Microvascular Research xxx (2014) xxx–xxx

Contents lists available at ScienceDirect

Microvascular Research

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

Altered skin flowmotion in hypertensive humans

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ARTICLE INFO

Article history:
Accepted 1 January 2014
Available online xxxx

ABSTRACT

Essential hypertensive humans exhibit attenuated cutaneous nitric oxide (NO)-dependent vasodilation. Using spectral analysis (fast Fourier transformation) we aimed to characterize the skin flowmotion contained in the laser-Doppler flowmetry recordings during local heating-induced vasodilation before and after concurrent pharmacological inhibition of nitric oxide synthase (NOS) in hypertensive and age-matched normotensive men and women. We hypothesized that hypertensive subjects would have lower total power spectral densities (PSDs), specifically in the frequency intervals associated with intrinsic endothelial and neurogenic control of the microvasculature. Furthermore, we hypothesized that NOS inhibition would attenuate the endothelial frequency interval. Laser-Doppler flowmetry recordings during local heating experiments from 18 hypertensive (MAP: 108 ± 2 mm Hg) and 18 normotensive (MAP: 88 ± 2 mm Hg) men and women were analyzed. Within site NO-dependent vasodilation was assessed by perfusion of a non-specific NOS inhibitor (N^G-nitro-L-arginine methyl ester; L-NAME) through intradermal microdialysis during the heating-induced plateau in skin blood flow. Local heating-induced vasodilation increased total PSD for all frequency intervals (all $p < 0.001$). Hypertensives had a lower total PSD ($p = 0.03$) and absolute neurogenic frequency intervals ($p < 0.01$) compared to the normotensives. When normalized as a percentage of total PSD, hypertensives had reduced neurogenic ($p < 0.001$) and augmented myogenic contributions ($p = 0.04$) to the total spectrum. NOS inhibition decreased total PSD ($p < 0.001$) for both groups, but hypertensives exhibited lower absolute endothelial ($p < 0.01$), neurogenic ($p < 0.05$), and total PSD ($p < 0.001$) frequency intervals compared to normotensives. These data suggest that essential hypertension results in altered neurogenic and NOS-dependent control of skin flowmotion and support the use of spectral analysis as a non-invasive technique to study vasoreactivity.

Introduction

The cutaneous circulation is an accessible vascular bed which allows for non-to minimally invasive studies of endothelial, neurovascular, and vascular smooth muscle vasoreactivity in vivo (Holowatz et al., 2008; Minson, 2010; Roustit and Cracowski, 2013). A number of skin vasoreactivity tests have been used to study microvascular function in populations that are at risk for or currently have cardiovascular disease (CVD). Local skin heating coupled with laser-Doppler flowmetry is a common test of microvascular function which elicits highly reproducible skin blood flow responses that have been mechanistically well characterized. Local skin heating causes a hyperemic response that is predominantly reliant on endothelium-dependent nitric oxide (NO)-mediated vasodilation (~70%) (Bruning et al., 2012; Kellogg et al., 2008). This response has been studied in a range of healthy (Bruning et al., 2012; Kellogg et al., 2008; Minson et al., 2001) and diseased populations (Holowatz and Kenney, 2011; Smith et al., 2011).

Our laboratory has examined cutaneous vasoreactivity in unmedicated stage 1 essential hypertensive men and women without other co-morbidities. This subject cohort exhibited endothelial dysfunction during local skin heating marked by a reduction in endothelial nitric oxide synthase (eNOS)-dependent vasodilation (Smith et al., 2011). There is growing evidence that spectral analysis of the low frequency periodic oscillations in blood flux measurements using laser-Doppler flowmetry can provide non-invasive mechanistic information on microvascular control mechanisms (Kastrup et al., 1989; Salerud et al., 1983; Stefanovska et al., 1999). These periodic oscillations, or skin flowmotion, represent the influence of heart beat (0.6–2.0 Hz), respiration (0.15–0.6 Hz), myogenic (~0.05–0.15 Hz) (Stefanovska et al., 1999), neurogenic (~0.02–0.05 Hz) (Kastrup et al., 1989; Soderstrom et al., 2003), and endothelial (~0.0095–0.02 Hz) influences on vascular smooth muscle relaxation (Gustafsson et al., 1993; Kvandal et al., 2003; Kvernmo et al., 1999; Rossi et al., 2008a). Spectral analysis has been performed on populations with known microvascular dysfunction such as peripheral arterial obstructive disease (Rossi et al., 2005), chronic kidney disease (Rossi et al., 2008b), diabetes (Schmiedel et al., 2007), chronic smokers (Avery et al., 2009; Rossi et al., 2007), hypercholesterolemics (Rossi et al., 2009) and essential hypertensive men and women (Gryglewska et al., 2010a,b; Rossi et al., 2006). While these studies observed altered skin flowmotion responses, many of them utilized different skin vasoreactivity tests and subject populations in which the mechanisms mediating skin blood flow are not thoroughly understood. The one study that has examined cutaneous vasoreactivity during local

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0026-2862/$ – see front matter © 2014 Published by Elsevier Inc.
http://dx.doi.org/10.1016/j.mvr.2014.01.001

skin heating in essential hypertensive men and women did not control local skin temperature throughout the entire protocol and used a different rate of skin heating (Gryglewska et al., 2010a) from what has been used in mechanistic studies examining NOS-dependent vasodilation in hypertensive humans (Smith et al., 2011). Further, no study has examined skin flowmotion in essential hypertensive men and women during local skin heating with concurrent pharmacological inhibition of NOS.

The aim of the present study was to utilize spectral analysis to evaluate skin vasoreactivity in unmedicated essential hypertensive and age-matched normotensive men and women before and after NOS inhibition. We hypothesized that essential hypertension would result in reduced total power spectral density (PSD) around the frequency intervals of interest during local heating, due to reduced intrinsic endothelial and neurogenic signaling. Furthermore, we hypothesized that *within* site inhibition of NO production would result in reduced endothelial signaling in both essential hypertensive and normotensive subjects.

Materials and methods

Subjects

All experimental protocols were approved by the Institutional Review Board at The Pennsylvania State University and conformed to the guidelines set forth by the *Declaration of Helsinki*. Verbal and written consent were voluntarily obtained from all subjects prior to participation. We performed Fourier transform-based power spectral analysis on previously collected local skin heating studies in unmedicated essential hypertensive subjects from our laboratory (pilot data and (Smith et al., 2011)). Fast Fourier transformations (FFT) were applied to laser-Doppler recordings during local skin heating in 18 essential hypertensive and 18 age-matched normotensive men and women. Subject characteristics are presented in Table 1. Subject's blood pressures were classified in accordance with the guidelines set forth by the American Heart Association (Chobanian et al., 2003) during three separate visits and further explored using an ambulatory 24-hour blood pressure monitor. Subjects underwent a complete medical screening including a resting ECG, physical examination, lipid profile and blood chemistry (Quest Diagnostics, Pittsburgh, PA) and were otherwise healthy with the exclusion of stage 1 essential hypertension. All subjects were normally active, non-diabetic, non-smokers who were not taking any prescription medications with primary or secondary vascular effects, including antihypertensive pharmacotherapy, vitamins and supplements. Seventeen of the 18 essential hypertensive subjects were naïve to antihypertensive therapy, and 1 subject had stopped antihypertensive therapy >1 year prior to participating in the protocol. All of the premenopausal women (n = 4) were studied on days 2 to 7 of their menstrual cycle, and postmenopausal women (n = 14) reported that it had been ≥1 year since the cessation of their last menses.

Table 1
Subject characteristics.

	Normotensives	Essential hypertensives
Subjects (male, female)	(5, 13)	(13, 5)
Age (years)	53 ± 1	52 ± 1
BMI	25.3 ± 0.7	26.5 ± 0.5
Systolic BP (mm Hg)	113 ± 3	139 ± 2**
Diastolic BP (mm Hg)	76 ± 2	92 ± 2*
MAP (mm Hg)	88 ± 2	108 ± 2**
Fasting glucose ($mg \cdot dL^{-1}$)	91 ± 2	93 ± 2
HbA1c	5.5 ± 0.1	5.5 ± 0.1
HDL ($mg \cdot dL^{-1}$)	56 ± 4	57 ± 3
LDL ($mg \cdot dL^{-1}$)	107 ± 5	110 ± 5
Triglycerides ($mg \cdot dL^{-1}$)	94 ± 12	91 ± 16

Subject characteristics (mean ± SEM). BMI, body mass index; BP, blood pressure; MAP, mean arterial pressure; HDL, high-density lipoprotein; LDL, low-density lipoprotein. *p < 0.01, **p < 0.001 significantly different than normotensive men and women.

Local skin heating and assessment of skin blood flow

The local heating protocols were performed in a thermoneutral laboratory with the subject in a semi-supine position and the experimental arm at heart level. Intradermal microdialysis fibers (MD 2000, Bioanalytical Systems, West Lafayette, IN) were inserted into the ventral side of the forearm skin as previously described (Smith et al., 2011). Local heaters (SHO2, Moor Devon UK) were used to control skin temperature and mount the laser-Doppler flowmeter probes (MoorLAB, Moor Devon UK), which measured skin blood flux over the microdialysis sites. We performed Fourier transform-based power spectral analysis on control microdialysis sites from pilot data and a previously published study (Smith et al., 2011). The control site had lactated Ringer's solution perfused throughout insertion hyperemia (60–90 min), a baseline where local skin temperature was clamped at 33 °C (20 min), during standardized local skin heating to 42 °C (0.5 °C every 5 s), and post local skin heating to record at least 30 min of a stable, heat-induced hyperemic skin blood flow response (Johnson and Kellogg, 2010; Kellogg et al., 1999; Minson et al., 2001; Smith et al., 2011). The latter skin blood flow plateau is predominantly mediated by NO from eNOS (Bruning et al., 2012; Johnson and Kellogg, 2010; Kellogg et al., 2008; Minson et al., 2001). The NO-dependent vasodilation was assessed *within* each control site by perfusing 20 mM of a non-specific NOS inhibitor (N^G-nitro-L-arginine methyl ester (L-NAME); Tocris) through the microdialysis fiber until the skin blood flow was reduced for a stable 20 min period (L-NAME plateau). After the L-NAME plateau, local skin temperature was increased to 43 °C and 28 mM of sodium nitroprusside (SNP) was perfused to elicit maximal vasodilation for data normalization and to test endothelium-independent vasodilator responsiveness (Holowatz et al., 2005).

Power spectral density (PSD) analysis

The skin blood flow data was collected using a MoorLAB laser-Doppler flowmetry system. This system uses 785 nm solid state laser diodes as the laser light source. In the MoorLAB device, the laser-Doppler signal was band-pass filtered in the range of 20 Hz to 14.9 kHz. The skin blood flow data from the local heating protocols were digitized and saved at a sampling frequency of 40 Hz using WinDAQ software (Dataq Instruments, Akron, OH). These files were later converted to Microsoft Excel files and each skin blood flow response was divided into four periods: baseline, local heating plateau, L-NAME plateau, and SNP plateau for further analysis. The regions of interest converted into Microsoft Excel files were determined by visually assessing 600 s (24,001 data points) of stable, flat portions of the laser-Doppler recordings that were devoid of motion artifacts.

Estimating PSD

The frequencies of oscillations contained in the laser-Doppler recordings from the four periods were analyzed using Fourier transform-based power spectral analysis as described by Avery et al. (2009). All laser-Doppler recordings were measured in arbitrary flow units (AU) prior to analysis. Periodograms were derived from the FFT of the laser-Doppler recordings in MATLAB® (version 2013a) and the average of the periodograms was used to estimate the PSD (Avery et al., 2009). The power (in AU^2) was calculated around the 0.01 Hz (0.008–0.02 Hz), 0.04 Hz (0.02–0.05 Hz) and 0.1 Hz (0.05–0.15 Hz) frequency intervals, considered to correspond to endothelial, neurogenic, and myogenic activity, respectively (Avery et al., 2009; Meyer et al., 2003; Rossi et al., 2006). During a given period (i.e. baseline, local heating plateau, etc.) the frequency intervals of interest were

Please cite this article as: Bruning, R.S., et al., Altered skin flowmotion in hypertensive humans, Microvasc. Res. (2014), http://dx.doi.org/10.1016/j.mvr.2014.01.001