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

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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].

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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.