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Original article

Tetrahydrobiopterin improves diastolic dysfunction by reversing changes in myofilament properties

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ABSTRACT

Despite the increasing prevalence of heart failure with preserved left ventricular function, there are no specific treatments, partially because the mechanism of impaired relaxation is incompletely understood. Evidence indicates that cardiac relaxation may depend on nitric oxide (NO), generated by NO synthase (NOS) requiring the co-factor tetrahydrobiopterin (BH_4). Recently, we reported that hypertension-induced diastolic dysfunction was accompanied by cardiac BH_4 depletion, NOS uncoupling, a depression in myofilament cross-bridge kinetics, and S-glutathionylation of myosin binding protein C (MyBP-C). We hypothesized that the mechanism by which BH_4 ameliorates diastolic dysfunction is by preventing glutathionylation of MyBP-C and thus reversing changes of myofilament properties that occur during diastolic dysfunction. We used the deoxycorticosterone acetate (DOCA)-salt mouse model, which demonstrates mild hypertension, myocardial oxidative stress, and diastolic dysfunction. Mice were divided into two groups that received control diet and two groups that received BH_4 supplement for 7 days after developing diastolic dysfunction at post-operative day 11. Mice were assessed by echocardiography. Left ventricular papillary detergent-extracted fiber bundles were isolated for simultaneous determination of force and ATPase activity. Sarcomeric protein glutathionylation was assessed by immunoblotting. DOCA-salt mice exhibited diastolic dysfunction that was reversed after BH_4 treatment. Diastolic sarcomere length (DOCA-salt 1.70 ± 0.01 vs. DOCA-salt + BH_4 1.77 ± 0.01 μm, $P<0.001$) and relengthening (relaxation constant, τ, DOCA-salt 0.28 ± 0.02 vs. DOCA-salt + BH_4 0.08 ± 0.01, $P<0.001$) were also restored to control by BH_4 treatment. pCa_{50} for tension increased in DOCA-salt compared to sham but reverted to sham levels after BH_4 treatment. Maximum ATPase rate and tension cost (ΔATPase/ΔTension) decreased in DOCA-salt compared to sham, but increased after BH_4 treatment. Cardiac MyBP-C glutathionylation increased in DOCA-salt compared to sham, but decreased with BH_4 treatment. MyBP-C glutathionylation correlated with the presence of diastolic dysfunction. Our results suggest that by depressing S-glutathionylation of MyBP-C, BH_4 ameliorates diastolic dysfunction by reversing a decrease in cross-bridge turnover kinetics. These data provide evidence for modulation of cardiac relaxation by post-translational modification of myofilament proteins.

1. Introduction

Hypertension is the most common risk factor for diastolic dysfunction in humans, which can lead to heart failure with preserved ejection fraction [1]. This type of heart failure is increasing, and accounts for significant mortality and healthcare expenditures [1,2]. Current treatments for diastolic dysfunction are inadequate, partially because the mechanism of altered myocardial relaxation is incompletely understood [3]. Nitric oxide (NO) generated by NO synthase (NOS) is a critical modulator of cardiac relaxation [4], and NO bioavailability is regulated by tetrahydrobiopterin (BH_4) [5].

Under physiological conditions, NOS catalyzes the production of NO from L-arginine to modulate myofilament contractility through mechanisms that are not clear [6–9]. BH_4 depletion, leads to NOS uncoupling [5,10], the production of superoxide instead of NO, and diastolic dysfunction [5,11]. BH_4 supplementation reverses these effects. Recently, we have reported that diastolic dysfunction was characterized by altered myofilament properties and by S-glutathionylation of cardiac myosin binding protein-C (MyBP-C)[12]. S-glutathionylation

Abbreviations: A, mitral inflow late filling velocity in pulse-wave Doppler; BDM, 2,3-butanedione monoxime; BH_4, tetrahydrobiopterin; DOCA, deoxycorticosterone acetate; E, mitral inflow early filling velocity in pulse-wave Doppler; EF, ejection fraction; FS, fractional shortening; LV, left ventricle; MyBP-C, cardiac myosin binding protein-C; NEM, N-ethylmaleimide; NO, nitric oxide; NOS, nitric oxide synthase; PK, protein kinase; TDI, tissue Doppler imaging; cTnI, cardiac troponin I; TnT, troponin T.

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is an oxidative post-translational modification of protein cysteines by the addition of the anti-oxidant tripeptide glutathione [13–15]. We tested whether the improvement in diastolic dysfunction with BH_4 treatment correlated with changes in myofilament properties and in S-glutathionylation of cardiac MyBP-C.

We demonstrate that oral administration of BH_4 improves diastolic dysfunction, reverses the changes in actin-myosin cross-bridge cycling, and decreases S-glutathionylated MyBP-C. Our results support the hypothesis that oxidative post-translational modifications and associated modulation of myofilament properties is a molecular mechanism for diastolic dysfunction.

2. Methods

All protocols were in accordance with the guidelines of the Animal Care and Use Committee of the University of Illinois and comply with the laws of the United States of America.

2.1. Generation of DOCA-salt mouse model

Previously, we have shown that the DOCA-salt mouse model leads to mild hypertension, NOS uncoupling, myocardial oxidative stress, and diastolic dysfunction [10]. A gradual and mild elevation in blood pressure was induced by unilateral nephrectomy, subcutaneous implantation of a controlled release deoxycorticosterone acetate (DOCA) pellet (0.7 mg/d; Innovative Research of America, Sarasota, FL), and substituting drinking water with 1.05% saline. Control animals underwent a sham operation, had placebo pellet implantation, and received water without salt.

2.2. Administration of BH_4

Mice were divided into two groups which received a control diet (sham N = 7; DOCA-salt N = 10) and two groups which received a BH_4 supplemental diet of 5 mg BH_4/day (200 mg/kg/day, Research Diets Inc, New Brunswick, NJ; sham + BH_4 N = 8; DOCA-salt + BH_4

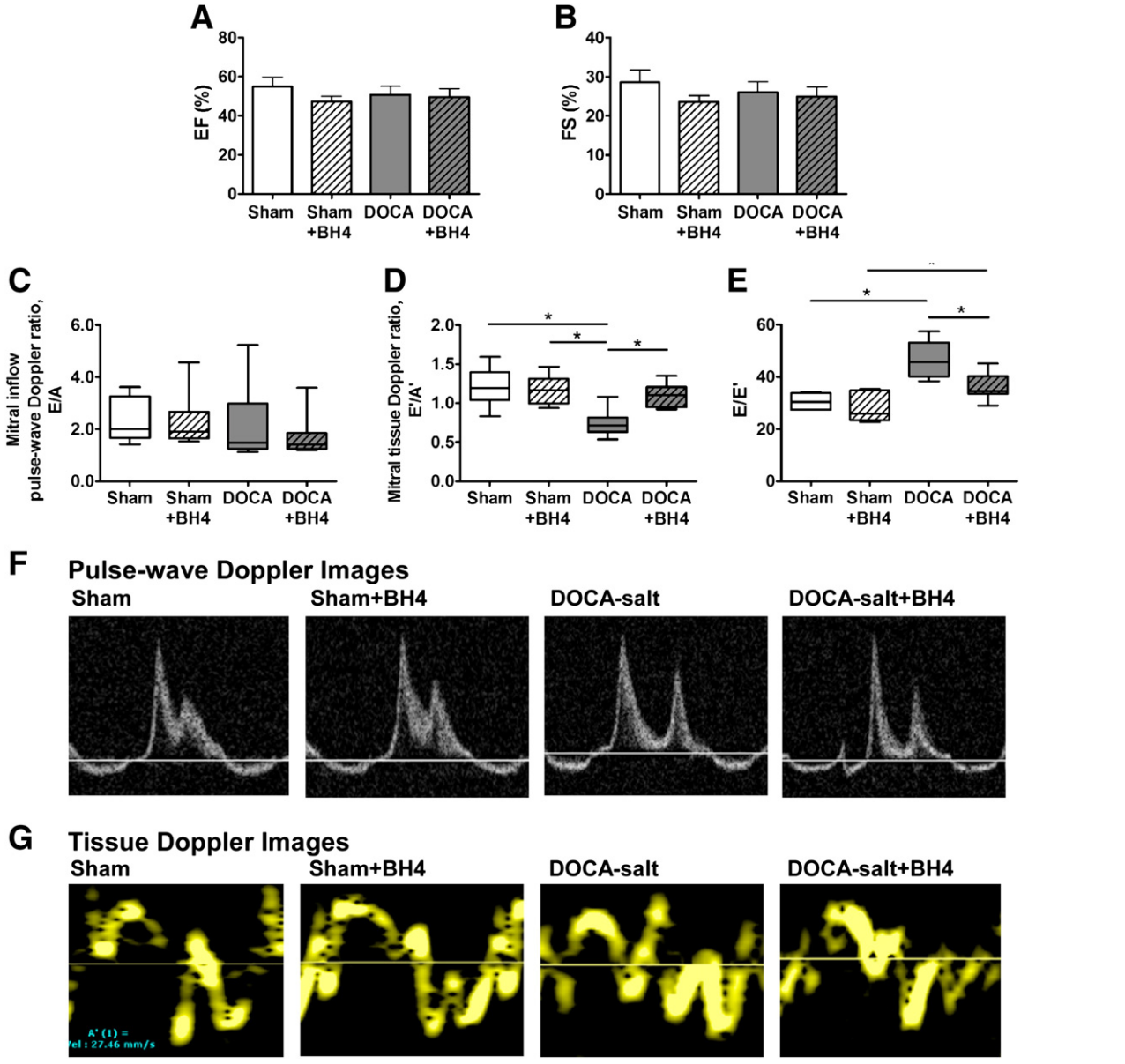

Fig. 1. Thoractic echocargiographic parameters in WT and DOCA-salt mice treated with or without BH_4. (A) Ejection fraction (%, EF) and (B) fractional shortening (%, FS) were determined in short axix M-mode view. (C) Mitral inflow pulse-wave Doppler ratio (E/A). (D) Mitral tissue doppler ratio, E′/A′. (E) E/E′. (F–G), Representative images from apical four chamber view of pulse-wave (F) and TDI (G). Data was represented mean ± SEM. N = 7–9 per group. Data were statistically analyzed using JMP statistical software by two-way ANOVA followed by Student's *t*-test. $^*P<0.05$.