Tissue-Specific Upregulation of Angiotensin-Converting Enzyme 1 in Spontaneously Hypertensive Rats Through Histone Code Modifications

Hae-Ahm Lee, Hyun-Min Cho, Dong-Youb Lee, Kee-Chul Kim, Hyung Soo Han, In Kyeom Kim

Abstract—The renin-angiotensin system has been implicated in the development of hypertension and damages several organs. The expressions of the components of a local renin-angiotensin system (RAS) in the hypertensive rats differ from those of the normotensive rats. We hypothesized that local tissue-specific upregulation of angiotensin-converting enzyme 1 (ACE1) in hypertension is caused by epigenetic changes. Adrenal gland, aorta, heart, kidney, liver, and lung tissues were excised from normotensive Wistar-Kyoto (WKY) rats and spontaneously hypertensive rats (SHRs). Ace1 mRNA and protein expressions were measured by real-time PCR and Western blot, respectively. Promoter methylation was revealed by bisulfite sequencing. Histone modifications, such as histone 3 acetylation (H3Ac), fourth lysine trimethylation (H3K4me3), and ninth lysine dimethylation (H3K9me2), were quantified by chromatin immunoprecipitation (ChIP), followed by real-time PCR. The expressions and associations of chromatin remodeling genes were analyzed by real-time PCR and ChIP, respectively. Local tissues from SHRs showed higher expressions of Ace1 mRNA and protein than those from the WKY rats. Ace1 promoter was mostly unmethylated in all of the tissues from both strains. The Ace1 promoter regions of SHR tissues were more enriched with H3Ac and H3K4me3, except in the lungs. The adrenal glands, hearts, and kidneys of SHRs showed less enrichment with H3K9me2. Valsartan treatment in SHRs decreased local Ace1 mRNA and protein expressions, which were accompanied by higher H3K9me2, as well as less H3Ac and H3K4me3. In conclusion, ACE1 is upregulated in local tissues of SHRs via histone code modifications. (Hypertension. 2012;59:621-626.) ● Online Data Supplement

Key Words: ACE1 ▪ hypertension ▪ chromatin remodeling ▪ epigenetic ▪ SHR

The renin-angiotensin system (RAS) plays a central role in the control of cardiovascular homeostasis, including blood pressure and fluid volume, and it is one of the most important pathogenesis genes in hypertension. Among the RAS components, angiotensin-converting enzyme (ACE) 1 catalyzes the conversion of the inactive decapeptide angiotensin (Ang) I to the active octapeptide Ang II. The actions of Ang II have been implicated in many cardiovascular conditions, such as hypertension, atherosclerosis, coronary heart disease, restenosis, and heart failure. ACE1, through catalysis of Ang-(1-7) to Ang-(1-5), inactivates the ACE2-Ang-(1-7)-Mas receptor axis, which acts as a buffer of RAS. The ACE1 inhibitors protect from end-organ damages by maintaining the ACE2-Ang-(1-7)-Mas axis. In addition, ACE1 inactivates bradykinin, neurotensin, and other vasoactive peptides. In part, this is because of the structure of ACE1, a protein composed of 2 independent catalytic domains denoted by the N and C domains. Two isoforms of ACE1 have been found, which are expressed from alternative promoters in a single gene. Somatic ACE is a glycoprotein with a molecular mass of 130 to 190 kDa that contains the 2 domains. The germinal isoform (90–110 kDa), which is restricted to the testicle, is identical to the C-domain of somatic ACE. ACE1 is present in most tissues in the form of a membrane-bound ectoenzyme; however, soluble forms of the enzyme are present in the lymph, blood plasma, and urine.

Early works primarily focused on systemic RAS; however, the discovery of all of the components of the RAS in tissues that can function locally has given rise to the notion that local RAS may play a significant role in the local control of circulation. Arterial ACE is upregulated in several animal models of hypertension in which the blood pressure was correlated with arterial ACE activity. ACE1 is also highly expressed in the other local tissues, including brain, heart, and kidneys, of the hypertensive animals than those of normotensive animals. Cardiac and renal RASs...
participate in cardiovascular homeostasis and pathogenesis of cardiovascular disorders, including left ventricle hypertrophy, vascular remodeling, and renal damage, which play a pivotal role in the occurrence of hypertensive complications. The serum ACE1 activity shows a poor correlation with blood pressure during the development of hypertension. In addition, hypertensive patients with normal or low levels of systemic ACE activity can be effectively treated with an ACE inhibitor. All of these results indicate that tissue ACE1 plays a more critical role than circulating ACE1 does in the regulation of blood pressure, as well as the progression of target organ damage. Although we have a tremendous amount of evidence regarding the role of ACE1 in established hypertension, how local Ace1 expression is regulated during the development of hypertension has not yet been elucidated.

Recently, some epidemiological evidence has reported that intrauterine stress may program the later development of the disease by reprogramming epigenetic changes, such as the promoter DNA methylation and microRNA expression. Moreover, somatic ACE expression is regulated by promoter methylation and histone acetylation in human cell lines and rat tissues. We hypothesized that local tissue-specific upregulation of ACE1 in spontaneously hypertensive rats (SHRs) is caused by epigenetic changes, such as DNA methylation or histone code modifications in the Ace1 promoter region.

Materials and Methods
Please see the online-only Data Supplement for our other Materials and Methods.

Animals
All of the experiments complied with the guiding principles for the care and use of animals approved by the institutional review board at Kyungpook National University School of Medicine and the National Institutes of Health Guide for the Care and Use of Laboratory Animals, and every effort was made to minimize both the number of animals used and their experience. Eighteen-week-old Wistar-Kyoto (WKY) rats and SHRs were purchased from SLC Co. (Shizuoka, Japan). Rats were intraperitoneally anesthetized with ketamine (150 mg/kg; Yuhan, Seoul, Korea) and xylazine (18 mg/kg; Bayer, Seoul, Korea). Aortas were mechanically deendothelialized by rolling the aortic rings with an edge of forceps inside. Left ventricular walls of hearts and cortices of adrenal glands and kidneys were taken for storage. Tissues were frozen in liquid nitrogen and stored at −80°C until further study. For valsartan treatment, rats were administered with 5 mg/kg per day of valsartan for 4 weeks.

Statistical Analysis
Data are expressed as mean ± SEM. Statistical significance was analyzed by an unpaired Student t test. A P value < 0.05 was interpreted as statistically significant.

Results
Local ACE1 Expression Levels in WKY Rat and SHR Tissues
All of the tissues from WKY rats and SHRs presented ACE1 protein with ~180 kDa, which is similar to somatic ACE1. ACE1 protein was more strongly expressed in the lungs of both WKY rats and SHRs than in the other tissues. SHR lungs were slightly increased with ACE1 than those of WKY rats, which had no significant difference. ACE1 protein levels were more increased in the adrenal glands, hearts, and kidneys of SHRs than those of WKY rats (Figure 1A). The ACE1 protein band was not detected in the livers of both strains. The aortas of both strains presented 2 ACE1 protein bands, 1 band was presented with ~180 kDa just like the somatic ACE1, which had no significant difference. The other band appeared at ~200 kDa (Figure 1A). The aortic ACE1 protein band ~180 kDa from SHRs was stronger than that of WKY rats, whereas the larger band was not significantly different. Ace1 mRNA was significantly higher in the tissues of SHRs than those of WKY rats, except in the lungs (Figure 1B).
Epigenetic Modifications in the Ace1 Promoter of WKY Rats and SHRs

The Ace1 promoter was mostly unmethylated in the adrenal glands, hearts, kidneys, and livers tissues and completely unmethylased in the aortas and lungs in either WKY rats or SHRs (Figure S2). Therefore, we focused on histone code modification, which is another epigenetic change.

Adrenal glands, aortas, hearts, and kidneys from SHRs showed a higher enrichment of Ace1 promoter on histone 3 acetylation (H3Ac) than those of WKY rats. The enrichments of the Ace1 promoter on H3Ac in the livers and lungs were not significantly different between WKY rats and those of SHRs (Figure 2A). Trimethylation of fourth lysine on histone 3 (H3K4me3) was significantly recruited in the Ace1 promoter of SHR tissues, such as aortas, hearts, kidneys, and livers, compared with those of WKY rats while not significantly different in the adrenal glands and lungs (Figure 2B). All of the SHR tissues showed less enrichment of ninth lysine dimethylation than those of WKY rats, except for the lungs (Figure 3).

Effect of Valsartan on ACE1 Expressions and Histone Code Modifications in the Ace1 Promoter of SHRs

Valsartan treatment reduced the blood pressure of SHRs to 40 mm Hg after 7 days. The reduced blood pressure was maintained for 4 weeks (Figure S1, available in the online-only Data Supplement). Valsartan treatment decreased the ACE1 protein in the hearts and kidneys, whereas it had little influence on the Ace1 mRNA expression. The graphs show mean ± SE from 4 independent experiments. Asterisks denote statistically significant (*P < 0.05 and **P < 0.01) differences between WKY and SHRs (n=4). Con, WKY; Val, valsartan administered.

Figure 2. Histone code modifications of the Ace1 promoter region in WKY rats and SHRs. Histone code modification was analyzed by chromatin immunoprecipitation (ChIP) assay. Soluble chromatin prepared from homogenized tissues with sonication was precipitated with specific antibodies in which DNA was extracted. Ace1 promoter enrichment of histone 3 acetylation (H3Ac; A), trimethylation of fourth lysine on histone 3 (H3K4me3; B), and dimethylation of ninth lysine on histone 3 (H3K9me2; C) were quantified by real-time PCR. The graphs show mean ± SEM from 4 independent experiments. Asterisks denote statistically significant (*P < 0.05 and **P < 0.01) differences between WKY and SHRs (n=4). Con, WKY; Val, valsartan administered.
Histone acetylation is functionally linked with H3K4 methylation (monomethylation, dimethylation). When histone was made hyperacetylated by treatment with HDAC1 on the Ace1 promoter region were well correlated with the expressions of ACE1 in the tissues of valsartan-treated rats (Figure 5A and 5B). The associations of KDM5A on the Ace1 promoter were decreased in the lungs of valsartan-treated rats (Figure 5C).

Discussion
In this study, we demonstrate that histone code modifications are important epigenetic factors that upregulate the ACE1 expression in the local tissues of SHRs. The local RASs or tissue RASs, such as those in adrenal glands, aortas, hearts, and kidneys of SHRs, have higher levels of Ace1 mRNA and protein than do those of WKY rats. Upregulation of Ace1 mRNA and protein in those tissues well coincided with enrichment of the Ace1 promoter with activating chromatin marks, such as H3Ac and H3K4me3.

The Ace1 promoter of local tissues of SHRs was simultaneously enriched with H3Ac and H3K4me3 (Figure 2). Histone acetylation is functionally linked with H3K4 methylation. When histone was made hyperacetylated by treatment with HDAC inhibitors, both the abundance and the degree of H3K4 methylation (monomethylation, dimethylation, and trimethylation) also increased. Several mammalian H3K4 methyltransferases have been reported, including Set1-complex of proteins associated with Set1 (COMPASS) and mixed lineage leukemia (MLL)-COMPASS-like complexes, either of which is capable of monomethylation, dimethylation, and trimethylation of H3K4. Two independent observations that the level of histone 3 acetylation determines the degree of H3K4 methylation and that histone acetylation is selectively targeted to chromatin, in which H3K4 has already been trimethylated, indicate that H3Ac and H3K4 methylations occur cooperatively in a given chromatin region.

We tried to investigate whether increased expression of ACE1 in SHRs was downregulated when BP was lowered. Because the ACE inhibitors might directly affect ACE expression, Ang type 1 antagonist valsartan was administered to decrease blood pressure in this study. ACE inhibitors are widely believed to suppress the RAS by inhibiting local generation of Ang II. However, a recent study demonstrated that ACE1 inhibitors do not completely suppress local formation of Ang II, because chymase also produces Ang II.

The valsartan treatment decreased blood pressure of SHRs in which Ace1 mRNA expression was downregulated in the heart and kidney but upregulated in the lungs (Figure 3). In accordance with a decrease in Ace1 mRNA expression, the recruitments of H3Ac and H3K4me3 in the Ace1 promoter region in the hearts and kidneys were also decreased but increased in the lungs (Figure 4). HDAC1 enrichments on the Ace1 promoter region were well correlated with Histone code modifications in the SHRs. Valsartan treatments significantly increased Ace1 mRNA, Ace1 protein, and KDM5A in the tissues from the SHR (Figure 4A and 4B). H3K4me3 was also decreased in the valsartan-treated hearts and kidneys of SHRs (Figure 4A and 4C).

Figure 4. Effect of valsartan treatment on histone code modifications in the SHRs. Valsartan was administered 5 mg/kg per day into the SHR (n=4) for 4 weeks. Histone code modification was analyzed by chromatin immunoprecipitation (ChiP) assay, as described previously. A, A representative picture of ChiP assay. Photos were captured by gel document system after PCR products were separated on 2% agarose gel. In Input 27 cycles and the other 30 cycles. Ace1 promoter enrichment of histone 3 acetylation (H3Ac, B), trimethylation of fourth lysine on histone 3 (H3K4me3; C), and dimethylation of ninth lysine on histone 3 (H3K9me2; D) were analyzed by real-time PCR. The graphs show mean±SEM from 4 independent experiments. Asterisks denote statistically significant (P<0.05) differences between control rats (n=4), Con; and valsartan-treated rats (n=4). Val;
studied in both WKY rats and SHRs (Figure S2). Although the Ace1 promoter region was mostly unmethylated in the lungs of WKY rats, intraperitoneal injection of DNA methyltransferase inhibitor increased the expression of Ace1 mRNA. It might be explained by the fact that DNA methyltransferase 3B affects histone modification by interaction with the hSNF2H chromatin remodeling enzyme, HDACs 1 and 2, and components of the histone methylation system.28 Moreover, an HDAC inhibitor also induced Ace1 mRNA expression.22 These data support the notion that histone modifications play a central role in the expression of Ace1 mRNA.

The expression patterns of ACE1 protein were similar to those of Ace1 mRNA except in the liver. The expression of ACE1 protein in adrenal glands, aortas, hearts, and kidneys were higher in SHRs than in WKY rats. However, the ACE1 protein was not detected in the livers of either strain, although Ace1 mRNA was detected in livers of both strains (Figure 1). The Ace1 mRNA level was not well correlated with the protein level in the fetal brain of rats with a low protein diet because of differential expressions of microRNAs targeting Ace1, such as mmu-mir27a and mmu-mir27b.15 Surprisingly, ACE1 enzyme activity was detected in the liver of rats with congenital hepatic fibrosis in which ACE1 enzyme activity in the liver might be related to its pathogenesis.29 The insertion/deletion (I/D) polymorphism of a 287-bp Alu element in intron 16 of the ACE gene affects serum ACE activity, resulting in higher myocardial infarction in the DD genotype.30 Moreover, the single nucleotide polymorphism rs7213516 in the Ace1 promoter also affects Ace1 mRNA expression.31 We speculate that epigenetic regulation of Ace1 mRNA expression is independent of genetic regulation of Ace1 mRNA expression by either II or DD mutant of the Ace1 gene, as well as polymorphisms in the Ace1 promoter.

Interestingly, 2 ACE1 bands (180 and \(\approx 200\) kDa) were detected only in the aortas by Western blot (Figure 1A). These 2 bands may result from posttranslational modifications; however, neither the mechanism nor influence on ACE activity was addressed in the present study. Because somatic ACE has 2 homologous and independent catalytic domains, the activity of large molecular weight ACE is worthwhile to be characterized. The expression levels of Ace1 mRNA in the lungs were similar between WKY rats and SHRs. When the blood pressure was decreased by valsartan, Ace1 mRNA expression was increased in the lungs as opposed to the other organs (Figure 3B). These findings suggest that ACE1 expression in the lungs is regulated in a different fashion as compared with the other local tissues. Although these results are interesting issues, they remain beyond the scope of our present study.

**Perspectives**

The present study reveals that histone code modifications in the Ace1 promoter upregulate ACE1 expression in the local tissues of SHRs. Specifically, the proximal promoter of Ace1 in local tissues of SHRs is enriched with activating histone codes, such as H3Ac and H3K4me3. However, the mechanism by which Ang type 1 blockade valsartan changes histone modification remains to be determined, for example,
reduction in blood pressure, inhibition of intracellular signaling through Ang type 1 receptor, or activation of intracellular signaling through Ang type 2 receptor/Mas receptor.

Because the actions of Ang II have been implicated in many cardiovascular conditions, it is of interest to elucidate the mechanism by which Ang II regulates the histone code modifications in various cardiovascular disease states. Recently, it was reported that Ang II increases acetyl-histone H3 and acetyl-histone H4 without affecting DNA methylation when it induces insulin-like growth factor type II receptor gene expression in H9c2 cardiomyoblast cells and pathologically hypertensive rat heart. Thus, further studies will be needed to clarify the relevance of epigenetic changes in the development of cardiovascular disease, including hypertension.

Sources of Funding
This research was supported by the Basic Science Research Program through the National Research Foundation of Korea, funded by the Ministry of Education, Science, and Technology (2011-0014066) and the Brain Korea 21 Project in 2011.

Disclosures
None.

References
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Hypertension, published online February 6, 2012;
Hypertension is published by the American Heart Association, 7272 Greenville Avenue, Dallas, TX 75231
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Print ISSN: 0194-911X. Online ISSN: 1524-4563

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Supplementary Materials and Methods

Blood pressure measurement

The blood pressure of the rats was measured by tail cuff method. Briefly, rats were preheated on a hotplate at 35 °C for 10 min and then placed in plastic restrainers. A cuff with a pneumatic pulse sensor was attached to the tail. Blood pressure values were recorded on a NIBP controller system (ADInstruments Pty Ltd, Castle Hill, NSW, Australia) with heating and were averaged from at least five consecutive readings obtained from each rat.

Protein extraction and western blot

The frozen tissues were homogenized in RIPA buffer containing protease inhibitors. Protein-matched samples (Bradford assay) were electrophoresed (SDS-PAGE), and then transferred to nitrocellulose (NC) membranes. The NC membranes were blocked with 5% skim milk in TBS (25 mmol/L Tris base and 150 mmol/L NaCl) for 2 h at room temperature, and then incubated with 0.2 μg/ml of ACE1 primary antibody (abcam, Cambridge, UK) at 4°C for overnight. Secondary antibody (1:2000 diluted) was incubated at room temperature for 1 h, and then washed three times, 10 min each in TBST. The target proteins were detected with ECL plus detection reagents (Amersham, Pittsburgh, PA, USA). The expression levels were quantified by an optical densitometry, ImageJ software.

Quantitative real-time RT-PCR

Tissues (about 100 mg) were homogenized in liquid nitrogen with a glass homogenizer. RNA was extracted by using Trizol Reagent (Invitrogen, Carlsbad, CA, USA) according to manufacturer’s recommendations. Total RNA (2 μg) was reverse-transcribed into cDNA by using RevertAid™ first strand cDNA synthesis (Fermentas, EU) in 20 μl reaction volume according to manufacturer’s instructions. Real-time -PCR was performed using ABI Prism 7000 sequence detection system (Applied Biosystems, Foster City, CA, USA). Ten micro liter of SYBR Green PCR master mix (TaKaRa, Japan), 4 μl of cDNA, and 200 nmol/L primer set were used for amplification in 20 μl reaction volume. All samples were amplified in triplicates in a 96-well plate and the cycling conditions were as follows: 2 min at 50°C, 10 min at 95°C, and 40 cycles at 95°C for 15 s followed by 1 min at 60°C. The relative mRNA expression level was determined by calculating the values of Δcycle threshold (ΔCt) by normalizing the average Ct value compared with its endogenous control (Gapdh) and then calculating 2-ΔΔCt values. All primer sets used in real-time PCR are shown in Supplementary table S1.

DNA bisulfite modification and sequencing

For DNA extraction, tissues were homogenized and incubated in a lysis buffer (0.5% SDS, 10 mmol/L Tris, and 20 μg/ml proteinase K, pH 8.0) at 50°C for 3 h. DNA was purified by phenol-chloroform and ethanol precipitation. The isolated DNA was modified by sodium bisulfite as described previously with a minor modification. After bisulfite modification, the CpG island (-178 ~ +105) was amplified by PCR, and the PCR products were cloned into T-easy vector (Promega, Madison, WI, USA). Five independent colonies were sequenced by an ABI3700 automated DNA sequencer in accordance with manufacturer’s instructions. Primer set used in bisulfite modified PCR is shown in Supplementary table S1.

Chromatin immunoprecipitation (ChiP) assay

ChiP analysis was performed according to the manufacturer’s instructions with minor modification using EZ ChiP (Upstate Biotechnology, Lake Placid, NY). In brief, tissues were fixed with 1% formaldehyde, and washed with ice-cold PBS. After homogenization, tissues were incubated with SDS lysis solution for 10 min on ice. The lysate were sonicated with 15 cycles of 100 amplitude of sonication for 10 s followed by cooling on ice for 50 s. The lysate were pre-cleared with protein G agarose beads for 2 h and 1~3 μg of antibodies (Upstate Biotechnology, Lake Placid, NY) were added and incubated at 4°C overnight.
Soluble chromatin captured by specific antibodies was harvested by protein G agarose bead. The beads were washed serially with a low-salt solution, high-salt solution, LiCl solution, and TE solution twice. The antibody-chromatin complexes were eluted from the beads with a solution containing 1% of SDS and 0.1 mol/L of NaHCO3. To reverse the crosslinking between DNA and chromatin, elutes were incubated at 65°C for 5 h after the addition of NaCl to a final concentration of 0.2 mol/L. The proteins were eliminated by digestion with proteinase K at 45°C for 2 h, and the DNA was purified with a spin column. A part of the promoter DNA fragments was amplified by real-time PCR. All samples were amplified in triplicates in a 96-well plate and the cycling conditions were as follows: 2 min at 50°C, 10 min at 95°C, and 40 cycles at 95°C for 15 s followed by 1 min at 60°C. The primer set used in ChIP assay is shown in Supplementary Table S1.
Supplementary Result

Effect of valsartan treatment on expressions and recruitments of chromatin remodeling proteins

After treatment of SHR with valsartan, we analyzed the expression levels of histone acetyltransferase [CREB-binding protein (Crebbp), Histone acetyltransferase 1 (Hat1), p300/CREB-binding protein (Pcaf)], histone deacetylase [histone deacetylase 1 (Hdac1), histone deacetylase 2 (Hdac2)], H3K4 methyltransferase complex subunit [Absent, small, or homeotic 2-like (Ash2l)], H3K9 methyltransferase [Euchromatic histone-lysine N-methyltransferase1 (Ehmt1)], lysine specific demethylase1, 4a, and 5a (Kdm1, Kdm4a, and Kdm5a). Valsartan treatment significantly decreased the histone acetyltransferases in the hearts (Fig. S3A-C), but increased histone deacetylases in their hearts and kidneys of SHR (Fig. S3E and F). On the other hand, Hat1 was increased (Fig. S3B), but Hdac1 was decreased in the lungs of the valsartan-treated SHR (Fig. S3E). The expressions of Ash2l were not affected by valsartan treatment (Fig. S3D). Ehmt1 was significantly increased in the hearts and kidneys whereas it was decreased in the lungs of valsartan-treated SHR (Fig. S3G). Hdac2 expression was significantly elevated in the heart of valsartan-treated SHR (Fig. S3F). Kdm1 was decreased in the hearts, kidneys, and lungs of valsartan-treated SHR (Fig. S3H). Kdm4a was decreased only in the lungs of valsartan-treated SHR (Fig. S3I). Kdm5a was significantly increased the mRNA expressions and Ace1 promoter associations in the hearts and kidneys of valsartan-treated SHR (Fig. 5 and S3J).
References

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Supplementary Figures

S1. Effect of valsartan administration on the blood pressure in hypertensive rats SHR rats were administrated valsartan (5 mg/kg/day) for 4 weeks. Blood pressure was measured by tail-cuff method. The line graphs represent means ± SE from three independent rats. Asterisks denote statistically significant (*P<0.05) differences between control group and valsartan administrated group.
S 2. *Ace1* promoter methylation status in WKY and SHR. Genomic DNA (2 μg) extracted from the tissues was modified by bisulfite and amplified by PCR including CpG island. After sequencing the PCR product, the remaining cytosine was considered as methylated cytosine. (A) The positions of CpG island, bisulfite sequencing, and ChIP analysis are shown schematically in which the numbers indicate the nucleotide location starting from transcription start site (TSS; +1). ChIP; Chromatin immunoprecipitation, BSP; Bisulfite sequencing PCR. (B) The methylation maps of *Ace1* promoter from WKY and SHR. ○; unmethylated, and ●; methylated
S 3. Effect of valsartan administration on expressions of chromatin remodeling genes. Each gene expression was measured by real-time PCR. Data were presented as means (n=4) of normalized value of $2^{\Delta\Delta Ct}$±SE. Asterisks denote statistically significant (*$P<0.05$) differences between control and valsartan-administered SHR. Con; control, Val; valsartan-administered. Ash2l; Absent, small, or homeotic 2-like, Crebbp; CREB-binding protein, Ehmt1; euchromatic histone-lysine N-methyltransferase1, Hat1; histone acetyltransferase1, Hdac; histone deacetylase, Kdm; lysine-specific demethylase, Pcaf1; p300/CBP associated factor1