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Visualizing oxidative stress-induced depression of cardiac vagal baroreflex by MRI/DTI in a mouse neurogenic hypertension model

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ABSTRACT

A clinical hallmark of hypertension is impairment of the cardiac vagal baroreflex, which maintains stable blood pressure and heart rate under physiological conditions. There is also evidence that oxidative stress in the brain is associated with neurogenic hypertension. We tested the hypothesis that an augmented superoxide level in the nucleus tractus solitarii (NTS), the terminal site of baroreceptor afferents, contributes to the depression of cardiac vagal baroreflex by disrupting the connectivity between the NTS and the nucleus ambiguus (NA), the origin of the vagus nerve, during neurogenic hypertension. An experimental model of neurogenic hypertension that employed intracerebroventricular infusion of angiotensin II in male adult C57BL/6 mice was used. Based on tractographic evaluations using magnetic resonance imaging/diffusion tensor imaging of the medulla oblongata in the brain stem, we found that the connectivity between the NTS and NA was disrupted in neurogenic hypertension, concurrent with impairment of the cardiac vagal baroreflex as detected by radiotelemetry. We further found that the disrupted NTS–NA connectivity was reversible, and was related to oxidative stress induced by augmented levels of NADPH oxidase-generated superoxide in the NTS. We conclude that depression of the cardiac vagal baroreflex induced by oxidative stress in the NTS in the context of neurogenic hypertension may be manifested in the form of dynamic alterations in the connectivity between the NTS and NA.

Introduction

The ability to maintain a stable blood pressure and heart rate is essential for survival in humans and animals, and the cardiac vagal baroreflex is one of the most fundamental feedback mechanisms in this homeostatic process. Augmented afferent discharges from arterial baroreceptors upon the detection of an increase in blood pressure will induce the nucleus tractus solitarii (NTS) to normalize the blood pressure by reducing heart rate via excitation of the nucleus ambiguus (NA), which sends inhibitory signals to the heart by way of the vagus nerve (Dampney, 1994; Spyer, 1994). Thus, a clinical hallmark of hypertension is impairment of the cardiac vagal baroreflex (Chaves et al., 2000; Luoh and Chan, 1998; Wong et al., 2002).

An increasing body of evidence suggests that oxidative stress is involved in the pathogenesis of hypertension (Chan et al., 2006; Kishi et al., 2004; Tai et al., 2005). One of the primary producers of reactive oxygen species (ROS) in the cell is the membrane-associated NADPH oxidase (Griendling et al., 2000; Mohazzab et al., 1994). Activation of NADPH oxidase is a multi-step process that is initiated by serine phosphorylation of the cytosolic regulatory $p47^{phox}$ subunit (Touyz et al., 2002). Following translocation to the membrane, the activated $p47^{phox}$ subunit is associated with the membrane-bound $gp91^{phox}$ and $p22^{phox}$ subunits to bring forth enzymatic activity (Deleo et al., 1996). The ROS, particularly superoxide anion ($O_2^{\bullet-}$), is an important intracellular messenger for angiotensin II (Ang II), a key mediator of hypertension. Thus, Ang II increases the activity of NADPH oxidase in the vasculature (Griendling et al., 1994) via activation of $p47^{phox}$ subunit (Li et al., 2004). Ang II also enhances $O_2^{\bullet-}$ production in the central nervous system (Gao et al., 2004; Zimmerman et al., 2002, 2004); the pressor effects induced centrally by Ang II are also mediated by NADPH oxidase-generated $O_2^{\bullet-}$ (Chan et al., 2007; Gao et al., 2004; Zimmerman et al., 2004).

Clinically, the angiotensin subtype 1 receptors (AT1R) are specifically targeted by angiotensin receptor blockers in antihypertensive

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therapy (Jarvis, 2012). Several characteristic effects, including a reduction in the cardiac vagal baroreflex (Chaves et al., 2000; Luoh and Chan, 1998; Wong et al., 2002) and an augmentation of Ang II-elicited oxidative stress in the brain (Chan et al., 2007; Glass et al., 2007; Wang et al., 2004, 2006), have been observed in neurogenic hypertension, in which chronic elevation of the 24-h average blood pressure is not caused primarily by a vascular or renal defect. Intracerebroventricular (i.c.v.) administration of Ang II reduces baroreflex sensitivity (BRS) (Elghozi and Head, 1990; Michelini and Bonagamba, 1990; Sun et al., 2009), and one of the targets of the octapeptide is the NTS (Healy et al., 1984; Weyhenmeyer and Phillips, 1982). Thus, microinjection of Ang II into the NTS promotes hypertension (Chaves et al., 2000; Katsunuma et al., 2003; Wong et al., 2002) and inhibits cardiac vagal baroreflex (Luoh and Chan, 1998; Wong et al., 2002) via the AT1R (Luoh and Chan, 1998). The downstream signaling after activation of AT1R involves NADPH oxidase-derived $O_2^{\bullet-}$ (Glass et al., 2007; Wang et al., 2004, 2006), engaging at least the $p47^{phox}$ subunit (Glass et al., 2007).

Close scrutiny of the literature revealed that visualization of alterations in connectivity within the cardiac vagal baroreflex circuit by diffusion tensor imaging (DTI) in the context of neurogenic hypertension has not been performed. Also unknown is whether depression of the cardiac vagal baroreflex and NADPH oxidase-derived $O_2^{\bullet-}$ induced by Ang II at the NTS are causally related. Based on an animal model (Elghozi and Head, 1990; Michelini and Bonagamba, 1990; Sakai, 2007) that involves i.c.v. infusion of Ang II, the guiding hypothesis of the present study is that an augmented $O_2^{\bullet-}$ level induced by Ang II in the NTS contributes to the depression of cardiac vagal baroreflex by disrupting the connectivity between the NTS and NA during neurogenic hypertension. Our results validated this hypothesis by showing that oxidative stress induced by augmented levels of NADPH oxidase-generated $O_2^{\bullet-}$ in the NTS disrupted reversibly the connectivity between the NTS and NA in a mouse model of neurogenic hypertension, concurrent with impairment of the cardiac vagal baroreflex.

Materials and methods

Ethics statement

All experimental procedures carried out in this study were approved by the Institutional Animal Care and Use Committee of the Kaohsiung Chang Gung Memorial Hospital, and were in compliance with the guidelines for animal care and use set forth by that committee.

Animals

Male adult C57BL/6 mice (26 ± 3 g) purchased from the Experimental Animal Center of the National Science Council, Taiwan were used. Animals were housed in an Association for Assessment and Accreditation of Laboratory Animal Care (AAALAC) International-accredited Center for Laboratory Animals under temperature control (24–25 °C) and 12-h light–dark cycle (lights on at 07:00 h). Standard laboratory rat chow and tap water were available ad libitum.

Magnetic resonance imaging (MRI) and DTI

Sequential MRI/DTI acquisition was performed under isoflurane anesthesia in a 9.4 T horizontal-bore animal MR scanning system (Biospec 94/20, Bruker, Ettingen, Germany). This scanning system is made up of a self-shielded magnet with a 20-cm clear bore and a BGA-12S gradient insert (12-cm inner diameter) that offered a maximal gradient strength of 675 mT m^{-1} and a minimum slew rate of 4673 T m^{-1} s^{-1}. It is also equipped with a modified high performance transmitter–receiver surface cryo coil for signal detection from the mouse head. As a routine, high resolution T_2-weighted sagittal anatomical reference images were first recorded, using multislice turbo rapid acquisition with refocusing echoes (Turbo-RARE) sequence with the following parameters: field of view = 18.0 mm × 15.0 mm; matrix dimension = 384 × 320 pixels; spatial resolution = 46.8 μm × 46.8 μm; slice thickness = 200 μm; interslice distance = 200 μm; echo time =12.6 ms; effective echo time = 25.3 ms; repetition time = 3000 ms; rare factor = 4; refocusing flip angle = 180°; number of averages =7; total acquisition time = 28 min. Based on orientation of landmark structures from the sagittal images (Fig. 1A), T_2-weighted coronal anatomical reference imaging (Fig. 1B) was performed on 10 adjacent slices from a restricted area of the brain stem that covered the medullary portion of the NTS and NA, using Turbo-RARE sequence acquisition with the following parameters: field of view = 12.0 mm × 12.0 mm; matrix dimension = 256 × 256 pixels; spatial resolution/pixel =47 μm × 47 μm; slice thickness = 200 μm; interslice distance =200 μm; echo time = 12.5 ms; effective echo time = 25 ms; repetition time = 3000 ms; rare factor = 4; number of averages = 12; acquisition time = 38 min.

Using identical spatial dimension as in the T_2-weighted coronal reference imaging, the connectivity between key brain stem nuclei in the cardiac vagal baroreflex circuit was subsequently evaluated using the spin echo-planar imaging-DTI sequence in the coronal plane covering the same ten 200-μm slices without gap (Fig. 1C). The parameters for acquisition were: field of view = 12.0 mm × 12.0 mm; matrix dimension = 128 × 128 pixels; spatial resolution/pixel = 93.8 μm × 93.8 μm; slice thickness = 200 μm; interslice distance = 200 μm; echo time = 24 ms; repetition time = 2500 ms; number of diffusion directions = 46; optimized b value/direction = 1500 s/mm^2; gradient duration = 4.1; gradient separation = 10.3; number of averages = 15; acquisition time = 33 min 45 s.

Post-processing of images

Post-processing of the image data entailed an analysis of fiber tractography and determination of DTI indices, using the National Taiwan University DSI studio (http://dsi-studio.labsolver.org). At each time-point, the fiber tracts between the NTS and NA on both sides were selected for tractographic evaluation. In addition, regions-of-interest (ROIs)-based analysis was performed to quantify two DTI indices, fractional anisotropy (FA) and number of fiber tracts (Jiang et al., 2006). In brief, based on the bilateral locations of the NTS and NA in the T_2-weighted coronal images (Fig. 1B), ROIs were marked manually on corresponding areas in the FA map of the brain stem (Fig. 1C). Values of FA, which ranged from 0 (isotropy) to 1 (maximum anisotropy), were derived from the standard deviation of the three eigenvalues (λ_1, λ_2, λ_3) of the diffusion ellipse of probability density function. To calculate fiber numbers, the tracts that passed through both NTS and NA were counted using the streamline tracking method.

Chronic i.c.v. infusion of Ang II by osmotic minipump

I.c.v. infusion of Ang II (Sigma-Aldrich, St. Louis, MO, USA) (Chan et al., 2007) was delivered by an osmotic minipump (Alzet 2001; Alzet Corp, Cupertino, CA, USA) (Sakai, 2007) for 7 days at 7.5 μg/h and a rate of 0.5 μL/h. Control infusion of artificial cerebrospinal fluid (aCSF) served as the volume and vehicle control.

Radiotelemetric recording of blood pressure

Blood pressure of mice was recorded under a conscious state in their home cages using implantable blood pressure telemeters (TA11PA-C10; Data Sciences International, Minneapolis, MN, USA) (Baudrie et al., 2007; Laude et al., 2008). The transmitted blood pressure signals were digitized and processed by an algorithm based on feature extraction to detect and measure the characteristics of blood pressure cycles. Using this acquisition software (Notocord-hem