ligand (Fuqua et al., 2000; Wang et al., 2001). Glucocorticoid receptor is acetylated by CLOCK/BMAL1, which reduces its transcriptional activity (Nader et al., 2009), whereas it is deacetylated by HDAC2 (Ito et al., 2006). In the present study, MR interacted with HDAC1 and HDAC2 regardless of Aldo exposure (Supplemental Fig. 4).

Treatment of HDACi resulted in decreased recruitment of MR and Pol II on the target genes in vivo (Fig. 5). Ligand-bound MR is transported into the nucleus, where it binds to specific hormone response elements (Drouin et al., 1992).

**Fig. 6.** VPA increased acetylation of MR. MR acetylation was investigated by western blot (WB) with anti–acetyl-lysine antibody after immunoprecipitation (IP) with anti-MR antibody (A and B), or by WB with anti-MR antibody after IP with anti–acetyl-lysine antibody (C and D). A representative immunoblot (A and C) and densitometry (B and D) show that MR acetylation was increased by VPA treatment. Data show the mean ± S.E. of six independent experiments. **P < 0.01 versus WKYs.

**TABLE 2**
Genes downregulated by VPA treatment

<table>
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<tr>
<th>VPA/Vehicle</th>
<th>Gene Symbol</th>
<th>Gene Description</th>
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<tr>
<td>0.350</td>
<td>Thbs4</td>
<td>Thrombospondin 4</td>
</tr>
<tr>
<td>0.363</td>
<td>Snp17</td>
<td>Sumo1/sentrin/SMT3 specific peptidase 17</td>
</tr>
<tr>
<td>0.377</td>
<td>Cnn1</td>
<td>Camello-like 2</td>
</tr>
<tr>
<td>0.389</td>
<td>Spic</td>
<td>Spi-C transcription factor (Spi-1/PU.1 related)</td>
</tr>
<tr>
<td>0.401</td>
<td>Tagln</td>
<td>Transgelin</td>
</tr>
<tr>
<td>0.420</td>
<td>Vsig4</td>
<td>V-set and immunoglobulin domain containing 4</td>
</tr>
<tr>
<td>0.439</td>
<td>Crf1</td>
<td>Cytokine receptor-like factor 1</td>
</tr>
<tr>
<td>0.454</td>
<td>RGD1562641</td>
<td>Similar to hypothetical protein</td>
</tr>
<tr>
<td>0.457</td>
<td>Chrdl1</td>
<td>Kohjirin</td>
</tr>
<tr>
<td>0.464</td>
<td>Bmp2</td>
<td>Bone morphogenetic protein 2</td>
</tr>
<tr>
<td>0.472</td>
<td>Olf1012</td>
<td>Olfactory receptor 1012</td>
</tr>
<tr>
<td>0.474</td>
<td>Cd55</td>
<td>Cd55 molecule</td>
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<td>RT1-M6-1</td>
<td>RT1 class 1, locus M6, gene 1</td>
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<td>0.493</td>
<td>Calpain1</td>
<td>Calpain 1, basic, smooth muscle</td>
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<td>0.497</td>
<td>RGD1564972</td>
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<tr>
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<td>Tlcd2</td>
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<td>0.499</td>
<td>Myot</td>
<td>Myotilin</td>
</tr>
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</table>
Walthier et al., 2005), which are located up to 10 kb upstream or downstream from the transcriptional start site of target genes and regulates transcriptional activity (Wang et al., 2004; van der Laan et al., 2008). Taken together, these results suggest that HDAC increases the transcriptional activity by deacetylation of MR in SHR, whereas HDACi prevents action of MR by acetylation of MR in SHR.

Histone code modifications are important mechanisms for epigenetic regulation. Expression of genes is epigenetically regulated by DNA methylation as well as histone modifications, including methylation, acetylation, phosphorylation, and ubiquitylation (Kouzarides, 2007). In particular, hyperacetylated histones by treatment with HDAC inhibitor increased the degree of H3Ac, which is linked with H3K4 methylation. Thus, H3K4 methylation (monomethylation, dimethylation, and trimethylation) is also increased (Nightingale et al., 2007). We previously reported that expression of angiotensin-converting enzyme (ACE), a component of the renin-angiotensin system, is regulated by histone acetylation (Lee et al., 2012). Expression of ACE1 was upregulated by enrichment of activating chromatin marks such as H3Ac and H3K4me3 on ACE1 promoter. Thus, HDAC inhibition had been expected mostly to increase the expression of genes through increased histone acetylation; however, gene expression profiles elucidated by microarray analyses show that, among the 27,997 genes identified, 73 were upregulated and 106 were downregulated in the hearts of SHR on VPA treatment (Tables 2 and 3). The number of downregulated genes was much more than that of upregulated genes in SHR after VPA treatment. Although VPA treatment increased enrichment of H3Ac and H3K4me3 on promoters of MR target genes in WKY and SHR (Fig. 4), actual expression of MR target genes was decreased by VPA treatment (Fig. 3). These findings suggest that enrichment of the promoters with H3Ac and H3K4me3 is not a sufficient condition for gene expression in the hearts of SHR.

MR expression is detected in epithelial and nonepithelial tissues, which implies roles in physiology and pathophysiology (Viengchareun et al., 2007). MR activation by mineralocorticoid or glucocorticoid produces oxidative stress and vascular inflammation, which eventually contributes to development of heart failure (Young et al., 2007). MR expression levels in epithelial cells plays a central role in controlling sodium transport. For example, Aldo and DOCA significantly induced Na+-K+-ATPase subunit α1, glucocorticoid-induced leucine zipper, and serum and glucocorticoid-regulated kinase 1 in vitro and in vivo (Cardinale et al., 2010). In hearts, Aldo not only induces genes encoding extracellular matrix proteins, such as TNX and collagen IV, but also those related to inflammation such as ORM-1 and PAI-1 (Fejes-Toth and Naray-Fejes-Toth, 2007). MR blockers spironolactone and eplerenone significantly reduce morbidity and mortality rates in patients with heart failure. Therefore, HDACi and MR antagonists may represent novel strategies for treating cardiac hypertrophy and fibrosis.

In summary, this study presents a mechanism by which HDACi attenuates cardiac hypertrophy and fibrosis through MR acetylation in SHR. Acetylation of MR decreases not only the recruitment of MR and Pol II but also expression of MR target genes that regulate hypertrophy and fibrosis. Therefore, HDACi may be a potential therapeutic target for the treatment of cardiac hypertrophy and fibrosis.

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Authorship Contributions

Participated in research design: Kang, Lee, Kim.
Completed experiments: Kang, Song.
Contributed new reagents or analytic tools: Lee, Seok.
Performed data analysis: Kang, Song, Kim.
Wrote or contributed to the writing of the manuscript: Kang, Kim, Kurz.

References


