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Spectral analysis of reflex cutaneous vasodilatation during passive heat stress

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ABSTRACT

Previous work has demonstrated that spectral analysis is a useful tool to non-invasively ascertain the mechanisms of control of the cutaneous circulation. The majority of work using spectral analysis has focused on local control mechanisms, with none examining reflex control. Skin blood flow was analysed using spectral analysis on the dorsal aspect of the forearm of 7 males and 7 females during passive heat stress, with mean forearm and local temperature at the site of measurement maintained at thermoneutral (33 °C) to minimize the effect of local control mechanisms. Participants were passively heated to ~1.2 ± 0.1 °C above baseline rectal temperature ($d = 4.0$, $P < 0.001$) using a water-perfused, tube lined suit, with skin blood flow assessed using a laser-Doppler probe with an integrated temperature monitor. Spectral analysis was performed using a Morlet wavelet on the entire data set, with median power extracted during 20 min of data during baseline (normothermia) and hyperthermia. Passive heat stress significantly increased laser-Doppler flux above baseline ($d = 4.7$, $P < 0.001$). Spectral power of the endothelial nitric oxide-independent (0.005–0.01 Hz; $d = 1.1$, $P = 0.004$), neurogenic (0.2–0.05 Hz; $d = 0.6$, $P = 0.025$), myogenic (0.05–0.15 Hz; $d = 1.5$, $P = 0.002$), respiratory (0.15–0.4 Hz; $d = 1.4$ $P = 0.002$), and cardiac (0.4–2.0 Hz; $d = 1.1$, $P = 0.012$) frequency intervals increased with passive heat stress. In contrast, the endothelial nitric oxide-dependent frequency interval did not change (0.01–0.02 Hz; $d = 0.3$, $P = 0.09$) with passive heat stress. These data suggest that cutaneous reflex vasodilatation is neurogenic in origin and not mediated by endothelial-nitric oxide synthase, and are congruent with invasive examinations of reflex cutaneous vasodilatation.

1. Introduction

An increase in core temperature elicits cutaneous vasodilatation independent of local skin temperature (Caldwell et al., 2016; Johnson et al., 1976; Mallette et al., 2016). Reflex (neural) control of blood flow in non-glabrous (hairy) skin is achieved through two branches of the sympathetic nervous system: a noradrenergic system that mediates vasoconstriction, and a cholinergic branch that elicits vasodilatation (Johnson et al., 2014). The initial vasodilatation from whole-body heating occurs due to a reduction in sympathetic vasoconstrictor tone (Edholm et al., 1957; Fox and Edholm, 1963; Johnson et al., 2014), with further increases in blood flow due to the influence of the sympathetic cholinergic system (Johnson et al., 2014; Kellogg et al., 1995). Reflex vasodilatation requires sympathetic neurotransmitters, including acetylcholine, vasoactive intestinal peptide (VIP), pituitary adenylyl cyclase activating polypeptide (PACAP), and neuronal-nitric oxide synthase (NOS) (Bennett et al., 2003; Kellogg et al., 1995, 2008b, 2009, 2010, 2012); however, the precise roles of these mediators in reflex vasodilatation remain unclear (Johnson et al., 2014).

Spectral analysis of the laser-Doppler signal offers a non-invasive method to examine the underlying mechanisms of skin blood flow control (Bračič and Stefanovska, 1999; Hodges and Del Pozzi, 2014; Podtaev et al., 2008, 2015; Stefanovska et al., 1999). While intradermal microdialysis is a powerful and well-established tool that allows for a sophisticated examination of the mechanisms of skin blood flow control, it reduces the magnitude of reflex vasodilatation in response to passive heat stress (Hodges et al., 2009). Thus, non-invasive approaches that do not alter the vasomotor response may provide valuable insight into the regulatory factors of cutaneous vasodilatation during passive heating.

Maintaining a constant local temperature throughout the experiment is important, as local temperature changes have an immediate impact on endothelial, myogenic, and neurogenic function (Podtaev et al., 2008), yet it is not commonly performed in studies examining reflex vasodilatation (Brunt et al., 2013; Kellogg et al., 2009; Wong et al., 2004). This is important, as the mechanisms of vasodilatation differ between

Abbreviations: NOS, nitric oxide synthase; $\overline{T}_{sk}$, mean body skin temperature; PACAP, pituitary adenylyl cyclase activating polypeptide; VIP, vasoactive intestinal peptide.

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local and reflex stimuli (Kellogg et al., 2008a, 2008b, 2009). Previous studies using spectral analysis have only examined local temperature changes (Del Pozzi et al., 2016; Hodges and Del Pozzi, 2014; Podtaev et al., 2008, 2015) or the responses to drug applications (Kvandal et al., 2003, 2006). Therefore, this study used spectral analysis – along with a combination of passive, whole-body heating while maintaining local temperature in a thermoneutral state (33 °C) – to examine the mechanisms of reflex vasodilatation. It was hypothesized that hyperthermia: 1) would lead to increased power in the neurogenic, myogenic, respiratory, and cardiac frequency bands, but 2) would not change the spectral power in either the endothelial nitric oxide-independent or endothelial nitric oxide-dependent frequency bands.

2. Methods

2.1. Ethical approval

This study was approved by the Bioscience Research Ethics Board of Brock University (REB #13-283) and conformed to the standards set forth by the Declaration of Helsinki. All participants were informed of the experimental protocol as well as the associated risks prior to participating. Verbal and written consent was obtained from each participant.

2.2. Participants

An *a priori* power analysis with means and standard deviations of the neurogenic frequency interval from previous work (Podtaev et al., 2015) indicated that a minimum of 8 participants would be required for an α of 0.05 and a β of 0.20. To account for a smaller expected effect from whole-body heating compared to local heating, 14 healthy active individuals (7 males and 7 females) were included. Participants were free of any diagnosed metabolic or cardiovascular diseases, and did not smoke or take any prescribed medication other than oral contraceptives. Females were tested during the first 10 days of the onset of self-reported menses, or during the placebo/no pill phase if on oral contraceptives (LH phase) (Gagnon et al., 2013; Mallette et al., 2016). The mean ($\pm$SD) for age, height, mass, body fat percentage, and peak oxygen uptake ($\dot{V}O_{2peak}$) for all participants were: 23 $\pm$ 2 years, 171.9 $\pm$ 7.3 cm, 64.6 $\pm$ 8.4 kg, 15 $\pm$ 6%, and 53.2 $\pm$ 6.5 ml·kg^{-1}·min^{-1}.

2.3. Experimental design

All participants completed a familiarization and an experimental session. Participants were instructed to avoid strenuous exercise and caffeine 12 h prior, and alcohol consumption 24 h prior to each experimental session. Additionally, participants were advised to have a light meal 1 to 2 h before each experimental session, and instructed to drink water *ad libitum*. During the familiarization session, height, mass, body fat percentage, and aerobic capacity were determined. Height and mass were measured with standard laboratory equipment. Body fat percentage was calculated using a 7-site (triceps, sub-scapula, mid-axilla, supra-illiac crest, chest, abdomen, and thigh) skinfold measurement (Jackson and Pollock, 1978; Jackson et al., 1980) with manual calipers (Harpenden, Bay International, West Sussex, UK). Aerobic fitness was determined through open-circuit spirometry (Gas Analyzer, ADInstruments; Labview software; Colorado Springs, CO, USA) during a progressive running test to volitional exhaustion on a treadmill (Trackmaster TMX22, Full Vision Inc., Newton, KS, USA).

Participants arrived at the laboratory (~22 °C, ~35% relative humidity) at 0800 h for each experimental session. Euhydration was confirmed using a refractometer (PAL-10S, Atago, USA) and defined as a urine specific gravity ≤1.020 (Casa et al., 2000). If euhydration was confirmed, participants were instructed to self-insert a rectal thermistor (Mon-A-Therm Core, Mallinckrodt Medical, St. Louis, MO, USA) 12 cm beyond the anal sphincter to assess rectal temperature (Lee et al., 2010; Mead and Bonmarito, 1949). T-type thermocouples (PVC-T-24-190, Omega Environmental Inc., Laval, QC, CAN) were taped (Transpore™, 3 M, St. Paul, MIN, USA) to the chest, thigh, upper arm, and calf to calculate mean skin temperature ($\bar{T}_{sk}$), calculated using the weighted average of four thermocouples (Ramanathan, 1964).

Participants then dressed in a three-piece liquid conditioning garment (BCS 4 Cooling System, Allen Vanguard, Ottawa, ON, CAN), consisting of 1/8″ diameter Tygon tubing sewn into a stretchable hood, jacket, and pants; the face, forearm, hands, and feet were not covered by the garment. Males wore only shorts and females wore shorts and a sports bra under the liquid conditioning garment to maximize the amount of skin exposed to the heated tubing to facilitate heat transfer to the body. To minimize evaporative heat loss, a polyvinyl rain suit was worn over the liquid conditioning garment. Water temperature of the liquid conditioning garment was manipulated by adding hot or cold water, maintained at a constant temperature by a temperature controller (Model 5202, Polyscience, Niles, IL, USA) and pumped (MED-ENG, Pembroke, CAN) at a flow rate of ~1.5 l·min^{-1}.

The dorsal surface of the forearm – not covered by the liquid conditioning garment – was equipped with three thermocouples (Omega Environmental Inc., Laval, QC, CAN) located on the proximal, middle, and distal portion to calculate mean forearm temperature. Red blood cell flux was assessed with laser-Doppler flowmetry (5010 LDPM, Perimed, Järfälla, Sweden) and used to provide an index of skin blood flow (Johnson et al., 1984; Öberg, 1989; Saumet et al., 1988). A combined laser-Doppler flux and skin heater probe (Probe 451, Perimed) was taped (PF 105-3, Perimed and Transpore™, 3 M) to the dorsal surface of the forearm. Thermocouples and the laser-Doppler probe were held in place with nylon wrapping (BurnNet, Glenwood Laboratories, Oakville, ON, CAN). Participants sat semi-recumbent in a Kore-Kooler® Rehab Chair (DQE, Fishers, IN, USA) with feet elevated to the height of the pelvis. The hand and forearm was immersed in a temperature-controlled water bath that was insulated with solar blankets throughout the experiment.

2.4. Experimental protocol

The protocol began by circulating ~32 °C water through the liquid conditioning garment to obtain a $\bar{T}_{sk}$ of ~33 °C, and the forearm bath was adjusted to make mean forearm skin temperature ~ 33 °C. Once laser-Doppler flux values were stable, 20 min of baseline (normothermia) data were collected. Following baseline measurements, participants were heated by increasing the water temperature circulating through the liquid conditioning garment from ~32 °C to ~49.5 °C to elicit a 1.2 °C rise in rectal temperature above baseline values (rectal temperature from the last 60 s baseline). During passive heating, forearm temperature was ~34 °C. Upon reaching an increase in rectal temperature of 1.2 °C, 20 min of hyperthermic data were collected.

Heart rate was continuously obtained from R-R intervals using a 3-lead electrocardiogram (Bio Amp, ADInstruments, Colorado Springs, CO, USA) and calculated online using Lab Chart (version 8, ADInstruments, Colorado Springs, CO, USA). Blood pressure was recorded at the left ankle before and after the normothermic and hyperthermic assessment using a manual sphygmomanometer.

2.5. Data processing

Laser-Doppler (flux and probe temperature) and temperature (rectal, whole-body skin, and mean forearm skin temperature) data were collected at 40 Hz and R-R intervals at 1 kHz (PowerLab, ADInstruments. Colorado Springs, CO, USA), respectively, and stored on a personal computer to be analysed and processed offline using MATLAB (The MathWorks Inc., Natick, MA, USA). Laser-Doppler flux data were collected with a time-constant of 0.2 s.