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Inhibition of Histone Deacetylation Blocks Cardiac Hypertrophy Induced by Angiotensin II Infusion and Aortic Banding

Hae Jin Kee, PhD; Il Suk Sohn, MD, PhD; Kwang Il Nam, MD, PhD; Jong Eun Park, BS; Yong Ri Qian, MD; Zhan Yin, PhD; Youngkeun Ahn, MD, PhD; Myung Ho Jeong, MD, PhD; Yung-Jue Bang, MD, PhD; Nacksung Kim, PhD; Jong-Keun Kim, MD, PhD; Kyung Keun Kim, MD, PhD; Jonathan A. Epstein, MD; Hyun Kook, MD, PhD

Background—A number of distinct stress signaling pathways in myocardium cause cardiac hypertrophy and heart failure. Class II histone deacetylases (HDACs) antagonize several stress-induced pathways and hypertrophy. However, cardiac hypertrophy induced by transgenic overexpression of the homeodomain only protein, HOP, can be prevented by the nonspecific HDAC inhibitors trichostatin A and valproic acid, suggesting that alternate targets that oppose class II HDAC function might exist in myocardium. We tested the effects of several HDAC inhibitors, including a class I HDAC-selective inhibitor, SK-7041, on cardiac hypertrophy induced by angiotensin II (Ang II) treatment or aortic banding (AB).

Methods and Results—Cardiac hypertrophy was induced by chronic infusion of Ang II or by AB in mice or rats and evaluated by determining the ratio of heart weight to body weight or to tibia length, cross-sectional area, or echocardiogram. Cardiac hypertrophy induced by Ang II or AB for 2 weeks was significantly reduced by simultaneous administration of trichostatin A, valproic acid, or SK-7041. Echocardiogram revealed that exaggerated left ventricular systolic dimensions were relieved by HDAC inhibitors. HDAC inhibitors partially reversed preestablished cardiac hypertrophy and improved survival of AB mice. The expressions of atrial natriuretic factor, α -tubulin, β -myosin heavy chain, and interstitial fibrosis were reduced by HDAC inhibition.

Conclusions—These results suggest that the predominant effect of HDAC inhibition, mainly mediated by class I HDACs, is to prevent cardiac hypertrophy in response to a broad range of agonist and stretch stimuli. (*Circulation*. 2006;113:51-59.)

Key Words: angiotensin ■ aortic banding ■ histone deacetylases ■ hypertrophy

In contrast to hyperplasia, which involves proliferation of cells, cardiac hypertrophy, an increase in the size of cardiac muscle cells, is often a primary adaptive response to exogenous physiological or pathological signals, such as myocardial infarction, hypertension, aortic stenosis, and valvular dysfunction. These stimuli in animal models and human diseases have generally been thought to induce prohypertrophic gene expression. Reactivation of fetal gene programs and repression of some adult cardiac genes are closely related to deterioration of heart function.¹

Clinical Perspective p 59

Posttranslational modifications of histones play an important role in transcriptional regulation.² In particular, acetyla-

tion of histone tails, mediated by histone acetyltransferases, is associated with activation of gene expression in myocardium.³ Conversely, repression of gene expression can be induced by histone deacetylation, which is mediated by a family of histone deacetylases (HDACs). HDACs are divided into 3 subfamilies on the basis of secondary structure.⁴ Class II HDACs, characterized by an amino-terminal domain that can interact with myocyte enhancer factor 2, have been strongly implicated as repressors of cardiac hypertrophic gene expression. HDAC5 and HDAC9, both class II HDACs, are highly expressed in myocardium, and inactivation of these genes promotes cardiac hypertrophy.⁵ These results might suggest that HDAC inhibition would promote hypertrophy. However, when isolated myocytes in primary culture are treated with chemical HDAC inhibitors,

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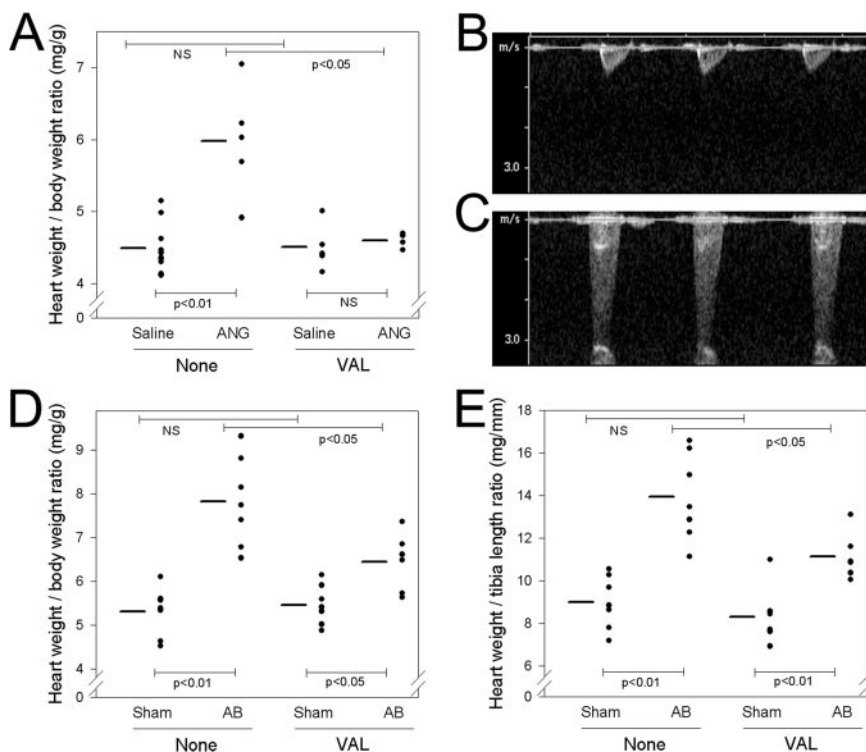


Figure 1. Effects of VAL, an HDAC inhibitor, on cardiac hypertrophy. A, VAL prevented cardiac hypertrophy induced by Ang II infusion (ANG). Mice were treated with Ang II for 2 weeks, with or without administration of VAL. HW/BW ratios are shown. Horizontal bars indicate the mean values, and dots represent individual animals. B and C, Typical Doppler echocardiogram showing the establishment of an appropriate pressure gradient in the AB model. Blood flow velocity was measured at the proximal (B) and distal (C) portions of constricted aorta. Flow velocity was markedly increased, which indicates narrowing of the aortic lumen. D to E, VAL also prevented cardiac hypertrophy induced by AB. HW/BW (D) and HW/TL (E) ratios were evaluated.

gene expression characteristic of hypertrophic signaling is paradoxically inhibited.⁶

Recently, we have shown that cardiac hypertrophy induced by transgenic overexpression of the homeodomain only protein, HOP, a transcriptional corepressor in the developing heart,⁷ can be prevented by treatment with the nonspecific HDAC inhibitors trichostatin A (TSA) and valproic acid (VAL). TSA also repressed hypertrophy induced by chronic infusion of isoproterenol, a β -adrenergic agonist.⁸ In the present study we sought to determine whether HDAC inhibition would block hypertrophic responses induced by both receptor-mediated agonists and stretch. Our results suggest that nonspecific as well as class I-selective HDAC inhibitors may prove to be useful pharmacological agents for the prevention or treatment of cardiac hypertrophy and heart failure.

Methods

In Vivo Hypertrophy Models

Five- to 8-week-old adult male CD1 mice ($n=225$) and 6-week-old adult male Sprague-Dawley rats ($n=40$) were purchased from Daehan Biolink (Daejeon, Korea) and housed individually in plastic cages in a temperature-controlled room. The experimental protocol was approved by the Chonnam National University Medical School Research Institutional Animal Care and Use Committee. All surgical procedures and echocardiography were performed with the animals under anesthesia with ketamine (16.65 mg/kg IM) and xylazine (7.77 mg/kg IM).

Angiotensin II (Ang II) infusion was done at 1.3 mg/kg per day for 14 days. For preparation of aortic banding (AB), the left chest was opened at the second intercostal space with the animal under anesthesia. Partial aortic constriction was performed by ligating the ascending aorta with a 22-gauge needle with a 7-0 braided polyester suture. The increased blood flow velocity was confirmed by Doppler echocardiogram (Figure 1B and 1C). Sham-operated animals underwent the same surgical procedure without constricting the aorta.

Establishment of hypertrophy was confirmed by echocardiography by measuring left ventricular (LV) wall thickness and dimensions, ratios of heart weight to body weight (HW/BW), and capacitance measurement in isolated cardiomyocytes after euthanasia.

Blood pressures of mice were evaluated by direct cannulation of the common carotid artery, connected to a polygraph (Grass 7E) through a pressure transducer. After the blood pressure was stabilized, the mean arterial pressure was recorded.

Implantation of osmotic minipumps and intraperitoneal administration of TSA (0.6 mg/kg/d) and VAL (0.71% in drinking water, ad libitum) were described previously.⁸ SK-7041 (SK) (In2Gen Co), dissolved in dimethyl sulfoxide, was administered 30 mg/kg per day IP 5 times a week for 2 weeks as described previously.⁹ The dose of HDAC inhibitors used has been reported to inhibit endogenous HDAC activity,⁹⁻¹³ and daily administration of TSA or VAL for a given period was well tolerated because it did not affect overall body weight or HW/BW ratio in control animals compared with those injected with vehicle alone (Figures 1A, 1D, 2A, 2D). TSA, VAL, or SK did not affect cardiac contractility (Table 1).

Evaluation of Regression of Cardiac Hypertrophy

Regression of cardiac hypertrophy was evaluated by HW/BW or heart weight to tibia length (HW/TL) ratios and by echocardiogram. Cross-sectional areas of cardiomyocytes were also examined. For each experimental group, hearts from 4 to 6 animals were mounted after coronal section and stained with hematoxylin-eosin. Ten cardiomyocytes in LV free wall were randomly chosen, and cross-sectional areas were measured with Multi Gauge software (Fuji Photo Film Co) by a blinded investigator.

Echocardiographic studies were performed with a 15-MHz linear array transducer system (Acuson Sequoia c512, Siemens).⁸ Two-dimensionally guided M-mode of LV at the papillary level was obtained from the parasternal long-axis view. For each mouse, measurements were made from at least 4 beats. LV cavity dimension and wall thickness were measured, and percent change in LV dimension (fractional shortening [FS]) (LV%FS) was calculated as follows: $LV\%FS = [(LVd - LVs) / LVd] \times 100$, where LVd is LV dimension at end-diastole and LVs is LV dimension at end-systole. For the estimation of flow velocity, pulsed-wave Dopp-

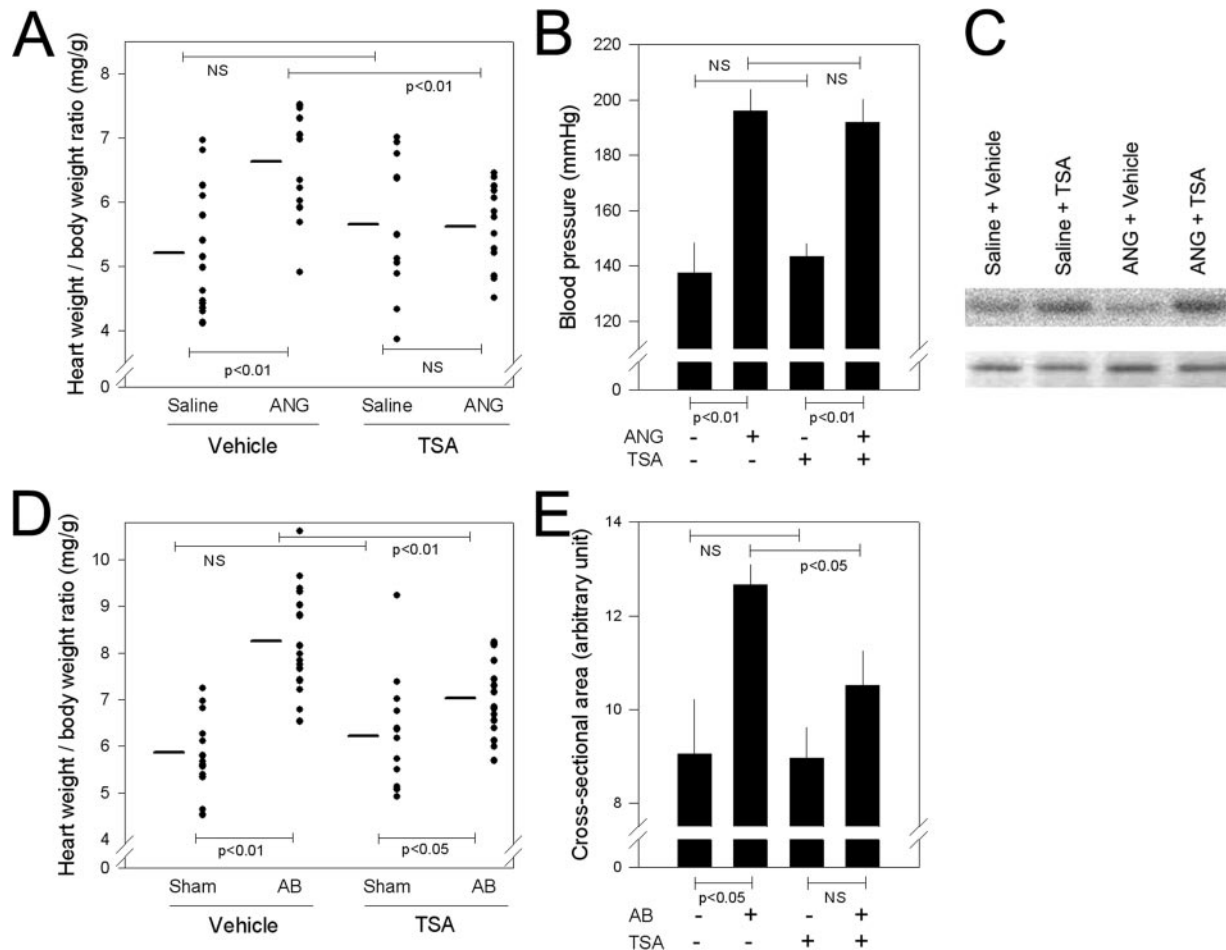


Figure 2. Effects of TSA, an HDAC inhibitor, on cardiac hypertrophy. A, TSA attenuated cardiac hypertrophy induced by Ang II (ANG). B, Effects of TSA administration on blood pressure. Ang II significantly increased blood pressure, but this increase as well as the basal level was not affected by TSA. C, TSA treatment increased acetylation of histone H4. Top, Representative Western blot analysis for acetylated histone H4 in the heart treated with TSA or vehicle is shown. Bottom, Loading control is shown by Ponceau S staining of nuclear fraction. D, TSA blocks AB-induced increase in heart weight. E, Changes in cross-sectional areas of cardiomyocytes in the LV free wall also indicate that TSA reduced individual cardiomyocyte volumes.

ler sample volume was placed on the aorta just proximal and distal to the constriction (Figure 1B and 1C).

To determine whether preexisting cardiac hypertrophy could be affected by HDAC inhibition, 42 AB mice aged 8 weeks were randomly divided into 4 groups. The first group was euthanized at 2 weeks after operation, and hypertrophy was confirmed (AB2w group). TSA was administered daily to the second group from the

next day of AB (AB+TSA2w group). The third group was maintained for 5 weeks without TSA (AB5w group). In the last group, 2 weeks after AB operation, daily injection of TSA was started and maintained for 3 additional weeks (AB2w+TSA3w group). For each experimental condition, age-matched mice with sham operation served as control. The long-term effect of HDAC inhibition was also evaluated by administration of VAL for 7

TABLE 1. Echocardiographic Parameters and Heart Weight in AB Mice Hearts Treated With 3 HDAC Inhibitors for 2 Weeks

	Control	AB	AB+VAL	AB+TSA	AB+SK
IVS, mm	0.76±0.03	1.11±0.02†	0.94±0.04‡	0.84±0.03§	0.82±0.03§
LVd, mm	3.98±0.03	4.28±0.11*	4.20±0.19	4.20±0.10	4.12±0.14
PWT, mm	0.73±0.03	1.09±0.04†	0.94±0.06	0.82±0.02§	0.77±0.03§
LVSd, mm	2.62±0.01	2.69±0.12	2.69±0.21	2.58±0.12	2.57±0.12
FS, %	34.17±0.44	37.28±2.06	35.85±5.38	38.56±1.41	37.73±0.71
HW, g	0.17±0.01	0.26±0.02†	0.21±0.01‡	0.20±0.01‡	0.18±0.01§

Data are mean±SEM of 4 to 6 mice. IVS indicates end-diastolic interventricular septal thickness; LVd, LV end-diastolic dimension; PWT, posterior wall thickness; LVSd, LV end-systolic dimension; and HW, heart weight.

* $P<0.05$, † $P<0.01$ vs control.

‡ $P<0.05$, § $P<0.01$ vs AB.

weeks, and survival rate as well as echocardiogram was examined.

Reverse Transcription–Polymerase Chain Reaction, Immunoblotting, and Histology

Reverse transcription–polymerase chain reaction (RT-PCR), immunoblotting, immunostaining, and Masson's trichrome staining of tissues were described previously.⁸ Transcript levels of heart-specific genes were examined.⁷ Total RNA was isolated with TriZol reagent (Invitrogen Life Technologies), and 1 μ g of RNA underwent reverse transcription reaction with Superscript first strand synthesis system for RT-PCR kit (Invitrogen Life Technologies). Quantification of the mRNA amounts was further confirmed with SYBR Green PCR kit (Applied Biosystems, Inc). Relative expression levels of genes were compared with those of glyceraldehyde-3-phosphate dehydrogenase (GAPDH) by the $2^{-\Delta\Delta C_t}$ method.¹⁴

For Western blot, anti-atrial natriuretic factor (ANF) (1:1000), anti- α -tubulin antibody (1:1000, Zymed, South San Francisco, Calif), or anti-actin antibody (1:1000, Sigma, St Louis, Mo) was used. Nuclear fractions were extracted from isolated hearts with nuclear extraction kit (Panomics Inc), and the loaded protein amounts were checked by Ponceau S staining after separation on SDS-PAGE gel. The level of acetylation of histone H4 was assessed by immunoblotting with anti-acetyl histone H4 antibody (1:2000, Upstate Biotechnology Inc, Lake Placid, NY), following the manufacturer's protocol.

For immunohistochemistry, primary antibody dilutions of ANF (Biosign, Saco, Maine) and type I collagen (Abcam, Cambridge, UK) were 1:200 and 1:100, respectively. Cardiomyocyte apoptosis was evaluated by FragEL DNA fragmentation Detection kit with colorimetric TdT enzyme (Calbiochem), following the manufacturer's protocol.

Results

Blockade of Cardiac Hypertrophy by Nonspecific HDAC Inhibitors

In previous work, we have shown that cardiac hypertrophy induced by constant infusion of isoproterenol can be significantly attenuated by TSA.⁸ We sought to determine whether nonspecific HDAC inhibition would also prevent the hypertrophy induced by an unrelated agonist or by pressure overload. Ang II is known to induce cardiac hypertrophy by activating receptor-mediated signaling cascades, including those involving mitogen-activated protein kinases, phosphatidylinositol 3-kinase, Akt, and janus-activating kinases.¹⁵ We treated CD1 mice with Ang II (1.3 mg/kg per day), which, as expected, induced cardiac hypertrophy, and HW/BW ratio was increased by 27% compared with the vehicle group. Concomitant treatment with VAL markedly reduced the hypertrophic response to Ang II (Figure 1A).

To determine whether cardiac hypertrophy induced by pressure overload in mice or rats could be prevented by HDAC inhibition, we treated AB mice or rats with TSA or VAL. AB induced a marked increase in flow velocity (Figure 1B and 1C) and LV wall thickness (Figure 3C and 3D) and a 46% increase in HW/BW ratio compared with sham-operated mice. VAL administration significantly attenuated this increase to 18% (Figure 1D), which was also evidenced by changes in HW/TL ratio (Figure 1E).

The Ang II–induced cardiac hypertrophy, as assessed by HW/BW ratio, was also significantly attenuated by TSA (Figure 2A), an unrelated agent with HDAC inhibitory activity.¹⁰ Blockade of cardiac hypertrophy by TSA in Ang II–treated animals raised the possibility that TSA might

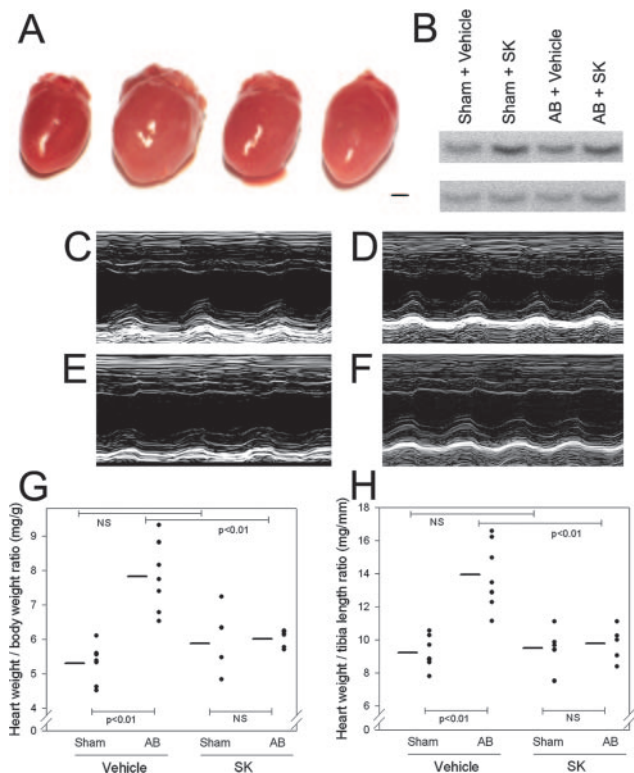


Figure 3. SK, a class I HDAC-selective inhibitor, blocks AB-induced cardiac hypertrophy. A, From the left, hearts from sham+vehicle, AB+vehicle, sham+SK, and AB+SK are shown. Note that cardiac enlargement by AB was abolished by administration of SK for 2 weeks, whereas SK did not affect the sham-operated heart. Bar=1 mm. B, Acetylation status of histone H4 is shown with loading control. C to F, Typical echocardiogram showing the LV from mice treated with sham+vehicle (C), AB+vehicle (D), sham+SK (E), and AB+SK (F). Increased LV wall thickness in AB mice is significantly reduced by SK treatment. The values are shown in Table 1. G and H, SK completely blocked the increase in HW/BW (G) and HW/TL (H) ratios by AB.

ameliorate the hypertension induced by Ang II, and thus the blockade of the hypertrophy might have been caused secondarily by reduction of afterload rather than by direct cardiac effects. To rule out this possibility, blood pressure was measured. As expected, Ang II markedly increased the blood pressure; however, TSA did not affect the increased or basal level of blood pressure (Figure 2B).

To determine whether regression of cardiac hypertrophy by HDAC inhibitors correlated with increases in histone acetylation in the cardiomyocytes, the effect of TSA treatment on histone H4 acetylation was determined by immunoblot analysis with an anti-acetyl histone H4 antibody. As shown in Figure 2C, TSA increased the acetylation of histone H4 of cardiomyocytes, whereas the total amount of histone was not changed.

Cardiac hypertrophy induced by AB, as shown by HW/BW ratio, was also significantly relieved by TSA (Figure 2D), which was further confirmed by changes in individual cardiomyocyte volume, measured by cross-sectional area (Figure 2E).

We performed similar experiments in rats and observed a comparable protective effect when AB rats were treated with

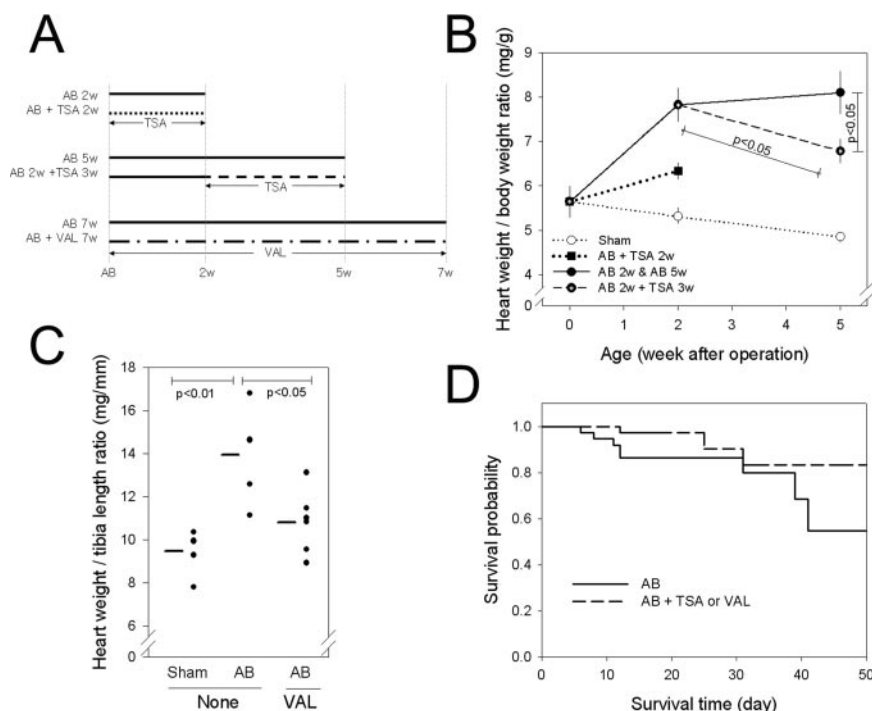


Figure 4. HDAC inhibition reverses preestablished cardiac hypertrophy. A, Experimental protocols to investigate the reversibility of cardiac hypertrophy. AB-operated mice were randomly divided into 4 groups and treated with HDAC inhibitors or vehicle as shown. In addition, age-matched mice with sham operation were provided as control. To observe reversibility, 2 weeks after AB when the development of AB was confirmed, daily injection of TSA was started and maintained for 3 weeks more. B, TSA reverses preestablished cardiac hypertrophy. TSA administration to mice relieved the preestablished cardiac hypertrophy as well as prevented further progression. In contrast, in AB5w mice with no TSA administration, cardiac hypertrophy was progressive. LV wall thickness and HW/TL ratio are provided in Table 2 and Figure II in the online-only Data Supplement, respectively. C, Long-term effects of VAL on cardiac hypertrophy induced by AB. Long-term administration of VAL also reduces HW/TL ratio. D, Kaplan-Meier plot reveals improved survival in the HDAC inhibitor-treated mice. In some mice, AB caused premature death due to heart failure, reducing the survival to 54.7% at 40 days after operation. However, the mortality rate was reduced in the mice administered TSA or VAL.

TSA or VAL. AB significantly increased the HW/BW ratio by 47%, but this increase was significantly blunted to 25% by TSA administration (Figure I in the online-only Data Supplement; see <http://circ.ahajournals.org/cgi/content/full/113/1/51/DC1>).

Prevention of Cardiac Hypertrophy by SK-7041, a Class I HDAC-Selective Inhibitor

The present observations as well as the previous reports^{6,8} are somewhat unexpected and at odds with the previous findings that class II HDACs inhibit cardiac hypertrophy in response to various exogenous stimuli.^{4,5} However, the lack of specificity of HDAC inhibitors used led us to postulate that class I HDACs oppose class II HDACs in regulating cardiac hypertrophy and that the activity of class I HDACs predominates in the myocardium such that nonspecific HDAC inhibition results in blockade of hypertrophic pathways. Thus, we examined the effects of a selective class I HDAC inhibitor in our model.

Recently, novel hybrid HDAC inhibitors were synthesized by combining the hydroxamic acid of TSA and the pyridyl ring of MS-275, a synthetic benzamide derivative. One of these new HDAC inhibitors, 3-(4-substituted phenyl)-N-hydroxy-2-propenamide (SK-7041 [SK]),¹³ was found to have high selectivity to HDAC1 and HDAC2, both belonging to the class I HDAC family.⁹ To test whether cardiac hypertrophy induced by pressure overload could be prevented by class I-selective HDAC inhibition, we treated AB mice with SK. AB-induced cardiac enlargement was almost completely prevented by SK, as shown in Figure 3A, and the reduction of LV dimension was confirmed by M-mode echocardiogram (Figure 3C to 3F, Table 1). Histone acetylation status in SK-treated hearts is shown in Figure 3B. SK

increased the histone H4 acetylation in both sham- and AB-operated groups (top panel).

AB increased HW/BW ratio by 46% in the vehicle-treated group; however, SK completely abolished the increase (to 2%) compared with the sham+SK group (Figure 3G). Because SK could induce mild weight loss in some mice, we measured tibia length and calculated HW/TL ratio. Similarly, the 51% increase in HW/TL ratio in the AB group was reduced to 3% by SK (Figure 3H). Table 1 shows the changes of LV dimensions as well as heart weight after administration of 3 HDAC inhibitors to AB mice. Although all 3 inhibitors significantly reduced the thickness of the interventricular septum and posterior wall, the reduction was greatest in the SK-treated group.

Regression of Preestablished Cardiac Hypertrophy and Long-Term Evaluation

We next examined whether HDAC inhibition can reverse preexisting cardiac hypertrophy, which might be of greater clinical significance. As expected, AB increased HW/BW

TABLE 2. Regression of Preestablished Cardiac Hypertrophy

	Control Group	AB5w Group	AB2w+TSA3w Group
IVS, mm	0.67±0.03	1.11±0.02†	0.84±0.04§
LVDd, mm	3.54±0.12	4.32±0.04*	3.95±0.21
PWT, mm	0.65±0.03	1.07±0.04†	0.84±0.04§
LVSD, mm	2.16±0.19	2.83±0.13*	2.52±0.15
FS, %	39.12±3.84	34.51±2.78	36.40±1.12
HW, g	0.15±0.01	0.27±0.02†	0.19±0.01‡

Abbreviations are as defined in Table 1.

*P<0.05, †P<0.01 vs control.

‡P<0.05, §P<0.01 vs AB5w group.

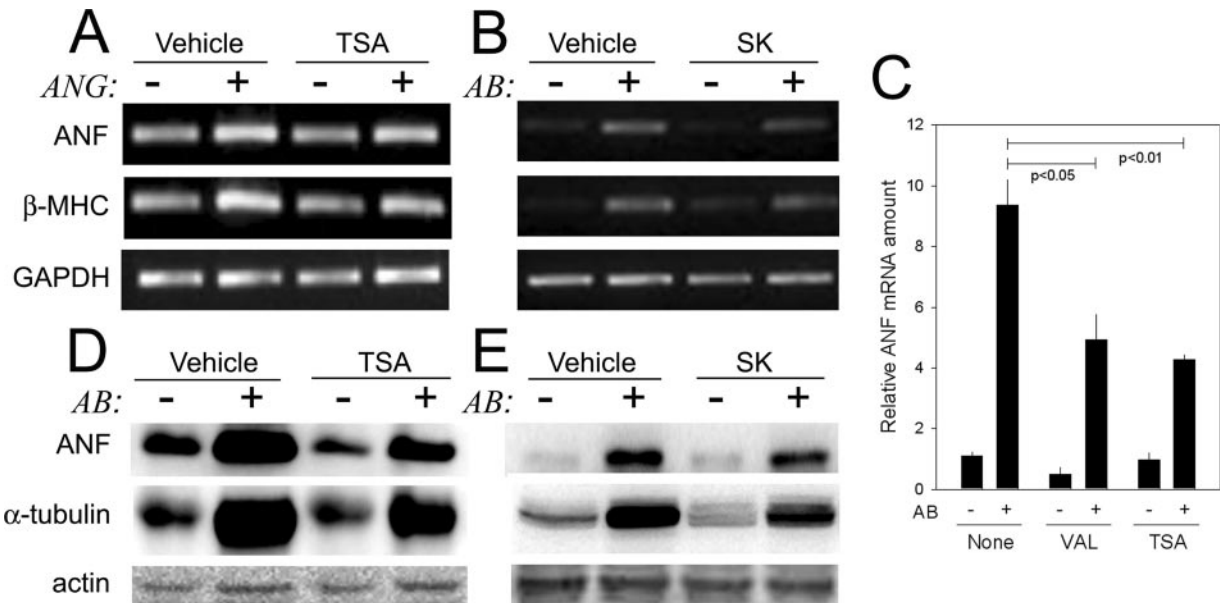


Figure 5. Attenuated expression of cardiac hypertrophy markers by HDAC inhibitors. A, Representative RT-PCR analysis of heart mRNA from control, Ang II (ANG), TSA, and TSA+ANG mice as indicated. The transcript levels of ANF or β -MHC were evaluated. In each lane, equal amounts of RNA were loaded as determined by GAPDH transcript level. B, Effects of SK on expression of ANF and β -MHC. SK significantly reduced the increase in transcript levels induced by AB. C, Quantitative analysis of changes of ANF mRNA amounts by real-time PCR. Three to 4 hearts from each experimental condition were evaluated. Each sample was measured in duplicate. Relative amount of ANF mRNA was calculated by comparison with that of GAPDH mRNA. Both VAL and TSA significantly reduced the ANF mRNA level. D and E, Representative immunoblot analysis of ANF, α -tubulin, and actin in the mice hearts treated with TSA (D) or SK (E). In each lane, equal amounts of proteins were loaded as determined by bicinchoninic acid assay and actin expression.

ratio over a 2-week period, and this increase was significantly attenuated by simultaneous administration of TSA (Figure 4B). Maintenance of a cohort of AB mice with vehicle treatment for the subsequent 3 weeks resulted in a further increase in HW/BW ratio, suggesting that cardiac hypertrophy is progressive. Interestingly, however, TSA administration for a 3-week period after 2 weeks of AB relieved the preexisting cardiac hypertrophy and prevented further progression; HW/BW ratio was significantly reduced compared with AB2w as well as AB5w mice (Figure 4B). Reversal of preestablished cardiac hypertrophy was further confirmed by measuring HW/TL ratio, in which the level of regression appeared even more dramatic (Figure II in the online-only Data Supplement). The regression of the heart weight paralleled the reduction of LV wall thickness (Table 2).

The long-term effects of HDAC inhibition on cardiac hypertrophy and survival were evaluated in AB mice treated with or without VAL or TSA for 7 weeks. HW/TL in AB mice was increased by 41% over that in age-matched controls; however, this increase was blunted to 19% by VAL. Figure III in the online-only Data Supplement also shows similar changes in HW/BW. Some AB mice died suddenly and were found to have enlarged hearts with severe myocardial fibrosis. Kaplan-Meier analysis revealed that the survival rate was 54.7% at 40 days after surgery. However, administration with TSA or VAL improved the survival rate to 83.3% (Figure 2E).

Hypertrophic Markers

Cardiac hypertrophy reactivates the fetal gene program, characterized by a switch from α -myosin heavy chain (α -

MHC) to β -MHC gene expression in the adult murine heart.^{1,16} β -MHC transcript levels as well as ANF transcripts were increased in Ang II and AB mice (Figure 5A and 5B). These increases were blunted by administration of TSA (Figure 5A) or SK (Figure 5B). The changes in ANF mRNA levels were confirmed by quantification, and both VAL and TSA significantly blunted the increase (Figure 5C).

In addition to ANF, α -tubulin is another marker of cardiac hypertrophy and cardiac protein expression increases in response to diverse hypertrophic stimuli.¹⁷ As expected, AB increased the protein levels of ANF and α -tubulin in mouse hearts. However, this increase was significantly blocked by treatment with TSA (Figure 5D) or SK (Figure 5E). Similar effects with regard to ANF and α -tubulin expression were seen in rat heart samples after AB with and without TSA (Figure IV in the online-only Data Supplement).

We examined expression of ANF and deposition of type I collagen¹⁸ by immunohistochemistry after AB and Ang II, with and without TSA. Staining for both ANF and type I collagen was significantly enhanced in samples from AB rats (Figure 6B and 6E) compared with sham controls (Figure 6A and 6D). These changes in gene expression were normalized by TSA (Figure 6C and 6F). In AB mice, SK treatment also normalized the changes in ANF (Figure 6G to 6I).

AB for 2 and 7 weeks (Figure 6K and 6N) caused significant interstitial collagen deposition, as demonstrated by Masson's trichrome staining. However, fibrosis was reduced in AB mice treated with SK for 2 weeks or VAL for 7 weeks (Figure 6L and 6O). Interestingly, in some mice with SK treatment, no evidence of interstitial fibrosis was seen (data not shown).

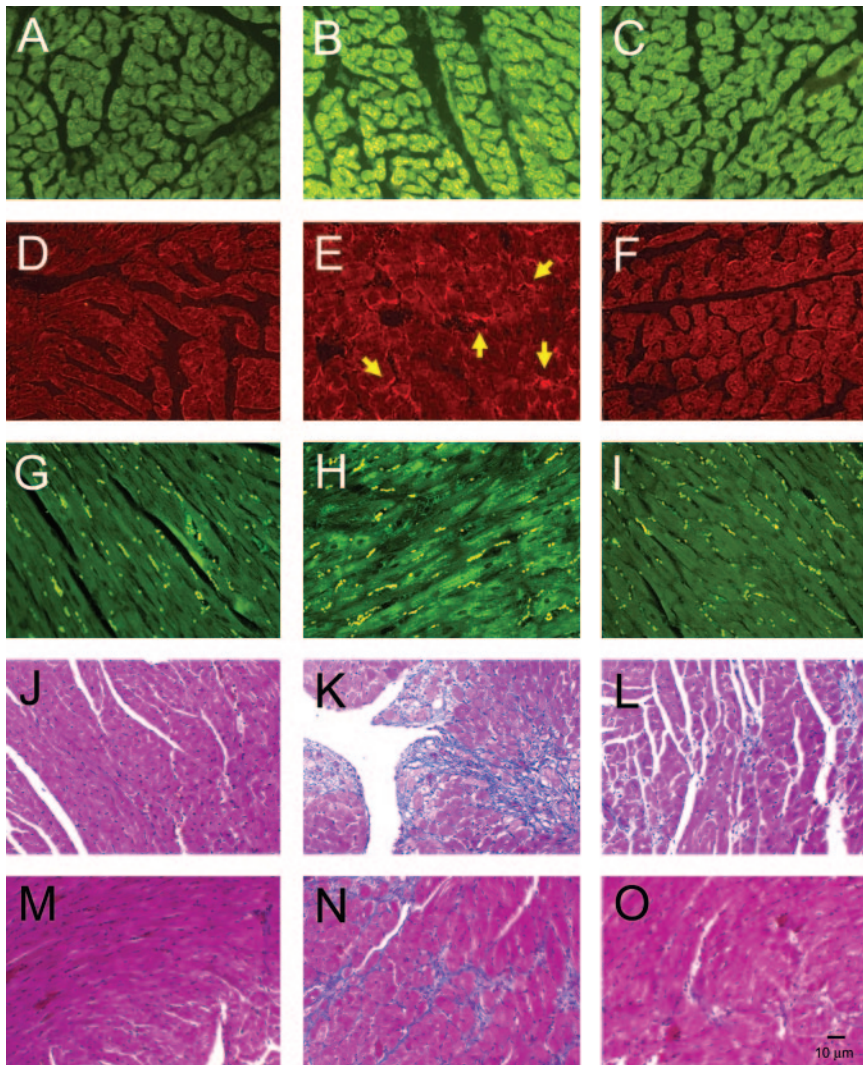


Figure 6. Visualization of cardiac markers and fibrosis in AB hearts with and without HDAC inhibitors. A to C, Indirect immunofluorescence detection of ANF in sham (A), AB+vehicle (B), and AB+TSA (C) rat hearts. Expression of ANF (small green dots) was increased in the cytoplasm of cardiomyocytes in AB+vehicle (B) but reduced by TSA (C). D to F, Type I collagen deposition detected by immunohistochemistry. Interstitial collagen deposition was increased by AB (E, yellow arrows), but this increase was blunted by TSA (F). G to I, Visualization of ANF in sham (G), AB+vehicle (H), and AB+SK (I) mice hearts. J to O, Masson's trichrome staining in mice hearts. Interstitial fibrosis (blue staining) was increased by AB for 2 weeks (K) or 7 weeks (N), and these increases were blocked by SK (L) or VAL (O).

Previous reports that HDAC inhibitors, including SK, induce apoptosis of transformed cells^{9,19,20} and thereby can be used for cancer treatment^{9,21} raise the possibility that apoptosis in cardiomyocytes may occur in our animal models, leading to reduction of cardiac mass. However, terminal deoxynucleotidyl transferase-mediated dUTP nick-end labeling (TUNEL) staining showed no apparent increase in cardiomyocyte apoptosis in TSA- or SK-treated animals compared with the vehicle group (data not shown).

Discussion

Our results clarify that nonspecific HDAC inhibitors can function to attenuate cardiac hypertrophic changes induced by diverse stimuli including receptor-mediated agonists and pressure overload. In addition, we have shown that a class I HDAC-selective inhibitor prevents cardiac hypertrophy and that administration of HDAC inhibitors can induce regression of preestablished cardiac hypertrophy in at least some animal models.

Cardiac hypertrophy induced by transgenic overexpression of HOP or by chronic infusion of either isoproterenol or Ang II is prevented or significantly attenuated by concomitant treatment with TSA. Likewise, hypertrophy induced by AB,

in which stretch-activated ion channels, integrins, and sarcomeric Z disks are involved in mediating hypertrophic signaling,²² is blocked by TSA or VAL. These results suggest that HDAC activation is a part of the final common pathway induced by diverse hypertrophic agonists and that inhibition of this step may serve as a therapeutic target for preventing or reversing cardiac hypertrophy and subsequent heart failure. Indeed, in this study, along with improved LV dimensions, as determined by echocardiogram, better survival in the TSA- or VAL-treated group in AB mice supports their beneficial effects on the transition of cardiac hypertrophy to heart failure. In addition, the regression of preestablished cardiac hypertrophy further raises the potential attractiveness for the therapeutic application of HDAC inhibitors.

It is unlikely that reduction in cardiac hypertrophy in the Ang II model was caused by relief of Ang II-induced hypertension, and we suggest that the primary target organ of HDAC inhibitors is heart rather than blood vessels. Likewise, cardiomyocyte apoptosis does not appear to be responsible for the reduction of heart mass. Previous reports^{6,23} have shown that TSA, VAL, or sodium butyrate, an alternate HDAC inhibitor, does not significantly affect the survival of primary cultured rat cardiomyocytes, and HDAC inhibitors in

this study did not increase apoptosis in cardiomyocytes *in vivo*. Although HDAC inhibitors have significant proapoptotic effects in transformed cells,¹⁹ Park et al⁹ clearly showed that untransformed epithelial cells are much more resistant to SK-induced apoptosis than are cancer cells.

To date, at least 17 distinct HDACs have been reported in humans. Members of all 3 classes of HDACs are expressed in the heart, and, recently, class III HDACs were reported to be endogenous inhibitors of apoptosis in the heart.²⁴ TSA or VAL inhibits both class I and class II HDACs. Given that class II HDACs function to antagonize cardiac hypertrophy,^{4,5} the antihypertrophic effect of nonspecific HDAC inhibitors is more likely to be mediated by class I HDACs. In this study we clearly demonstrate that class I HDACs in cardiomyocyte are prohypertrophic because SK, a class I HDAC-selective inhibitor, blocked the development of cardiac hypertrophy.

Given the proposed antagonistic actions of class I and class II HDACs, why do nonspecific HDAC inhibitors result in antihypertrophic effects? The most plausible explanation is that class I HDAC activity predominates with regard to hypertrophic gene expression in the heart. Indeed, the enzymatic activity of class II HDACs is not required for the transcriptional repression of prohypertrophic genes in the heart, as a splice variant of HDAC9 that lacks a deacetylase domain is highly effective in repressing cardiac hypertrophy.^{5,25} These reports further support our conclusion that the net functional role of HDAC activity, probably mediated by class I HDACs, in cardiomyocytes is prohypertrophic.

Cell proliferation/growth arrest mechanisms are known to contribute to the regulation of cardiac hypertrophy.²⁶ In cancer cells, for example, HDAC inhibitors relieve histone compaction to upregulate p21,²⁷ which has been implicated as a repressor of hypertrophy.²⁸ It is still unclear, however, whether HDACs may affect other prohypertrophic signal transduction cascades, because several nonhistone targets, such as tubulin and GATA transcription factor,^{23,29,30} which can affect cardiac remodeling, have been implicated. For example, in primarily cultured rat cardiomyocytes, TSA was shown to increase α -MHC expression but to decrease cardiac tubulins. Although early growth response gene 1 (EGR-1) was elucidated as a primary mediator of the responses,²³ direct evidence for the functional involvement of EGR-1 in *in vivo* hypertrophy remains to be demonstrated.

This work relies entirely on the use of pharmacological inhibitors of HDAC activity. Although we have attempted to use several different chemical inhibitors, it remains possible that the effects that we observed on cardiac hypertrophy are produced by effects of the drugs unrelated to their abilities to inhibit HDAC catalytic function. Indeed, alternative cellular functions for HDACs remain possibly unrelated to the regulation of chromatin structure as described above, and unappreciated functions of the pharmacological agents that we have used may exist. Nevertheless, the cardiac effects of these agents are of interest given the increasing likelihood of more widespread clinical use for noncardiac indications, including the treatment of neoplasia.^{21,31} Therefore, as well as development of novel HDAC inhibitors with selectivity and low toxicity, additional preclinical testing is indicated to

validate the use of these inhibitors for prevention or reversal of cardiac hypertrophy and failure in humans.

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Disclosures

None.

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CLINICAL PERSPECTIVE

Although many stress signals can induce cardiac hypertrophy and subsequent congestive heart failure in humans and animal models, few interventions have been identified that can block hypertrophic signaling in cardiac myocytes or that are able to induce regression of preestablished hypertrophy. Recently, alterations in chromatin structure and global gene transcription, which are regulated by enzymes that add or remove acetyl groups from core histones, have been implicated in the regulation of cardiac hypertrophy. Removal of acetyl groups and resulting compaction of chromatin and repression of transcription are mediated by histone deacetylases (HDACs). Chemical HDAC inhibitors are being actively developed by the pharmaceutical industry and are in clinical trials for the treatment of cancer. In this report it is demonstrated that chemical HDAC inhibitors can significantly attenuate the response of mice and rats to a broad spectrum of hypertrophic stimuli including pressure overload and receptor-mediated agonists such as angiotensin II. Partial regression of preestablished hypertrophy can also be produced by HDAC inhibition. Because prior data suggested that inhibition of class II HDACs would promote hypertrophy, the finding that broad-spectrum HDAC inhibitors prevent hypertrophy suggests that the targets of these drugs in the heart are class I HDACs; this hypothesis is supported by data from this study in which a class I specific inhibitor is used. This work suggests that inhibition of class I HDAC activity in the heart should be considered a therapeutic target for the treatment or prevention of cardiac hypertrophy and heart failure worthy of further validation and investigation.