Biochemical and Biophysical Research Communications 463 (2015) 1012–1020

Contents lists available at ScienceDirect

Biochemical and Biophysical Research Communications

journal homepage: www.elsevier.com/locate/ybbrc

Tetrahydrobiopterin reverse left ventricular hypertrophy and diastolic dysfunction through the PI3K/p-Akt pathway in spontaneously hypertensive rats

Peng Chang [a, 1], Qiongying Wang [a, 1], Han Xu [a], Mina Yang [a], Xin Lin [a], Xiuli Li [a], Zhengyi Zhang [b], Xiaowei Zhang [a], Feng Zhao [a], Xu Zhao [a], Feng Bai [a], Jing Yu [a, *]

[a] Department of Cardiology, Lanzhou University Second Hospital, Gansu, China
[b] Department of General Medicine, Lanzhou University Second Hospital, Gansu, China

ARTICLE INFO

Article history:
Received 1 June 2015
Accepted 8 June 2015
Available online 18 June 2015

Keywords:
Tetrahydrobiopterin
Hypertrophy
Diastolic dysfunction
Phosphorylated Akt
Cardiac oxidation

ABSTRACT

Hypertension induced hypertrophy and diastolic dysfunction and is associated with cardiac oxidation and reduced NO production. We hypothesized that tetrahydrobiopterin (BH4) can regulate the phosphatidylinositol 3-kinase/protein kinase B (PI3K/Akt) signaling pathway and reverse cardiac hypertrophy and diastolic dysfunction in spontaneously hypertensive rats. Ten-week-old male spontaneously hypertensive rats (SHR) and age-matched normotensive control Wistar-Kyoto (WKY) rats were divided into five groups, WKY, WKY + BH4, SHR, SHR + BH4 and SHR + VAL. In SHR, diastolic dysfunction was accompanied by concentric hypertrophy, cardiac oxidation, and reduced cardiac BH4 and NO production. Four-week BH4 and valsartan administration reversed hypertrophy and improved diastolic function. BH4 and valsartan blunted the expression of hypertrophy markers α-skeletal actin (α-SA) and β-myosin heavy chain (β-MHC). Only BH4 reduced hypertension and induced myocardial fibrosis and expression of transforming growth factor-β1 (TGF-β1). BH4 reduced cardiac oxidant stress and increased NO production. Exogenous BH4 increased phosphorylated Akt levels and increased Bcl-2 expression.

In conclusion, less BH4 and reduced NO increases myocardial hypertrophy and cardiac oxidative stress, which exacerbates diastolic dysfunction. Exogenous BH4 ameliorates cardiac hypertrophy and diastolic dysfunction through the PI3K/p-Akt pathway. BH4 may be a potent therapy for hypertension with diastolic dysfunction.

1. Introduction

Hypertension is one of the most common diseases in primary care and an important preventable contributor to disease and death [1,2]. Hypertension-induced hypertrophy increases the risk for heart failure [3], especially left ventricular (LV) diastolic dysfunction, i.e., delayed relaxation and augmented diastolic stiffness. There are no specific treatments for diastolic dysfunction, partly because of a relative lack of a mechanistic understanding of this disorder [4–7]. Recently, Silberman et al. demonstrated that LV diastolic dysfunction was associated with cardiac oxidation and reduced NO production [8].

Nitric oxide (NO), an endothelium-derived relaxing factor [9], is an important regulator of vascular tone and blood pressure and is synthesized by endothelial NO synthase 6 (eNOS6) [10]. Blocking eNOS with pharmacological inhibitors causes significant peripheral vasoconstriction and elevated blood pressure [11]. Tetrahydrobiopterin (BH4) is a cofactor of nitric oxide synthase (NOS). Nitric oxide (NO) bioavailability is reduced during the early stages of hypertension [12–14].

The role of myocardial apoptosis has been recognized in abnormal cardiac function [15]. Cardiac oxidation has been linked to progression of left ventricular hypertrophy and diastolic dysfunction [16]. The phosphatidylinositol 3-kinase (PI3K) signaling pathway is an important regulatory pathway implicated in the regulation of cell proliferation, angiogenesis and apoptosis [17,18].

* Corresponding author. Department of Cardiology, Lanzhou University Second Hospital, No. 82 Cuiyingmen, Lanzhou, Gansu 730030, PR China.
E-mail address: yujing2304@126.com (J. Yu).
[1] These authors contributed equally to this work.

http://dx.doi.org/10.1016/j.bbrc.2015.06.051

The present study aimed to confirm whether BH4 supplement up-regulates phosphorylated Akt levels and reverses cardiac hypertrophy and diastolic dysfunction in spontaneously hypertensive rats.

2. Methods

2.1. Animals

Ten-week-old male spontaneously hypertensive rats (SHR) and age-matched normotensive control Wistar-Kyoto (WKY) rats were obtained from Vital River Laboratory Animal Technology Co., Ltd (Beijing, China). Rats were housed in a 22 ± 2 °C room with a 12:12-h light/dark cycle (lights on at 07:00) and access to food and tap water *ad libitum*.

All experiments were conducted in accordance with the National Institutes of Health's Guide for the Care and Use of Laboratory Animals (National Academy Press, revised 1996). All experiments were performed under sodium pentobarbital anesthesia, and all efforts were made to minimize suffering.

2.2. Drug preparation

The rats were divided into five groups. WKY + BH4 (n = 16) and SHR + BH4 (n = 16) groups received BH4 200 mg/kg/d (Schircks Laboratories, Jona, Switzerland) by gavage for four weeks. WKY and SHR control (n = 16) groups received the same volume of sterile water as vehicle. The SHR + VAL group (n = 16) were received valsartan 30 mg/kg/day by gavage for four weeks.

2.3. Blood pressure measurement

After four weeks of treatment, we measured blood pressure using the standard tail-cuff method with a NIBP (non-invasive blood pressure) controller system (ADInstruments Pty Ltd., Castle Hill, NSW, Australia). A programmable sensor attached to a tail cuff was used to monitor tail pulse waves and measure blood pressure when the pulse waves became stable and rhythmic. Systolic blood pressure (SBP) and diastolic blood pressure (DBP) were averaged from five recordings.

2.4. Cardiac function and geometry assessed by echocardiography

Rats were anesthetized with sodium pentobarbital (50 mg/kg, i.p.), maintained at 37 °C, and studied by echocardiography (Vivid E9, GE, Pittsburgh, PA, USA; Probe i13L Intraoperative Linear Probe). M-mode images in the parasternal long-axis and LV short-axis views at the midpapillary level were taken. Measurements were averaged from five consecutive beats at end-expiration. Baseline images prior to treatment were acquired in all groups. After four weeks of treatment, the rats underwent echocardiography again.

2.5. Hemodynamic measurements

The Mikro-Tip® pressure volume (PV) catheter and MPVS Ultra™ system (Millar Instruments, Houston, TX, USA) were used to assess left ventricular function. Body temperature was maintained at 37 °C using a rectal thermometer probe and DC temperature control module (FHC, New Brunswick, ME, USA).

2.6. Measurement of cardiac BH4 and BH2

Hearts were rapidly excised and stored in liquid nitrogen. BH4, BH2 and biopterin were assessed with HPLC analysis (System GOLD, Beckman Coulter) using a differential oxidation method described previously [19,20] in homogenized heart samples. Data were analyzed using 32 Karat chromatography software (Beckman Coulter) and are expressed as nmol/mg tissue.

2.7. Measurement of NO generation

NO was measured in myocardium using the NO detection kit (Biovision, Mountain View, CA, USA) according to the manufacturer's instructions. The optical density values of the samples were measured at 540 nm on a spectrophotometer (Beckman DU530, Beckman Coulter, Brea, CA, USA).

2.8. Cardiac cyclic guanosine monophosphate, malondialdehyde and superoxide dismutase

Oxidative stress markers, including cyclic guanosine monophosphate (cGMP), superoxide dismutase (SOD) and malondialdehyde (MDA), were measured in left ventricular myocardium tissue using ELISA kits (Nanjing Jiancheng Bioengineering Institute, Nanjing, China) according to the manufacturer's instructions.

2.9. Quantitative real-time polymerase chain reaction (PCR)

Heart tissues were homogenized in liquid nitrogen, and total RNA was extracted using Trizol reagent (Invitrogen, Carlsbad, CA, USA). Quantitative RT-PCR was performed with an Applied Biosystems Prism 7900 Sequence Detection System (Applied Biosystems, Foster City, CA, USA). All primers for RT-PCR of β-myosin heavy chain (β-MHC), α-skeletal actin (α-SA) and transforming growth factor-beta 1 (TG-Fβ1) were designed by TaKaRa (TaKaRa, Dalian, China). The mRNA levels of β-MHC, α-SA and TGF-β1 were normalized to that of GAPDH.

2.10. Western blot analysis

The frozen left ventricular tissue tissues were homogenized in RIPA buffer containing protease inhibitors. The BCA Protein Array Kit (Pierce, Rockford, IL, USA) was used for protein quantification. The NC membrane was blocked with 5% skim milk in Tris-buffered saline with primary antibody against phosphatidylinositol 3-kinase

Table 1
Body weight, heart weight and blood pressure.

Parameters	WKY (n = 6)	WKY + BH_4 (n = 6)	SHR (n = 6)	SHR + BH_4 (n = 6)	SHR + VAL (n = 6)
BW (g)	305 ± 11	299 ± 15	269 ± 8*	244 ± 10*	247 ± 8*
HW (mg)	784 ± 32.2$^{\#}$	789 ± 27.9	821 ± 36.2*	755 ± 20.5$^{\#\#}$	747 ± 17.7$^{\#\#}$
HW/BW (mg/g)	2.57 ± 0.10$^{\#\#}$	2.65 ± 0.17$^{\#\#}$	3.30 ± 0.19**	3.11 ± 0.18$^{\#}$	3.02 ± 0.07$^{\#\#}$
SBP (mmHg)	122 ± 6.8$^{\#\#}$	120 ± 7.6$^{\#\#}$	209 ± 9.4**	198 ± 8.9$^{**\#}$	171 ± 9.0$^{**\#\#}$
DBP (mmHg)	76 ± 5.3$^{\#\#}$	75 ± 7.1$^{\#\#}$	113 ± 11.1**	102 ± 4.1$^{*\#\#}$	96 ± 9.2$^{*\#\#}$
HR (bpm)	388 ± 16	396 ± 16	398 ± 12	395 ± 20	398 ± 13

HW/BW: heart weight to body weight, SBP: systolic blood pressure, DBP: diastolic blood pressure, HR: heart rate. Values are presented as the mean ± S.D.
$^{*}P < 0.05$ and $^{**}P < 0.01$ vs. WKY, $^{\#}P < 0.05$ and $^{\#\#}P < 0.01$ vs. SHR.

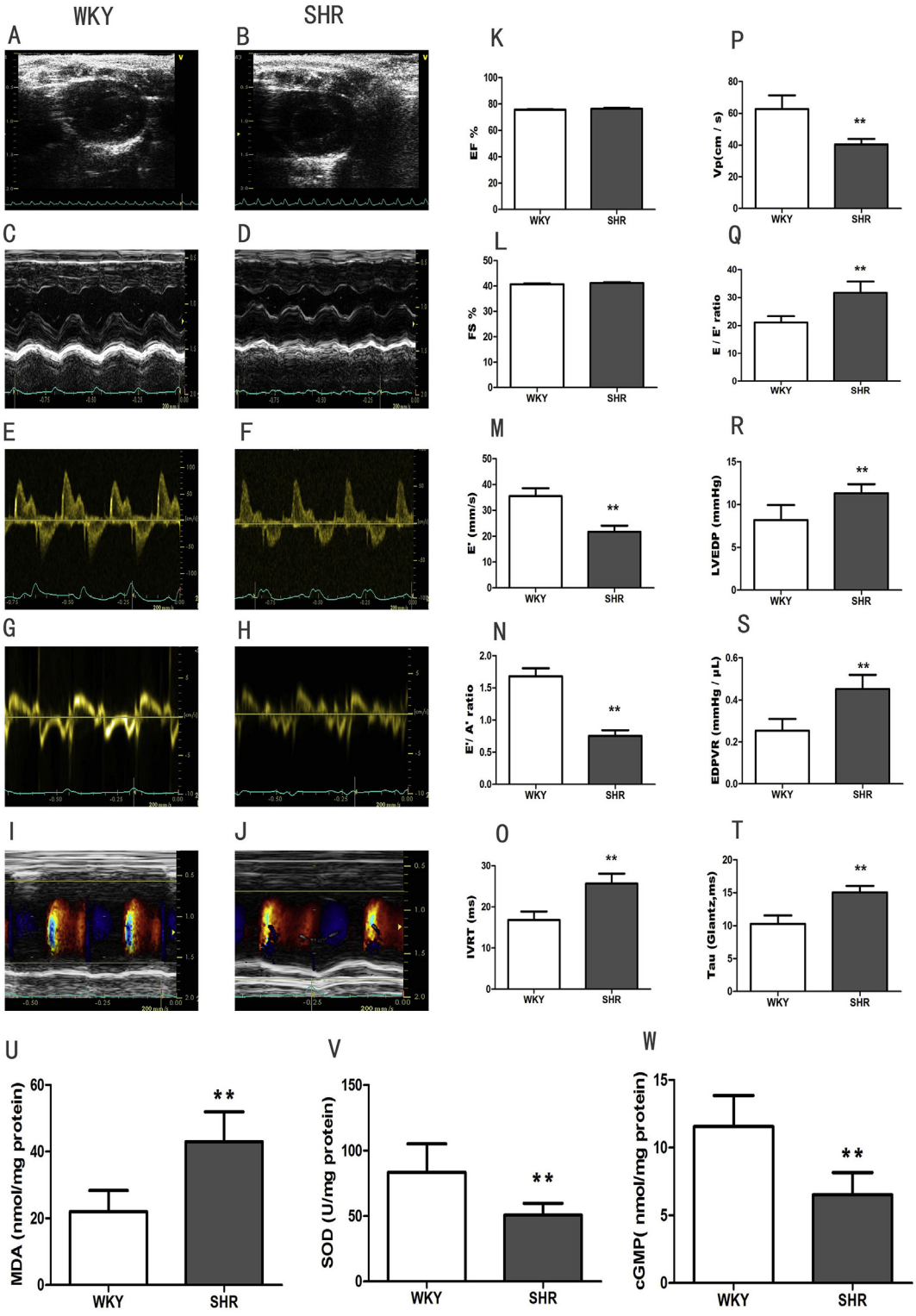

Fig. 1. Hypertrophy and diastolic dysfunction in hypertensive rats was associated with cardiac oxidation. A and B: Echocardiographic assessment by B-type echo at a mid-LV short-axis view. C and D: Echocardiographic assessment with M-mode. E and F: Transmitral flow Doppler. G and H: Mitral annulus tissue Doppler. I and J: LV inflow propagation velocity (Vp) with M-mode. Twelve-week-old SHR exhibited greater hypertrophy and worsening diastolic dysfunction. K–Q: EF, ejection fraction; FS, fractional shortening; E′, mitral annulus longitudinal velocity tissue Doppler early filling; A′, mitral annulus longitudinal velocity tissue Doppler late filling; IVRT, isovolumetric relaxation time; and Vp, LV inflow propagation velocity. R, S and T: Invasive hemodynamic assessment of LV diastolic dysfunction. LVEDP: LV end-diastolic pressure; EDPVR, end-diastolic pressure–volume relationship; and Tau (Glantz), time constants for isovolumic relaxation. n = 6 per group for echocardiographic and hemodynamic assessments. U–W: Cardiac oxidative stress in SHR and WKY, n = 12 per group. cGMP: cyclic guanosine monophosphate, SOD: superoxide dismutase, MDA: malondialdehyde. Data are presented as the mean ± SD. $^{*}P < 0.05$ and $^{**}P < 0.01$ vs. WKY, $^{\#}P < 0.05$ and $^{\#\#}P < 0.01$ vs. SHR.

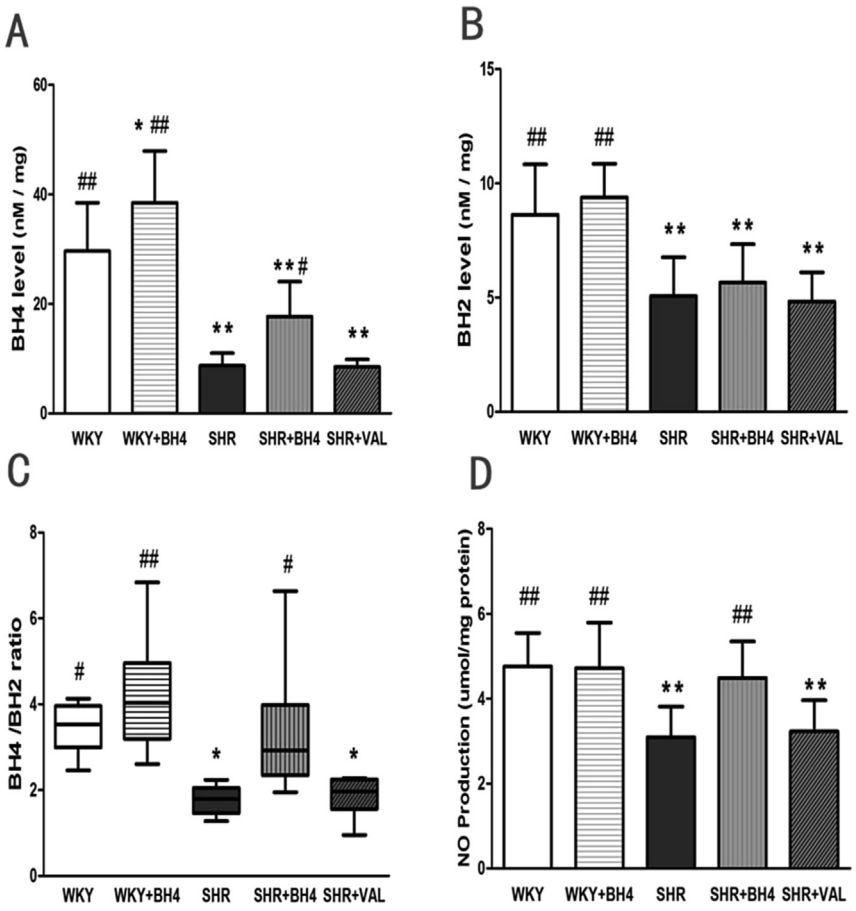

Fig. 2. Less cardiac BH4 and reduced NO in hypertensive rats with diastolic dysfunction. A–C: Cardiac BH4 and BH2 levels and BH4/BH2 ratio. D: Cardiac NO production. Data are presented as the mean ± SD.$^{*}P < 0.05$ and $^{**}P < 0.01$ vs. WKY, $^{\#}P < 0.05$ and $^{\#\#}P < 0.01$ vs. SHR.

(PI3K 110 kDa, 1:1000 diluted, Cell Signaling Inc.), total Akt (60 kDa, 1:1000 diluted, Cell Signaling Inc.) and phosphorylated Akt-Ser473 (60 kDa, 1:1000 diluted, Cell Signaling Inc.), Akt-Thr308 (60 kDa, 1:1000 diluted, Cell Signaling Inc.). Secondary antibody (1:2000 diluted) was incubated at room temperature for 1 h and then washed three times for 15 min. GAPDH (37 kDa, 1:5000, Santa Cruz) was used to control for protein loading. The target proteins were detected with ECL plus detection reagents (Amersham, Pittsburgh, PA, USA). Immunoreactive bands were visualized by enhanced chemiluminescence (Pierce, Rockford, IL). Protein expression was assessed by densitometric analysis using Image J software (NIH).

2.11. Immunohistochemistry

Fixed heart tissues were embedded in paraffin, and cardiac tissue slices were obtained from the mid-LV level. The frozen samples were cut into sections at a thickness of 6 μm. The sections were incubated for 3 h at room temperature with the antibody against vascular endothelial growth factor (VEGF), B cell lymphoma/lewkmia-2 (BCL-2) and Bcl-2-associated X protein (Bax) at a dilution of 1:100 and washed with PBS. The positive immunostained areas were quantified with an image analysis system (Olympus Optical, Tokyo, Japan).

2.12. Statistical analysis

All data are expressed as the mean ± S.D. Multiple groups were compared using one-way ANOVA, followed by the least significant difference test. A probability level of 0.05 or less was considered statistically significant. The statistical analysis was performed with the GraphPad Prism 5.0 statistical package program (GraphPad Software, Inc., La Jolla, CA, USA).

3. Results

3.1. Body weight, heart weight and blood pressure

After four weeks of treatment, body weight was measured in all groups. As shown in Table 1, body weight in the WKY rats was significantly heavier than the SHR rats, but no significant differences were observed between the SHR groups. Heart weight in the SHR group was significantly higher compared with the SHR + BH4 and SHR + VAL groups. Compared with the SHR group, BH4 and valsartan treatment induced a significantly lower heart weight to body weight ratio, but no significant differences in the ratio were observed between the BH4 and valsartan groups.

Both systolic and diastolic blood pressure was significantly higher in the SHR groups than the age-matched WKY rats. SBP was 209 ± 9.4 mmHg after vehicle administration in the SHR group but was significantly decreased to 171 ± 9.0 mmHg by valsartan and 198 ± 8.9 mmHg by BH4. DBP exhibited the same patterns as SBP in all groups (Table 1). Heart rate did not significantly change in each group.

3.2. Hypertensive rats exhibited hypertrophy, and diastolic dysfunction was associated with cardiac oxidation

Spontaneously hypertensive rats exhibited concentric hypertrophy and diastolic dysfunction with preserved systolic function,

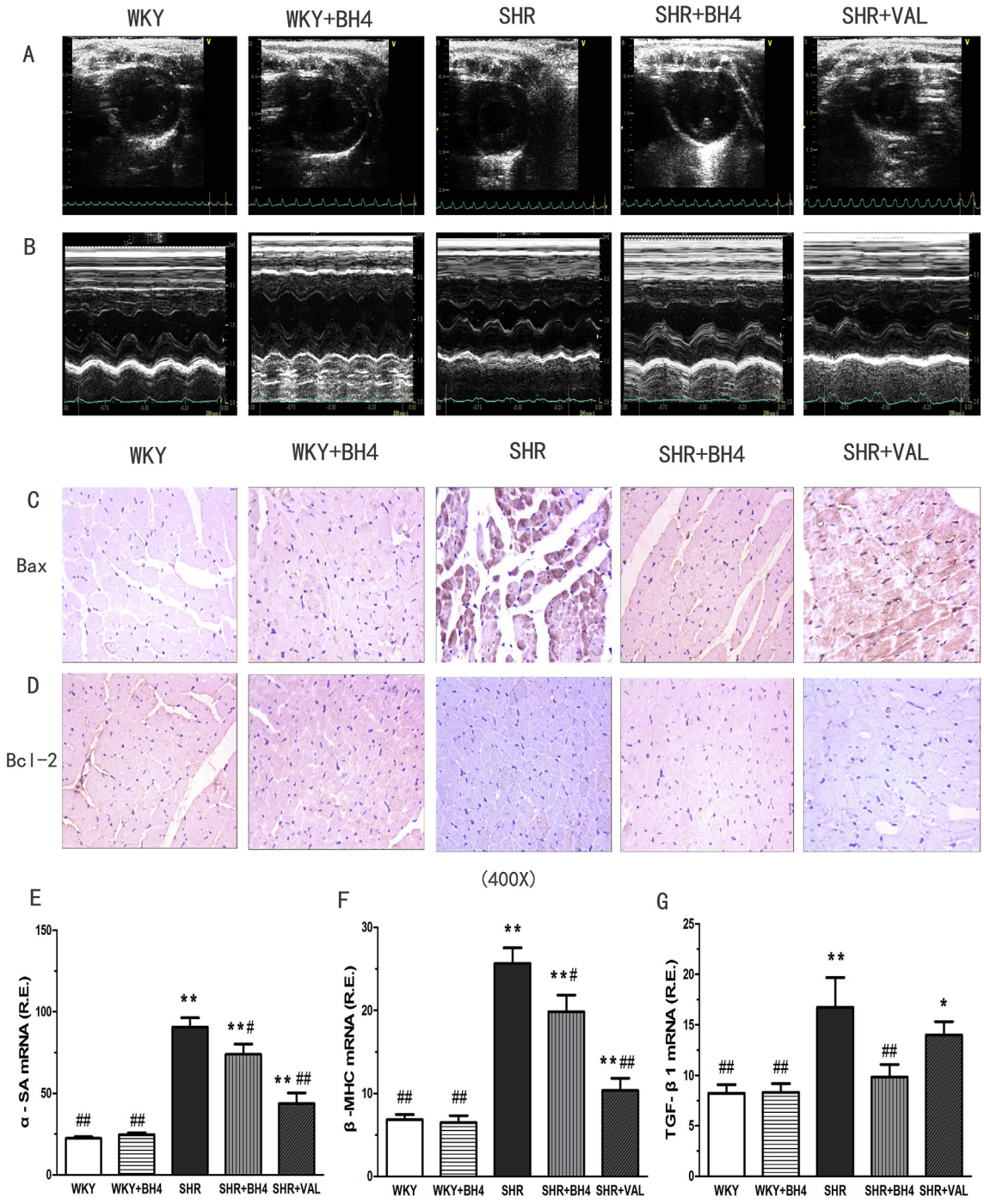

Fig. 3. BH4 reversed hypertrophic remodeling. A and B: Echocardiographic assessment with B-type and M-mode at a mid-LV short-axis view. C and D: Protein expression with immunohistochemical staining of Bax and Bcl-2 in myocardium (400×). E–G: TaqMan real-time polymerase chain reaction analysis of mRNA expression of genetic markers of myocardial hypertrophy, including α-SA (α-skeletal actin) and β-MHC (β-myosin heavy chain) mRNA levels. Data are presented as the mean ± SD. $^{*}P < 0.05$ and $^{**}P < 0.01$ vs. WKY, $^{\#}P < 0.05$ and $^{\#\#}P < 0.01$ vs. SHR.

as determined by transthoracic echocardiography (see Supplemental Data and Fig. 1E–J). The interventricular septal diastolic thickness (IVSd) and left ventricular posterior wall diastolic thickness (LVPWd) of SHR rats were significantly thicker than WKY rats in the LV M-Mode Protocol due to chronic high blood pressure (Fig. 1A–D). Comparing with age-matched WKY rats, the ejection fraction (EF%), fractional shortening (FS%), LVEDD and LVESD of SHR were not different (see Supplemental Data and Fig. 1K, L), suggesting that there was no morphologic or systolic functional damage in early age in spontaneously hypertensive rats.

SHR exhibited significant reductions in tissue mitral annulus early longitudinal (E′) velocities, as well as the ratio of early annulus to late annulus velocities (E′/A′). The ratio of early diastolic filling velocity to early diastolic mitral annulus velocity (E/E′) has reported to be best correlated with invasive hemodynamic measures of diastolic dysfunction [21]. SHR had a higher E/E′ compared with

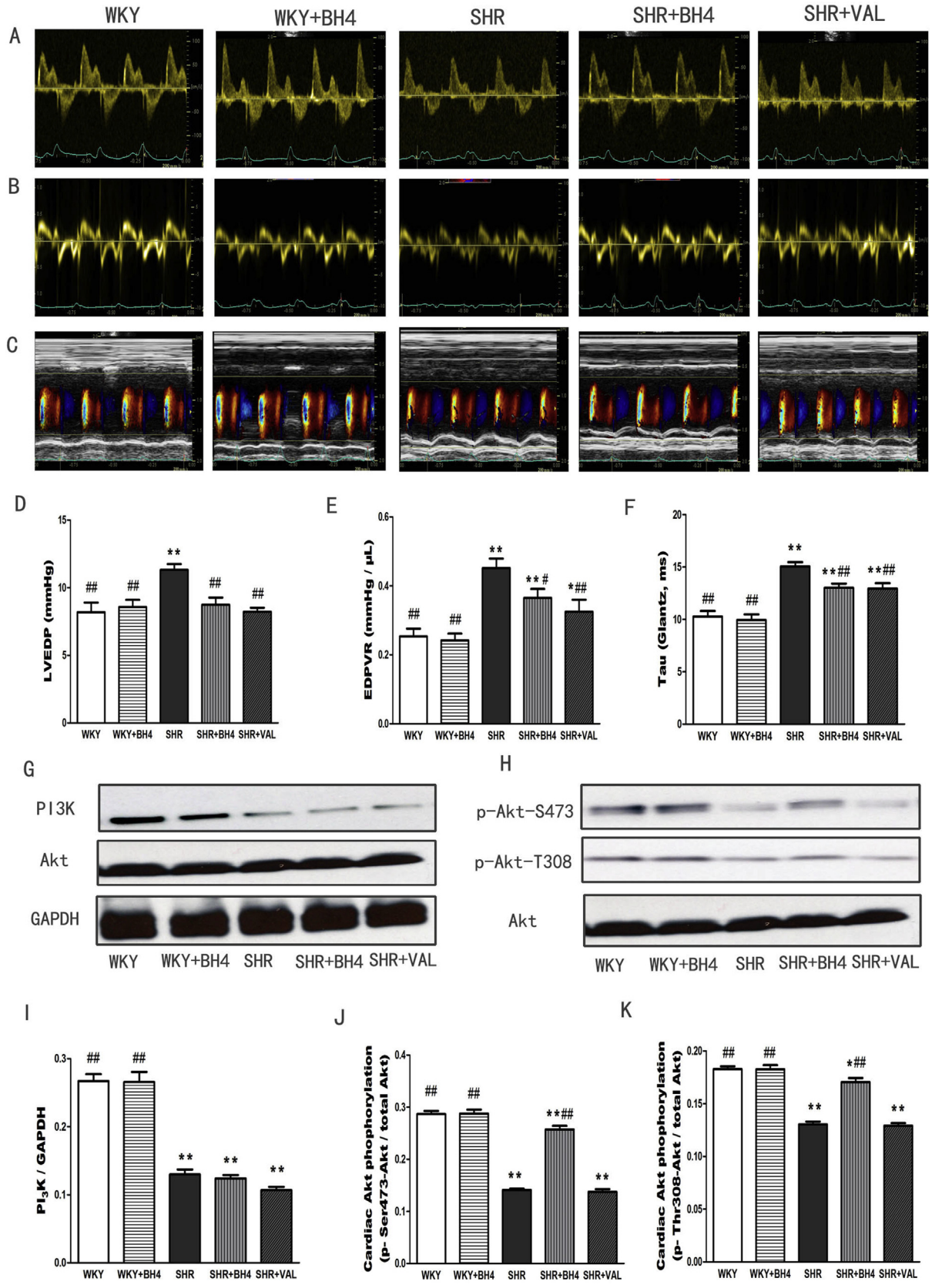

Fig. 4. BH4 restored LV diastolic function and up-regulated phosphorylated Akt levels. A–C: Cardiac diastolic function assessed by echocardiography (n = 6 per group). A: Mitral inflow patterns. B: Tissue Doppler mitral annular and late diastolic velocities. C: Color M-mode flow propagation with velocity (Vp). D–F: Cardiac diastolic function as assessed by hemodynamics. nD: LVEDP, LV end-diastolic pressure. E: EDPVR, end-diastolic pressure–volume relationship. F: Tau (Glantz), time constants for isovolumic relaxation. G–K: Western blot analysis for PI_3K and Akt expression and phosphorylation. G: PI_3K and Akt western blots. H: Phosphorylation of Akt at Ser473 and Thr308 western blots. I: Ratio of PI_3K to GAPDH. J: Ratio of phosphorylated S473-Akt to total Akt. K Ratio of phosphorylated T308-Akt to total Akt. Data are presented as the mean ± SD.$^{*}P < 0.05$ and $^{**}P < 0.01$ vs. WKY, $^{\#}P < 0.05$ and $^{\#\#}P < 0.01$ vs. SHR.

WKY rats, and LV isovolumetric relaxation time (IVRT) was significantly increased. SHR was also significantly reduced in LV inflow propagation velocity (Vp) compared with WKY controls (see Supplemental Data and Fig. 1M–Q).

Invasive hemodynamic evaluation confirmed the echocardiographic findings. As expected, LV end systolic pressure were elevated in the SHR and compared with WKY rats. Spontaneously hypertensive rats had prolonged time constants for isovolumic relaxation compared with WKY controls (Tau, Glanz: 10.27 ± 1.29 versus 15.05 ± 0.99, $P < 0.05$; see Supplemental Data and Fig. 1T). SHR had a steeper end-diastolic pressure–volume relation (EDPVR, 10.27 ± 1.29 versus 15.05 ± 0.99, $P < 0.05$; see Supplemental Data and Fig. 1S) compared with WKY rats. LV systolic function was preserved in SHR compared with WKY rats, as indicated by invasive indexes, including the peak rate of pressure increase (dP/dtmax) and slope of the end-systolic pressure–volume relation (ESPVR).

Concentric remodeling of the SHR LV resulted in diastolic dysfunction, as assessed by invasive hemodynamic and transthoracic echocardiography. Zhong et al. reported that Ang II induced myocardial hypertrophy diastolic dysfunction and oxidative stress [22]. Silberman et al. also found that diastolic dysfunction was associated with cardiac oxidation [8]. Consistent with these results, we observed that the activities of superoxide anion radical scavenging enzymes SOD and levels of cGMP in the SHR myocardium were dramatically reduced, whereas the concentration of MDA was increased compared with WKY rat (Fig. 1U–W).

3.3. Cardiac oxidation was related to less cardiac BH4 and reduced NO

After four weeks of treatment, we assayed cardiac BH4 content and its degradation 7,8-dihydrobiopterin (BH2) by HPLC. As shown in Fig. 2A–C, BH4 levels were remarkably lower in SHR hearts with diastolic dysfunction (8.70 ± 2.28 versus 29.63 ± 8.86; $P < 0.001$). Cardiac BH2 content in SHR was also decreased compared with WKY rats without diastolic dysfunction (5.06 ± 1.70 versus 8.63 ± 2.20; $P = 0.001$). The ratio of BH4 to BH2 was significantly lower in SHR compared with WKY control (1.77 ± 0.34 versus 3.45 ± 0.59; $P = 0.011$).

Tetrahydrobiopterin (BH4) is a crucial cofactor for NO production from nitric oxide synthase (NOS). The ratio of reduced BH4 to oxidized proteins reflects the availability of BH4 for NOS. We found that NO production was reduced in SHR compared with WKY control (3.09 ± 0.72 versus 4.76 ± 0.79; $P < 0.001$, Fig. 2D).

Administration BH4 to SHR increased cardiac BH4 ($P = 0.026$, SHR versus SHR + BH4) and the ratio of BH4 to BH2 ($P = 0.026$, SHR versus SHR + BH4).

As shown in Fig. 2D, total nitrite/nitrate content, which is representative NO production of in SHR myocardium, was elevated after BH4 administration ($P = 0.001$, SHR versus SHR + BH4). Thus, reduced NO production contributed to cardiac oxidation and resulted in LV diastolic dysfunction in hypertensive rats.

Previous reports and our data indicated that hypertension caused increased oxidative stress in the LV myocardium. Further, diastolic dysfunction was associated with cardiac oxidation. These data (Fig. 2) suggested that BH4 depletion limited NO production in SHR with diastolic dysfunction.

3.4. BH4 reversed hypertrophic remodeling and comparisons with valsartan

As previously described, spontaneously hypertensive rats exhibited concentric hypertrophic remodeling. Two-dimensional echocardiographic (Fig. 3A) and M-mode images (Fig. 3B) revealed that hypertrophic LV wall thickness in SHR was attenuated by treatment with both BH4 and valsartan. The anti-hypertrophic remodeling effect of valsartan was stronger than BH4 (Supplemental Data).

The expression of hypertrophy genes α-SA (α-skeletal actin; Fig. 3E) and β-MHC (β-myosin heavy chain; Fig. 3F) was significantly increased in hypertensive rats and decreased after treatment with BH4 and valsartan. The changes of α-SA and β-MHC mRNA expression were similar with morphometric results assessed by echocardiography. The twelve-week-old spontaneously hypertensive rats exhibited a mild degree of myocardial fibrosis. mRNA expression of TGF-β1 (transforming growth factor-β1), a fibrosis-associated gene, was increased in SHR and inhibited by BH4 ($P = 0.006$, SHR versus SHR + BH4; Fig. 3G). Valsartan had no effect on myocardial TGF-β1expression ($P = 0.154$, SHR versus SHR + VAL). These results demonstrated that BH4 and valsartan can suppress pathological hypertrophy and that BH4 can inhibit myocardial fibrosis.

The protein expression of Bax and Bcl-2 in the myocardium of WKY rats and SHR is shown in Fig. 3C and D. Compared with WKY rats, immunohistochemical analysis indicated that Bax expression was significantly increased and Bcl-2 expression was decreased in SHR myocardium. However, after BH4 administration, Bax and Bcl-2 expression was down-regulated and up-regulated, respectively ($P < 0.01$), in the WKY group compared with the SHR group. Bax and Bcl-2 expression in SHR + VAL rat myocardium was not significantly changed compared with the SHR group. These results suggested that the mechanisms by which BH4 and valsartan reversed hypertrophic remodeling were different.

3.5. BH4 restored LV diastolic function and up-regulated phosphorylated Akt levels

We assessed the expression of PI3K, Akt and phosphorylated Akt levels in cardiac tissues using Western blotting. PI3K expression was significantly decreased in SHR compared with WKY rats (Fig. 4G and I). Akt expression was not different between SHR and WKY rats, whereas BH4 and valsartan administration had no effect on this protein. Compared with control WKY rats, the amount of cardiac phosphorylated Akt for serine 473 and threonine 308 was decreased in SHR with diastolic dysfunction (Fig. 4G, H). We assessed whether BH4 and valsartan affected phosphorylated Akt levels. BH4 increased the serine and threonine Akt phosphorylation, whereas valsartan decreased serine and threonine Akt phosphorylation (Fig. 4). Phosphoinositide 3-kinase (PI3K) and its downstream effector Akt are involved in regulating cardiac cell proliferation, survival, and apoptosis. Phosphorylated Akt has been reported to preserve cardiac function [17].

In this present study, the invasive hemodynamic assessment (Fig. 4D–F) and echocardiographic measurement (Fig. 4A–C) confirmed that both BH4 and valsartan can restore the LV diastolic function in SHR. SHR with diastolic dysfunction were characterized by reduced E′/A′ ratio, augmented E/E′ ratio, lower Vp velocity, elevated LV end-diastolic pressure, increased end-diastolic pressure volume slope, and increased LV relaxation time constant, which were all rescued by treatment with BH4 and valsartan (Fig. 4A–F). However, our results demonstrated that only BH4 can up-regulate phosphorylated Akt levels in serine 473 and threonine 308 (Fig. 4H, J and K). These results showed that exogenous BH4 can improve diastolic dysfunction in SHR, partly by promoting the PI3K/phosphorylated Akt signaling pathway.

4. Discussion

Diastolic dysfunction associated with hypertension leads to cardiac hypertrophic remodeling. Our study demonstrated that

exogenous chronic BH4 and valsartan treatment for one month can sustain blood pressure reduction and reverse concentric hypertrophic remodeling in SHR. Compared with valsartan administration, the reduction of blood pressure and cardiac hypertrophy for BH4 was comparatively modest and did not induce changes in heart rate. Exogenous BH4 has been reported to ameliorate pre-existing advanced left ventricular hypertrophy/fibrosis and was more effective than tempol, a less targeted anti-oxidant [23].

Many studies have highlighted the important role of myocyte NOS uncoupling in cardiac hypertrophic remodeling and diastolic dysfunction [8,23–26]. This morphometric and functional defect was associated with reduced NOS NO production and cardiac oxidation. Our results were consistent with those of Moens and Takimoto et al. [23]. BH4 reversed chronic hypertrophic remodeling and diastolic dysfunction. In contrast, cGMP, SOD and NO production were increased in the SHR + BH4 group compared with control SHR. Thus, chronic supplementation of exogenous BH4 can alleviate oxidative stress in SHR hearts.

Cardiac hypertrophy has been reported to develop by 4 weeks of age in SHR, and diastolic dysfunction is evident at 2–3 months [27]. The present findings demonstrated that chronic persistent high blood pressure induced morphological, functional and molecular alterations in SHR hearts, leading to cardiac hypertrophy with decreased myocardial compliance and inhibited diastolic function. Both BH4 and valsartan decreased mRNA expression of α-SA and β-MHC, lowered blood pressure and improved diastolic function. We also found that BH4 can inhibit the expression of l TGF-β1 and myocardial fibrosis. Angiotensin receptor blockers can blunt cardiac hypertrophy and diastolic dysfunction progression [28], but BH4 reversed these heart abnormalities supported by detrimental anoxygenic properties.

In this present study, LV diastolic dysfunction was accompanied by impaired activation of the PI3K/p-Akt signaling pathway. Akt is an important regulator of the downstream effects of PI3K, which mediates cell proliferation and survival. Recent studies have shown that PI3K/Akt signaling pathway activation prevented cardiomyocyte apoptosis and protected the myocardium from ischemic injury.

Previous studies have shown that in the adult heart, short-term Akt activation can induce cardiac hypertrophy and does not affect contractile function, which can enhance myocardial vascular endothelial growth factor secretion and promote coronary angiogenesis [29]. Akt increases the expression of sarcoplasmic reticulum calcium ATPase 2 (SERCA2) and improves cardiac function in mice [30,31]. Akt can promote cell survival and improve cardiac function through phosphorylation of multiple downstream targets due to its pro-survival effects [32–34].

The present study showed that the protective effects of BH4 were associated with the PI3K/p-Akt pathway. Exogenous BH4 can activate phosphorylated Akt expression for serine 473 and threonine 308 in SHR with cardiac hypertrophy and diastolic dysfunction. BH4 regulated the Bcl-2 family and reduced cardiomyocyte apoptosis by activation of phosphorylated Akt, whereas valsartan can reverse cardiac hypertrophy and improve diastolic function without affecting phosphorylated Akt expression. The PI3K/Akt signaling pathway regulates growth and survival; activating Akt phosphorylation plays a crucial role in this pathway, and inhibition of the phosphorylation of Akt results in apoptosis [35–37].

Our study has several limitations. Although we found that BH4 prevents the progression of hypertrophic remodeling and cardiac function, this action is not necessarily the primary mechanism.

In summary, less BH4 and reduced NO increases myocardial hypertrophy and cardiac oxidative stress, which exacerbates diastolic dysfunction. This cardiac dysfunction and pathological remodeling can be reversed to some degree by BH4 treatment. BH4 may be a potent therapy for hypertension with diastolic dysfunction.

Authors' contribution

Peng Chang and Qiongying Wang contributed equally to this work.

Conflict of interests

The authors declare that there is no conflict of interest.

Acknowledgments

This study was supported by the National Natural Science Foundation of China (NSFC-81270332), Gansu Province Natural Science Foundation (1104FKCA150, 1205TCYA042), and the Gansu Administration of Traditional Medicine Foundation (GZK-2010-Z-1).

Appendix A. Supplementary data

Supplementary data related to this article can be found at http://dx.doi.org/10.1016/j.bbrc.2015.06.051.

Transparency document

Transparency document related to this article can be found online at http://dx.doi.org/10.1016/j.bbrc.2015.06.051.

References

[1] P.A. James, S. Oparil, B.L. Carter, et al., 2014 evidence-based guideline for the management of high blood pressure in adults: report from the panel members appointed to the Eighth Joint National Committee (JNC 8), J. Am. Med. Assoc. 311 (2014) 507–520.
[2] A.V. Chobanian, G.L. Bakris, H.R. Black, et al., The seventh report of the joint national committee on prevention, detection, evaluation, and treatment of high blood pressure: the JNC 7 report, J. Am. Med. Assoc. 289 (2003) 2560–2572.
[3] O.H. Cingolani, X.P. Yang, M.A. Cavasin, et al., Increased systolic performance with diastolic dysfunction in adult spontaneously hypertensive rats, Hypertens 41 (2003) 249–254.
[4] B.A. Borlaug, W.J. Paulus, Heart failure with preserved ejection fraction: pathophysiology, diagnosis, and treatment, Eur. Heart J. 32 (2011) 670–679.
[5] T.E. Owan, D.O. Hodge, R.M. Herges, et al., Trends in prevalence and outcome of heart failure with preserved ejection fraction, N. Engl. J. Med. 355 (2006) 251–259.
[6] D.D. Schocken, E.J. Benjamin, G.C. Fonarow, et al., Prevention of heart failure: a scientific statement from the American Heart Association councils on epidemiology and prevention, clinical cardiology, cardiovascular nursing, and high blood pressure Research; Quality of Care and outcomes Research Interdisciplinary Working Group; and functional genomics and Translational Biology Interdisciplinary Working Group, Circulation 117 (2008) 2544–2565.
[7] M. Ouzounian, D.S. Lee, P.P. Liu, Diastolic heart failure: mechanisms and controversies, Nat. Clin. Pract. Cardiovasc. Med. 5 (2008) 375–386.
[8] G.A. Silberman, T.H. Fan, H. Liu, et al., Uncoupled cardiac nitric oxide synthase mediates diastolic dysfunction, Circulation 121 (2010) 519–528.
[9] M.T. Ziolo, M.J. Kohr, H. Wang, Nitric oxide signaling and the regulation of myocardial function, J. Mol. Cell. Cardiol. 45 (2008) 625–632.
[10] R.F. Furchgott, J.V. Zawadzki, The obligatory role of endothelial cells in the relaxation of arterial smooth muscle by acetylcholine, Nature 288 (1980) 373–376.
[11] L.J. Ignarro, G.M. Buga, K.S. Wood, et al., Endothelium-derived relaxing factor produced and released from artery and vein is nitric oxide, Proc. Nat. Acad. Sci. 84 (1987) 9265–9269.
[12] E.R. Werner, N. Blau, B. Thöny, Tetrahydrobiopterin: biochemistry and pathophysiology, Biochem. J. 438 (2011) 397–414.
[13] M.A. Tayeh, M.A. Marletta, Macrophage oxidation of L-arginine to nitric oxide, nitrite, and nitrate. Tetrahydrobiopterin is required as a cofactor, J. Biol. Chem. 264 (1989) 19654–19658.
[14] N.S. Kwon, C.F. Nathan, D.J. Stuehr, Reduced biopterin as a cofactor in the generation of nitrogen oxides by murine macrophages, J. Biol. Chem. 264 (1989) 20496–20501.
[15] X. Wang, The expanding role of mitochondria in apoptosis, Genes. Dev. 15 (2001) 2922–2933.

[16] E. Takimoto, H.C. Champion, M. Li, et al., Oxidant stress from nitric oxide synthase-3 uncoupling stimulates cardiac pathologic remodeling from chronic pressure load, J. Clin. Invest. 115 (2005) 1221–1231.
[17] T. Ha, F. Hua, X. Liu, J. Ma, J.R. McMullen, T. Shioi, S. Izumo, J. Kelley, X. Gao, W. Browder, D.L. Williams, R.L. Kao, C. Li, Lipopolysaccharide-induced myocardial protection against ischaemia/reperfusion injury is mediated through a PI3K/Akt-dependent mechanism, Cardiovasc. Res. 78 (2008) 546–553.
[18] S. Dimmeler, I. Fleming, B. Fisslthaler, et al., Activation of nitric oxide synthase in endothelial cells by Akt-dependent phosphorylation, Nature 399 (1999) 601–605.
[19] B.M. Boulden, J.D. Widder, J.C. Allen, D.A. Smith, R.N. Al-Baldawi, D.G. Harrison, S.I. Dikalov, H. Jo, S.C. Dudley, Early determinants of H_2O_2-induced endothelial dysfunction, Free Radic. Biol. Med. 41 (2006) 810–817.
[20] U. Landmesser, S. Dikalov, S.R. Price, L. McCann, T. Fukai, S.M. Holland, W.E. Mitch, D.G. Harrison, Oxidation of tetrahydrobiopterin leads to uncoupling of endothelial cell nitric oxide synthase in hypertension, J. Clin. Invest. 111 (2003) 1201–1209.
[21] A.M. Faria, A. Papadimitriou, K.C. Silva, et al., Uncoupling endothelial nitric oxide synthase is ameliorated by green tea in experimental diabetes by re-establishing tetrahydrobiopterin levels, Diabetes 61 (2012) 1838–1847.
[22] J. Zhong, R. Basu, D. Guo, et al., Angiotensin-converting enzyme 2 suppresses pathological hypertrophy, myocardial fibrosis, and cardiac dysfunction, Circulation 122 (2010) 717–728.
[23] A.L. Moens, E. Takimoto, C.G. Tocchetti, et al., Reversal of cardiac hypertrophy and fibrosis from pressure overload by tetrahydrobiopterin: efficacy of recoupling nitric oxide synthase as a therapeutic strategy, Circulation 117 (2008) 2626–2636.
[24] E.M. Jeong, M.M. Monasky, L. Gu, et al., Tetrahydrobiopterin improves diastolic dysfunction by reversing changes in myofilament properties, J. Mol. Cell. Cardiol. 56 (2013) 44–54.
[25] I. Fleming, J. Bauersachs, R. Busse, Calcium-dependent and calcium-independent activation of the endothelial NO synthase, J. Vasc. Res. 34 (1997) 165–174.
[26] P.J. Andrew, B. Mayer, Enzymatic function of nitric oxide synthases, Cardiovasc. Res. 43 (1999) 521–531.
[27] M. Kokubo, A. Uemura, T. Matsubara, et al., Noninvasive evaluation of the time course of change in cardiac function in spontaneously hypertensive rats by echocardiography, Hypertens. Res. 28 (2005) 601–609.
[28] L.H. Opie, P.J. Commerford, B.J. Gersh, et al., Controversies in ventricular remodelling, Lancet 367 (2006) 356–367.
[29] I. Shiojima, K. Sato, Y. Izumiya, et al., Disruption of coordinated cardiac hypertrophy and angiogenesis contributes to the transition to heart failure, J. Clin. Invest. 115 (2005) 2108–2118.
[30] Y. Izumiya, I. Shiojima, K. Sato, et al., Vascular endothelial growth factor blockade promotes the transition from compensatory cardiac hypertrophy to failure in response to pressure overload, Hypertens 47 (2006) 887–893.
[31] Y.K. Kim, S.J. Kim, A. Yatani, et al., Mechanism of enhanced cardiac function in mice with hypertrophy induced by overexpressed Akt, J. Biol. Chem. 278 (2003) 47622–47628.
[32] S.J. Kim, M. Abdellatif, S. Koul, et al., Chronic treatment with insulin-like growth factor I enhances myocyte contraction by upregulation of Akt-SERCA2a signaling pathway, Am. J. Physiol. Heart Circ. Physiol. 295 (2008) H130–H135.
[33] Y. Hayakawa, M. Chandra, W. Miao, et al., Inhibition of cardiac myocyte apoptosis improves cardiac function and abolishes mortality in the peripartum cardiomyopathy of Galpha(q) transgenic mice, Circulation 108 (2003) 3036–3041.
[34] I. Shiraishi, J. Melendez, Y. Ahn, et al., Nuclear targeting of Akt enhances kinase activity and survival of cardiomyocytes, Circ. Res. 94 (2004) 884–891.
[35] T. Matsui, J. Tao, F. del Monte, et al., Akt activation preserves cardiac function and prevents injury after transient cardiac ischemia in vivo, Circulation 104 (2001) 330–335.
[36] Y. Li, Y. Jiang, Y. Wan, et al., Medroxyprogestogen enhances apoptosis of SKOV-3 cells via inhibition of the PI3K/Akt signaling pathway, Biomed. Res. 27 (2013) 43–50.
[37] A.J. Barber, M. Nakamura, E.B. Wolpert, et al., Insulin rescues retinal neurons from apoptosis by a phosphatidylinositol 3-kinase/Akt-mediated mechanism that reduces the activation of caspase-3, J. Biol. Chem. 276 (2001) 32814–32821.