Microvascular Research 97 (2015) 47–54

Contents lists available at ScienceDirect

Microvascular Research

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

To reheat, or to not reheat: that is the question: The efficacy of a local reheating protocol on mechanisms of cutaneous vasodilatation

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ARTICLE INFO

Article history:
Accepted 25 September 2014
Available online 2 October 2014

Keywords:
Local heating
Adrenergic
Laser-Doppler
Microdialysis
NOS
Skin

ABSTRACT

The aim of this study is to determine the effect of repeated bouts of local skin heating on the roles of nitric oxide synthase (NOS) and sympathetic nerves in cutaneous vasodilatation. In 3 repeated-heating protocols skin blood flux of the forearm and leg was measured using laser-Doppler flowmetry and data are presented as cutaneous vascular conductance (CVC; flux/blood pressure). Local heating was performed from 33 °C (thermoneutral) to 42 °C at 0.5 °C·10 s^{-1}, allowed to cool passively for ~60-min, then reheated at the same rate. In protocol 1, CVC was measured in response to repeated heating. In protocol 2, NOS was inhibited with N^G-nitro-L-arginine methyl ester (L-NAME) and in protocol 3, sympathetic nerve blockade was achieved with bretylium tosylate (BT), both infused *via* intradermal microdialysis. In protocol 1, there were no differences ($P > 0.05$) in CVC at either the forearm (88 ± 4 *vs.* 86 ± 4%max) or the leg (97 ± 4 *vs.* 96 ± 6%max) between heating bouts. In protocol 2, no differences ($P > 0.05$) in CVC were observed between heating bouts at L-NAME treated sites at either the forearm (55 ± 3 *vs.* 51 ± 4%max) or the leg (71 ± 3 *vs.* 70 ± 4%max) . In protocol 3, there were differences ($P < 0.001$) between BT treated sites when comparing the first and second bouts of heating for both the forearm (75 ± 3 *vs.* 88 ± 4%max) and the leg (79 ± 3 *vs.* 97 ± 4%max). The effect of sympathetic blockade on CVC responses to local heating was abolished following repeated bouts of heating. Consequently, it is our suggestion that when examining mechanisms of skin blood flow control, investigators use single bouts of local heating.

Introduction

The cutaneous circulation plays a prominent role in the control of body temperature (Johnson et al., 2014). *In vivo* studies have confirmed that humans are able to regulate body temperature remarkably well under a variety of environmental conditions by regulating the level of perfusion to the skin (Charkoudian, 2003; Hodges and Johnson, 2009; Johnson and Kellogg, 2010b; Kellogg, 2006). The management of local cutaneous perfusion is achieved through a combination of autonomic nerve activity (*e.g.* noradrenergic sympathetic nerves) (Hodges and Johnson, 2009) and local factors such as nitric oxide synthase (NOS) (Johnson and Kellogg, 2010a). These mechanisms have been studied at length, with heating protocols remaining relatively homogenous (Hodges and Johnson, 2009; Holowatz, 2008; Holowatz and Kenney, 2010; Johnson and Kellogg, 2010a; Johnson and Kellogg, 2010b; Johnson et al., 2014; Minson, 2010). However, some recently published studies have employed a heat then reheat protocol allowing for each individual skin site to serve as its own control (Medow et al., 2008; Stewart et al., 2007b). While a strength of this approach is that the protocol does appear to improve the problem of inter-site variability associated with the laser-Doppler (Johnson et al., 1984), it has been suggested that desensitization to local heating may occur at skin sites that have been previously heated (Ciplak et al., 2009; Frantz et al., 2012). The data are equivocal, but it is important to note that these laboratories have examined different skin sites, one using the forearm (Ciplak et al., 2009; Frantz et al., 2012) and the other using the calf (Medow et al., 2008; Stewart et al., 2007b). Additionally, differing rates of skin heating were employed and previous work has clearly demonstrated that the rate of skin heating affects how the cutaneous vasculature responds (Hodges et al., 2009b).

Recently, we have shown that regional differences in the contribution of nitric oxide synthase exist between the skin of the forearm and the leg (Del Pozzi et al., 2013). We found that the cutaneous vasodilator response to local skin warming was greater in the legs than in the forearms. However, the contribution of NO to the vasodilator response during local skin warming was similar in the forearms and legs. Additionally, Pawelczyk and Levine (2002) proposed that the legs of humans are exposed to increased pressures compared to the arms. Suggesting that this would result in altered adrenergic responsiveness (Pawelczyk and Levine, 2002). The authors found that the

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http://dx.doi.org/10.1016/j.mvr.2014.09.006
0026-2862/

vascular responsiveness between the forearms and the legs in response to exogenous α-receptor agonists was not uniform, with the legs presenting a greater sensitivity to α-receptor stimulation. Whether sympathetic function in the cutaneous circulation differs between the forearms and legs remains unknown.

These findings have prompted us to investigate the effect of repeated bouts of local skin heating (so called heat–reheat) on the roles of NOS and noradrenergic sympathetic nerves in cutaneous vasodilatation.

To achieve this we performed a series of 3 studies. In *protocol 1*, we examined the effect of a heat–reheat protocol on cutaneous vasodilatation at forearm and leg skin sites. Based on the work of Stewart and coworkers (Medow et al., 2011; Stewart et al., 2007a; Stewart et al., 2009; Stewart et al., 2011; Stewart et al., 2008a) we hypothesized that the vasodilator response to the second bout of heating would be the same as the first. In *protocol 2*, we examined the effect of NOS inhibition combined with the same (protocol 1) heat–reheating procedure. Based on previous data that demonstrated a desensitization of the cutaneous vasculature to NO during repeated bouts of local skin heating (Ciplak et al., 2009; Frantz et al., 2012), we hypothesized that the vasodilator response to the first bout of heating with NOS inhibition would be similar in comparison to the second bout of heating. In *protocol 3*, we blocked noradrenergic sympathetic nerves while employing the heat–reheating protocol. Based on our previous work in which we demonstrated a temporal influence of adrenergic inhibition (Hodges and Sparks, 2013), we hypothesized that sympathetic blockade would reduce the vasodilator response to the first bout of heating but not the second bout of heating.

Materials and methods

Participants

Ten volunteers (27 ± 4 years, 4 men and 6 women) participated in the study. Participants were asked to refrain from caffeine and alcohol for 24 h prior to testing. The participants were also instructed to show up for the testing following a 2 h fast, while being allowed to drink water *ad libitum*. The subject pool consisted of healthy, active individuals who were not tobacco users. As per our previous studies (Carter and Hodges, 2011; Del Pozzi et al., 2013; Hodges and Del Pozzi, 2014; Hodges et al., 2009b; Hodges and Sparks, 2013), all female subjects were using oral contraceptives and currently in the low hormone phase of their cycles, verified through self-report.

The current study was approved for completion by the local Institutional Review Board at The University of Alabama. Participants were fully informed of the experimental methods as well as the associated risks prior to their volunteering to be a participant. Verbal as well as written informed consent was obtained from each participant. The experimental protocols conformed to the guidelines set forth by the Declaration of Helsinki.

Measurements and instrumentation

The experimental sessions were performed over 3 separate days (one day for each testing protocol). All participants arrived for testing at the laboratory at 0700 h. All testing was conducted in a temperature controlled room at 22 °C ± 1 °C. All studies were performed with the participants laying supine on an adjustable gurney.

Skin temperature and skin blood flow

Skin temperature was monitored and manipulated using an integrated skin heater and temperature monitor (SH02, Moor Instruments Devon, UK). Red blood cell flux was measured as an index of skin blood flow by laser-Doppler-flowmetry (MoorVMS-LDF2, Moor Instruments, Devon, UK). Laser-Doppler flow (LDF) probes (VP12, Moor Instruments, Devon, UK) were placed in a small aperture in the skin heaters enabling the measurement of skin blood flow at the site of skin heating. In protocols 2 and 3, laser-Doppler probes and local heaters were placed directly above the microdialysis membranes.

Intradermal microdialysis

Intradermal microdialysis was used to administer pharmacological agents in protocols 2 and 3. As per our previous work in this area (Carter and Hodges, 2011; Del Pozzi et al., 2013; Hodges and Sparks, 2013; Tew et al., 2011d), custom built microdialysis fibers were placed intradermally using a 22-gauge needle. Fibers were placed on the dorsal aspect of the forearm and the lateral aspect of the calf (Del Pozzi et al., 2013). A 90-min trauma resolution period was allowed prior to data collection at skin sites instrumented with microdialysis fibers (Hodges et al., 2009a).

Pharmacological agents

Drugs were infused at all skin sites *via* intradermal microdialysis fibers and infused at a rate of 4 $\mu l \cdot min^{-1}$. Bretylium tosylate (BT) (US Pharmacopeia, Rockville, MD USA) was infused at a concentration of 10 mM (Carter and Hodges, 2011; Hodges et al., 2009b; Tew et al., 2011c). BT pre-synaptically blocks the release of sympathetic neurotransmitters (Haeusler et al., 1969). N^G-nitro-L-arginine methyl ester (L-NAME) (US Pharmacopeia, Rockville, MD USA) was administered at a concentration of 20 mM (Hodges et al., 2008; Hodges et al., 2009b; Hodges et al., 2006). As per our pilot work and previous studies, drugs were infused for a minimum of 60 min before collection of data. Sodium nitroprusside (SNP) was infused at all sites at a concentration of 58 mM for 35 min to elicit maximal cutaneous vascular conductance (CVC) at the end of protocols 2 and 3 (Carter and Hodges, 2011; Kellogg et al., 2009; Kellogg et al., 2010; Wong and Fieger, 2012).

Blood pressure

Through direct (Park and Guntheroth, 1970) and indirect (Sareen et al., 2012) methods it has previously been demonstrated that supine mean arterial pressure does not differ between the arm and the leg. Blood pressure was measured through oscillometry (BPTru Medical Devices, Coquitlam, BC Canada) in the contra-lateral arm every 5 min throughout the duration of the study protocol. Mean arterial pressure (MAP) was calculated as;

$$((2 \cdot \text{diastolic blood pressure} + \text{Systolic blood pressure}) \div 3).$$

Local heating and heat–reheat protocols

Local skin heaters were set at 33 °C (thermoneutral) prior to instrumentation and allowed to stabilize and then placed on the skin sites. After 10 min, and confirmation that the recordings were stable, baseline measurements were recorded at 33 °C for 10 min, local skin temperature was then increased to 42 °C at 0.5 °C·10 s^{-1}.No sensation of pain was reported by any participant as a result of the local skin heating protocol. All skin sites were maintained at 42 °C until a stable plateau in skin blood flow had been achieved, after which, all skin heaters were returned to 33 °C and the skin was allowed to passively cool for ~60 min (Stewart et al., 2008a; Stewart et al., 2008b), though some sites did take longer to return to stable baseline skin blood flow. After a second 10 min baseline was recorded at 33 °C, the reheat protocol was initiated, increasing local skin temperature as per the first heating protocol. All sites were maintained at 42 °C until a stable plateau had been achieved.