Myocardial Protection Against Pressure Overload in Mice Lacking Bach1, a Transcriptional Repressor of Heme Oxygenase-1

Shinji Mito, Ryoji Ozono, Tetsuya Oshima, Yoko Yano, Yuichiro Watari, Yoshiyuki Yamamoto, Andrei Brydun, Kazuhiko Igarashi, Masao Yoshizumi

Abstract—Bach1 is a stress-responsive transcriptional factor that is thought to control the expression levels of cytoprotective factors, including heme-oxygenase (HO)-1. In the present study, we investigated the roles of Bach1 in the development of left ventricular (LV) hypertrophy and remodeling induced by transverse aortic constriction (TAC) in vivo using Bach1 gene-deficient (Bach1−/−) mice. TAC for 3 weeks in wild-type control (Bach1+/+) mice produced LV hypertrophy and remodeling manifested by increased heart weight, histological findings showing increased myocyte cross-sectional area (CSA) and interstitial fibrosis (picro Sirius red staining), reexpressions of ANP, BNP, and βMHC genes, and echocardiographic findings showing wall thickening, LV dilatation, and reduced LV contraction. Deletion of Bach1 caused significant reductions in heart weight (by 16%), CSA (by 36%), tissue collagen content (by 38%), and gene expression levels of ANP (by 75%), BNP (by 45%), and βMHC (by 74%). Echocardiography revealed reduced LV dimension and ameliorated LV contractile function. Deletion of Bach1 in the LV caused marked upregulation of HO-1 protein accompanied by elevated HO activity in both basal or TAC-stimulated conditions. Treatment of Bach1−/− mice with tin-protoporphyrin, an inhibitor of HO, abolished the antihypertrophic and antiremodeling effects of Bach1 gene ablation. These results suggest that deletion of Bach1 caused upregulation of cytoprotective HO-1, thereby inhibiting TAC-induced LV hypertrophy and remodeling, at least in part, through activation of HO. Bach1 repressively controls myocardial HO-1 expression both in basal and stressed conditions, inhibition of Bach1 may be a novel therapeutic strategy to protect the myocardium from pressure overload. (Hypertension. 2008;51:1570-1577.)

Key Words: hypotrophy ■ HO-1 ■ mice ■ oxidative stress ■ Bach1 ■ remodeling

Cardiac hypertrophy has been regarded as a compensatory mechanism of the heart to maintain cardiac output during pathological states with sustained increases in hemodynamic load, but it is associated with a high risk of cardiac mortality because of its established role in the development of cardiac failure. Heme-oxygenase (HO) is an enzyme that degrades prooxidant heme to carbon monoxide and biliverdin/bilirubin. HO-1 is an inducible form and HO-2 is a constitutive form of the enzyme.1 HO-1, the activity of which is 10-fold greater than that of HO-2, is considered to be a stress-induced cytoprotective factor because (1) it is swiftly upregulated on exposure to cellular stress and (2) the catalytic products, carbon monoxide and biliverdin/bilirubin, have antiinflammatory2 and antioxidant3 actions, respectively. In the left ventricle (LV), stresses such as pressure overload cause generation of reactive oxygen species (ROS) and inflammatory reaction, which are thought to be involved in the underlying mechanisms of LV hypertrophy and the subsequent process of LV remodeling.4 HO-1 may be activated in the LV in such stressed conditions, attenuating the effects of prohypertrophic ROS signals and thereby inhibiting LV hypertrophy and LV remodeling. In support of this hypothesis, it has been reported that HO-1–deficient mice developed severe cardiac hypertrophy in a model of renovascular hypertension.5 It has been reported that pharmacological induction of HO-1 by cobalt protoporphyrin caused attenuation of angiotensin (Ang) II–induced cardiomyocyte hypertrophy in vitro and in vivo,6 whereas a controversial result has been also reported.7 Therefore, the roles of HO-1 in LV hypertrophy and LV remodeling are not fully understood. It is also not known how HO-1 itself is regulated in the course of LV hypertrophy and subsequent LV remodeling.

In this regard, it is important to understand the mechanism of transcriptional regulation of HO-1. HO-1 gene expression is controlled by 2 inducible enhancers carrying multiple Maf recognition elements (MARE), also known as stress-responsive elements. Bach1, a basic leucine zipper-type transcription factor, binds to MARE to repress transcription

Received October 8, 2007; first decision November 3, 2007; revision accepted March 26, 2008.
From the Departments of Cardiovascular Physiology and Medicine (S.M., M.Y.), Clinical Laboratory Medicine (R.O., T.O., Y. Yano), and Medicine and Molecular Science (Y.W., Y. Yamamoto, A.B.), Hiroshima University Graduate School of Biomedical Sciences, Hiroshima; and the Department of Biochemistry (K.I.), Tohoku University Graduate School of Medicine, Tohoku, Japan.
Correspondence to Ryoji Ozono, MD, PhD, 1-2-3 Kasumi, Minami-ku, Hiroshima 734-8551, Japan. E-mail ozono@hiroshima-u.ac.jp
© 2008 American Heart Association, Inc.
Hypertension is available at http://hyper.ahajournals.org
DOI: 10.1161/HYPERTENSIONAHA.107.102566
of HO-1, forming heterodimers with 1 of the small Maf proteins,\textsuperscript{8–11} whereas binding of transcription activators such as NF-E2–related factor 2 (Nrf2) leads to activation of HO-1 transcription.\textsuperscript{9,10} However, on exposure to oxidative stresses, Bach1 loses its DNA-binding activity, being exported out of the nuclei, which in turn makes MARE accessible to HO-1 activators.\textsuperscript{16,17} HO-1 expression is thereby despressed by oxidative stress, and subsequent nuclear accumulation of Nrf2 and other HO-1 activators leads to upregulation of HO-1 expression. Consistent with this, tissue expression of HO-1 was found to be markedly upregulated in Bach1 gene–deficient (Bach1\textsuperscript{−/−}) mice.\textsuperscript{9}

We have recently demonstrated that Bach1\textsuperscript{−/−} mice displayed markedly enhanced response of myocardial HO-1 expression after ischemia/reperfusion injury, resulting in a dramatic reduction in ischemia-induced myocardial cell death,\textsuperscript{18} indicating that the absence of Bach1 is beneficial for the heart exposed to transient ischemic stress. However, the roles of Bach1 in the heart exposed to long-lasting hemodynamic stress such as pressure overload are still uncertain. In the present study, we investigated the roles of Bach1 and HO-1 in LV hypertrophy and remodeling induced by pressure overload in vivo in Bach1\textsuperscript{−/−} mice.

**Methods**

The detailed methods are described in the online supplement available at http://hyper.ahajournals.org.

**Animals and Study Groups**

We used Bach1\textsuperscript{−/−} mice backcrossed onto C57BL/6J mice.\textsuperscript{9,18} Mice of this strain were regarded as wild-type (Bach1\textsuperscript{+/+}) controls. A transverse aortic constriction (TAC) were performed in male mice at ages of 8 to 10 weeks.\textsuperscript{19} The mice were randomly allocated to TAC groups (TAC-Bach1\textsuperscript{+/+} and TAC-Bach1\textsuperscript{−/−} groups) or sham operation groups (sham-Bach1\textsuperscript{+/+} and sham-Bach1\textsuperscript{−/−} groups) and maintained until 3 weeks after the surgery. To block HO activity, we intraperitoneally injected tin-protoporphyrin IX (SnPP) at a dose of 1 mg/kg body weight 24 hours before the TAC operation and every 2 days throughout the study periods.

**Assessment of Cardiac Geometry and Function**

Cardiac geometry and function were evaluated 3 weeks after induction of TAC in anesthetized animals using an echocardiography system (Toshiba SSA 550A).\textsuperscript{18,20} LV pressure was directly measured by 1.4F Millar pressure catheter (Millar Instruments).\textsuperscript{21}

**Tissue Weight and Histological Analysis**

The mice were euthanized 3 weeks after TAC, then heart weight (right and left ventricular weight) and lung weight were measured. Tissue sections were stained with hematoxylin and eosin or with 0.1% Sirius red F3BA saturated in picric acid.\textsuperscript{22} We assessed myocardocyte cross-sectional area (CSA), perivascular fibrosis index (a percent ratio of perivascula fibrosis area to artery area), and myocardial total collagen content (a percent rate of Sirius-red stained collagen area to total myocardial area) in digitalized microscopic images.\textsuperscript{18,20,22,23}

**Real-Time Polymerase Chain Reaction Analysis for Cardiac Gene Expression**

Cardiac gene expressions (2 days after surgery), including those of natriuretic peptide precursor type A (ANP), natriuretic peptide precursor type B (BNP), βmyosin heavy chain (βHMHC), ferritin heavy chain (H-FT), and ferritin light chain (L-FT), were assessed by real-time polymerase chain reaction (PCR).\textsuperscript{9,18,22}

**Western Blot and HO Activity**

HO-1 protein expression was evaluated 2, 7, and 21 days after surgery by Western blot analysis.\textsuperscript{18} HO activity in microsomes was determined in LV tissues 2 days after surgery as previously described.\textsuperscript{18}

**Statistical Analysis**

Results are expressed as means±SEM. Statistical differences among the groups were assessed with ANOVA followed by Fisher’s PLSD test. \(P<0.05\) was considered to indicate statistical significance.

**Results**

**Effect of TAC on HO-1 Protein Expression in the LV**

We investigated the effects of TAC on HO-1 protein levels in the LVs of Bach1\textsuperscript{+/+} and Bach1\textsuperscript{−/−} mice 2, 7, and 21 days after TAC by Western blotting. An anti–HO-1 antibody detected a band of approximately 32 kDa in size (Figure 1A). This band disappeared when the antibody was preincubated with recombinant HO-1 protein (SPP-730, Stressgen), indicating the validity of this antibody for immunoblot analysis.
Effects of TAC on Body Weight, Blood Pressure, and Heart Rate

Table S1 shows the effects of TAC for 3 weeks on body weight, heart rate, and blood pressure in Bach1+/+ and Bach1−/− mice. TAC or SnPP treatment did not significantly affect mouse growth (body weight), heart rate, or tail cuff blood pressure in either strain of mice.

Attenuation of Pathological LV Hypertrophy and Remodeling in Bach1−/− Mice

As shown in Figure 3, TAC for 3 weeks caused approximately 2-fold increases in the ratio of heart weight to body weight (HW/BW) in TAC-Bach1+/+ mice. In TAC-Bach1−/− mice, HW/BW was significantly (P<0.05) decreased by 16% compared with that in TAC-Bach1+/+ mice, indicating that Bach1 ablation resulted in attenuation of LV hypertrophy. After treatment with SnPP, however, the difference between HW/BWs of TAC-Bach1+/+ mice and TAC-Bach1−/− mice was abolished, suggesting that attenuation of TAC-induced HW/BW in TAC-Bach1−/− mice was mediated by increased activity of HO (Figure 2). SnPP treatment slightly increased HW/BW even in TAC-Bach1−/− mice, but the change did not reach statistical significance.

The extent of pulmonary congestion was estimated by the rate of lung weight to body weight (LW/BW; Figure S1). TAC caused an approximately 2-fold increase in LW/BW in TAC-Bach1+/+ mice, suggesting that TAC-induced LV hypertrophy was associated with pulmonary congestion. This increase in LW/BW was attenuated by 29% in TAC-Bach1−/− mice, suggesting that Bach1 is involved in the mechanism of TAC-induced pulmonary congestion. However, SnPP treatment caused significant elevation of LW/BW in both of TAC-Bach1+/+ mice and TAC-Bach1−/− mice and failed to diminish the difference between the 2 groups, suggesting that was higher in Bach1−/− mice than in Bach1+/+ mice either before or after TAC. Furthermore, SnPP treatment effectively inhibited HO activity both in TAC-Bach1+/+ mice and TAC-Bach1−/− mice.

In the sham-Bach1+/+ mice, HO-1 expression was absent, but it was already upregulated in the sham-Bach1−/− mice (Figure 1B). In the TAC-Bach1+/+ mice, HO-1 level was increased on day 2 after TAC and then decreased, disappearing 21 days after TAC (Figure 1B and 1C). In the TAC-Bach1−/− mice, HO-1 was similarly upregulated on day 2 after TAC, the expression level being 4- to 5-fold greater than that in the TAC-Bach1+/+ group. The high HO-1 level in this group was sustained until 21 days after TAC.

HO Activity

To determine whether the upregulation of HO-1 expression is associated with elevation in its enzymatic activity and to determine whether administration of SnPP effectively blocks HO activity, tissue HO activity was measured using LVs taken 2 days after TAC or the sham operation. As shown in Figure 2, HO activity was present in sham-Bach1+/+ mice in which HO-1 was undetectable, reflecting an activity of HO-2, a constitutive form of this enzyme. Correlating well with the protein levels of HO-1 (Figure 1B and 1C), HO activity level was inhibited HO activity both in TAC-Bach1−/− mice and TAC-Bach1−/− mice.
Tissues were obtained 3 weeks after surgery. The right end bars show the effect of concomitant SnPP treatment in the TAC-

Figure 4. Histological analysis. A, Cross sections of cardiomyocytes of LVs from Bach1+/+ and Bach1−/− mice that had undergone TAC or the sham operation. Paraffin sections were stained with hematoxylin and eosin (magnification ×400). B, Quantitative analysis of the effect of TAC or sham operation on cardiac myocyte cross-sectional area in Bach1+/+ and Bach1−/− mice (3 weeks after surgery). Two hundred cross-sections were counted per section as described in Methods. The right end bars shows the effect of concomitant SnPP treatment in the TAC- Bach1+/+ group and TAC-Bach1−/− group. Values are means±SEM (n=5 per treatment group).

Histological Analysis

Consistent with the increase in heart weight, TAC caused an increase in CSA by 2.3 fold in Bach1+/+ mice (Figure 4A and 4B). This effect of TAC was again significantly attenuated by 36% in TAC-Bach1−/− mice (P<0.05 versus TAC-Bach1+/+ mice). SnPP treatment had no significant effect on CSA of TAC-Bach1+/+ mice, whereas the same treatment significantly increased CSA in TAC-Bach1−/− mice, diminishing the difference between CSAs of the 2 strains, suggesting that the effect of Bach1 ablation on CSA was mediated by HO.

Sirius red staining showed increased interstitial fibrosis (Figure 4C through 4F) after 3 weeks of TAC both in Bach1+/+ and Bach1−/− mice. The fibrosis developed both in
the perivascular area (Figure 4C) and in the myocardial interstitial area (Figure 4D). In TAC-Bach1+/− mice, the perivascular fibrosis index was markedly increased, whereas Bach1 ablation significantly (P<0.05 versus TAC-Bach1+/− mice) reduced the index by 33% (Figure 4E). SnPP treatment had no effect on the perivascular fibrosis index in TAC-Bach1+/− mice, whereas the same treatment significantly increased the index in TAC-Bach1+/− mice, abolishing the difference between the 2 strains.

Next, we evaluated the total collagen content of the myocardium including the perivascular area (Figure 4F). Analogous to the results of perivascular fibrosis index, myocardial total collagen content was increased by approximately 6-fold in TAC-Bach1+/− mice, and it was attenuated by 37% in TAC-Bach1+/− mice. SnPP treatment abolished the difference between the total collagen contents of TAC-Bach1+/− mice and TAC-Bach1−/− mice. These results suggest that Bach1 ablation ameliorated TAC-induced myocardial fibrosis through upregulation of HO activity.

Echocardiography

Table 1 shows the results of echocardiography. Figure S2 shows the recordings of M mode echocardiography. There was no significant difference between LV diastolic posterior wall thicknesses (PWTd), LV dimensions (LVDd and LVDs), and % fractional shortenings (FS) of sham-Bach1+/+ and sham-Bach1−/− mice. TAC for 3 weeks in Bach1+/+ mice significantly (P<0.05 versus TAC-Bach1−/− mice) increased posterior wall thickness (PWT) by 8.2%, LVDd by 8.8%, and LVDs by 21% and significantly decreased %FS by 11%, suggesting that TAC caused LV hypertrophy and dilatation, thereby reducing LV contractile function. On the other hand, in TAC-Bach1−/− mice, both LVDd and LVDs were significantly (P<0.05) smaller by 4.5% and 12%, respectively, than those in TAC-Bach1+/− mice, suggesting that Bach1 ablation attenuated the TAC-induced LV dilatation and ameliorated LV contractile function. In TAC-Bach1−/− mice, PWTd tended to be smaller and %FS tended to be larger than those in TAC-Bach1+/− mice, although the differences between the 2 groups did not reach statistical significance.

SnPP treatment in TAC-Bach1+/− mice had no significant effects on PWTd, LVDd, LVDs, and %FS. On the other hand, SnPP treatment in TAC-Bach1−/− mice resulted in significant increases (P<0.05 versus TAC-Bach1−/− mice group) in LVDd and LVDs and a significant decrease (P<0.05 versus TAC-Bach1−/− mice group) in %FS, diminishing the difference between the 2 strains, suggesting that attenuation of TAC-induced LV remodeling and dysfunction in Bach1−/− mice was mediated by increased activity of HO.

Hemodynamic Measurements

To investigate the effects of Bach1 ablation on LV function, intracardiac pressures were directly measured by catheterization through the right carotid artery (Table 2). Figure S3 shows the trace recording of LV pressure. The data suggest that TAC either in Bach1+/+ mice or in Bach1−/− mice resulted in significant pressure overload (LVSP) with small variation. The extent of pressure overload was not different between the 2 strains. LV enddiastolic pressure (LVEDP), an index of ventricular preload, was significantly elevated in

Table 1. Effects of TAC for 3 Weeks on Echocardiographic Parameters

<table>
<thead>
<tr>
<th>Group</th>
<th>Sham-Bach1+/+</th>
<th>Sham-Bach1−/−</th>
<th>TAC-Bach1+/+</th>
<th>TAC-Bach1−/−</th>
<th>TAC-Bach1+/+ + SnPP</th>
<th>TAC-Bach1−/− + SnPP</th>
</tr>
</thead>
<tbody>
<tr>
<td>n</td>
<td>10</td>
<td>5</td>
<td>10</td>
<td>5</td>
<td>6</td>
<td>6</td>
</tr>
<tr>
<td>PWTd, mm</td>
<td>0.73±0.02</td>
<td>0.72±0.02</td>
<td>0.96±0.03</td>
<td>0.90±0.03†</td>
<td>1±0.04†</td>
<td>0.98±0.04†</td>
</tr>
<tr>
<td>LVDd, mm</td>
<td>3.08±0.02</td>
<td>3.08±0.04</td>
<td>3.35±0.03†</td>
<td>3.20±0.04‡</td>
<td>3.36±0.07§</td>
<td>3.32±0.06§</td>
</tr>
<tr>
<td>LVDs, mm</td>
<td>1.64±0.03</td>
<td>1.66±0.05</td>
<td>1.98±0.07*</td>
<td>1.74±0.08‡</td>
<td>2.14±0.08§</td>
<td>2.04±0.09§</td>
</tr>
<tr>
<td>%FS, %</td>
<td>46.3±1.12</td>
<td>45.2±1.50</td>
<td>41.0±1.83*</td>
<td>45.3±2.14†</td>
<td>36.2±1.32§</td>
<td>38.6±2.20§</td>
</tr>
</tbody>
</table>

PWTd indicates LV diastolic posterior wall thickness; LVDd, LV end-diastolic dimension; LVDs, LV end-systolic dimension; FS, fractional shortening; SnPP.

*P<0.05 vs Sham-Bach1+/+; †P<0.05 vs Sham-Bach1−/−; ‡P<0.05 vs TAC-Bach1+/+; §P<0.05 vs TAC-Bach1−/−. Values are means±SEM.

Table 2. Left Ventricular Hemodynamics at 3 Weeks After TAC and Sham Operation

<table>
<thead>
<tr>
<th>Group</th>
<th>Sham-Bach1+/+</th>
<th>Sham-Bach1−/−</th>
<th>TAC-Bach1+/+</th>
<th>TAC-Bach1−/−</th>
</tr>
</thead>
<tbody>
<tr>
<td>n</td>
<td>8</td>
<td>8</td>
<td>8</td>
<td>8</td>
</tr>
<tr>
<td>HR, bpm</td>
<td>431±26</td>
<td>490±21</td>
<td>453±28</td>
<td>516±24</td>
</tr>
<tr>
<td>LVSP, mm Hg</td>
<td>96±3.2</td>
<td>101±3.4</td>
<td>178±16.6*</td>
<td>200±9.4†</td>
</tr>
<tr>
<td>LVEDP, mm Hg</td>
<td>0.5±1.6</td>
<td>1.3±1.5</td>
<td>11.9±4.3*</td>
<td>7.7±1.7</td>
</tr>
<tr>
<td>Max dp/dt, mm Hg/s</td>
<td>9093±869</td>
<td>9876±1091</td>
<td>8799±1039</td>
<td>11474±569</td>
</tr>
<tr>
<td>Min dp/dt, mm Hg/s</td>
<td>6695±849</td>
<td>8300±882</td>
<td>8032±779</td>
<td>9893±1304</td>
</tr>
<tr>
<td>Contractility index</td>
<td>207±22.6</td>
<td>194±13.7</td>
<td>158±12.8§</td>
<td>220±12.9</td>
</tr>
<tr>
<td>Tau, ms</td>
<td>14.2±1.7</td>
<td>9.9±0.9</td>
<td>14.9±1.4</td>
<td>12.7±2.1</td>
</tr>
</tbody>
</table>

HR indicates heart rate; LVSP, left ventricular systolic pressure; LVEDP, left ventricular end-diastolic pressure; Max dp/dt, the steepest slope during the upstroke of the pressure curve; Min dp/dt, the steepest slope during the downstroke of the pressure curve; Contractility index; Max dp/dt divided by the pressure at the time of Max dp/dt; Tau, the exponential time constant of relaxation.

*P<0.05 vs Sham-Bach1+/+; †P<0.05 vs Sham-Bach1−/−; ‡P<0.05 vs TAC-Bach1−/−. Values are mean±SEM.
Bach1-supplemental Method) was significantly reduced in TAC-Bach1−/− mice, expressions of ANP, BNP, and βMHC were markedly elevated. The levels of TAC-induced upregulation were significantly lowered in TAC-Bach1−/− mice (ANP by 75%, BNP by 45%, and βMHC by 74% compared with the levels in TAC-Bach1+/+ mice), suggesting that Bach1 is involved in the mechanism of TAC-induced reprogramming of the cardiac gene expression pattern. SnPP treatment in TAC-Bach1−/− mice increased the expressions of ANP, BNP, and βMHC, diminishing the difference between the expression levels of TAC-Bach1+/+ mice and TAC-Bach1−/− mice, indicating that the effect of Bach1 ablation was mediated by upregulation of HO.

**Change in Iron Homeostasis in Bach1−/− Mice**

We investigated whether Bach1 ablation alters iron homeostasis because increased HO-1 may cause overproduction of harmful free iron (Figure S4A through S4C, please see http://hyper.ahajournals.org). Prussian blue staining of cardiac tissues of sham-Bach1+/+ and sham-Bach1−/− mice showed no excess iron deposition in the tissue (Figure S4A). Next, we determined mRNA levels of ferritin, including H-FT and L-FT, in sham-Bach1+/+ and sham-Bach1−/− mice. H-FT mRNA level was significantly higher in sham-Bach1−/− mice than in sham-Bach1+/+ mice (Figure S4B). There was no difference between L-FT mRNA levels in the two strains (Figure S4C).

**Discussion**

Bach1 is a transcription factor whose biological role and downstream mediators have not been fully elucidated. In the present study, we demonstrated that TAC-induced LV hypertrophy and associated interstitial fibrosis were significantly suppressed in Bach1-deficient mice. The mutant mice displayed enhanced HO-1 expression during a period of 3 weeks after TAC, and the cardioprotective effects of Bach1 ablation was almost completely abolished by administration of SnPP, an inhibitor of HO, suggesting that upregulation of HO-1 may be, at least in part, responsible for the cardioprotection.

There is ample evidence indicating that HO-1 is the major effector molecule under the control of Bach1. In the present study, SnPP almost completely abolished the cardioprotective effects of Bach1 ablation, whereas it had no effect in wild-type mice, providing a basis to conclude that HO-1 explains most of the effects of Bach1 ablation. However, we need to be careful about this interpretation because SnPP may have nongenetic actions on SnPP include inhibition of caspase 3, activation of soluble guanylyl cyclase in vitro, inhibition of stimulated cGMP generation in vivo, activation of generation of NO through iNOS, and inhibition of VEGF. Controversial results regarding the effects on guanylyl cyclase have been reported. Despite such reports, SnPP is the most specific, potent, and widely used inhibitor of HO. At least in the present experimental setting, SnPP effectively
inhibited cardiac HO activity (Figure 2) and had no significant action in wild-type animals (Figures 3 and 4, Table 1), supporting a dominant role of HO in the inhibition of LV remodeling in Bach1-deficient mice.

In the present study, we investigated the role of Bach1 in iron homeostasis as well as in HO-1 expression. Interestingly, it has recently been suggested that ferritin gene and thioredoxin reductase1 gene contain Bach1-binding element (MARE) and that Bach1 repressively controls transcription of these genes as it does for the HO-1 gene.32 Consistent with this observation, we demonstrated that H-FT mRNA level was significantly increased in sham-TAC mice compared to that in sham-Bach1+/− mice, although it remains to be determined whether the increase in ferritin mRNA level was mediated by a transcriptional or posttranscriptional mechanism.33 Prussian blue staining showed that there was no iron deposition in cardiac tissue of Bach1−/− mice (Figure S4A), suggesting that toxic iron produced by heme catalysis was effectively removed. These observations suggest that Bach1 coordinately regulates iron/oxygen/antioxidant metabolism and that deletion of Bach1 leads to cellular protection.

In the present study, myocardial HO-1 expression (Figure 1) and HO activity (Figure 2) were upregulated by TAC in Bach1+/− mice as well as in the Bach1−/− mice. Therefore, SnPP, an inhibitor of HO, could have some deteriorating effects on LV hypertrophy or remodeling inTAC-Bach1+/− mice. However, in the present study, SnPP had no significant effects on such parameters in the wild-type mice. This finding suggests that the extent of pressure overload-induced activation of HO in normal animals is not physiologically significant. Comparing the extents of HO-1 upregulation over the entire experimental period of 3 weeks (Figure 1), the increment of HO-1 in TAC-Bach1+/− mice is much smaller than that in TAC-Bach1−/− mice. The impact of SnPP treatment in normal animals may also differ by factor. For example, BNP expression, but not ANP and βMHC expressions, was increased by SnPP (Figure 5B).

Perspectives
Controlling LV hypertrophy and subsequent development of heart failure is a challenge in clinical medicine. Previous studies have demonstrated that introduction of HO-1 conferred tissue protection against ischemic damage in the heart.34,35 Accordingly, the results of the present study and previous studies6-7 suggest that HO-1 is an attractive target of antihypertrophic therapy in the LV exposed to hemodynamic stress. Based on the results of the present study, we propose that inhibition of Bach1 may be a novel and elegant therapeutic approach to effectively enhance HO-1 expression, avoiding the use of toxic heme and heavy metals, classic HO-1 inducers, or the use of a gene delivery technique, which remains problematic for clinical application.

Acknowledgments
The authors express great thanks to Dr Yulin Liao and Dr Hidetoshi Okazaki in Osaka University for instruction on the surgical procedure of TAC and for technical assistance in the hemodynamic study.

Sources of Funding
This study was supported by a Grant-in-Aid for Scientific Research (17590493) from the Ministry of Education, Japan and a grant from Takeda Science Foundation.

Disclosures
None.

References


Myocardial Protection Against Pressure Overload in Mice Lacking Bach1, a Transcriptional Repressor of Heme Oxygenase-1
Shinji Mito, Ryoji Ozono, Tetsuya Oshima, Yoko Yano, Yuichiro Watari, Yoshiyuki Yamamoto, Andrei Brydun, Kazuhiko Igarashi and Masao Yoshizumi

Hypertension. 2008;51:1570-1577; originally published online April 21, 2008; doi: 10.1161/HYPERTENSIONAHA.107.102566
Hypertension is published by the American Heart Association, 7272 Greenville Avenue, Dallas, TX 75231
Copyright © 2008 American Heart Association, Inc. All rights reserved.
Print ISSN: 0194-911X. Online ISSN: 1524-4563

The online version of this article, along with updated information and services, is located on the World Wide Web at:
http://hyper.ahajournals.org/content/51/6/1570

Data Supplement (unedited) at:
http://hyper.ahajournals.org/content/suppl/2008/04/21/HYPERTENSIONAHA.107.102566.DC1

Permissions: Requests for permissions to reproduce figures, tables, or portions of articles originally published in Hypertension can be obtained via RightsLink, a service of the Copyright Clearance Center, not the Editorial Office. Once the online version of the published article for which permission is being requested is located, click Request Permissions in the middle column of the Web page under Services. Further information about this process is available in the Permissions and Rights Question and Answer document.

Reprints: Information about reprints can be found online at:
http://www.lww.com/reprints

Subscriptions: Information about subscribing to Hypertension is online at:
http://hyper.ahajournals.org//subscriptions/