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The contribution of sensory nerves to the onset threshold for cutaneous vasodilatation during gradual local skin heating of the forearm and leg

Gary J. Hodges [a,1], Gregory W. McGarr [a,1], Matthew M. Mallette [a], Andrew T. Del Pozzi [b], Stephen S. Cheung [a,*]

[a] Environmental Ergonomics Laboratory, Department of Kinesiology, Brock University, St. Catharines, ON L2S 3A1, Canada
[b] Integrative Exercise Physiology Laboratory, School of Kinesiology, Ball State University, Muncie, IN 47306, USA

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ABSTRACT

During local skin heating, the temporal onset of vasodilatation is delayed in the leg compared to the forearm, and sensory nerve blockade abolishes these differences. However, previous work using rapid skin heating did not allow for determination of sensory nerve influences on temperature thresholds for vasodilatation. Two sites were examined on both the forearm and leg, one control (CTRL), and one treated for sensory nerve blockade (EMLA). Skin blood flux was monitored using laser-Doppler probes, with heaters controlling local skin temperature (T_{loc}). T_{loc} was increased from 32–44 °C (+1 °C·10 min^{-1}). Stimulus–response curves were constructed by fitting a four-parameter logistic function. EMLA significantly increased T_{loc} onset in the forearm (CTRL = 35.3 ± 0.4 °C; EMLA = 36.8 ± 0.7 °C) and leg (CTRL = 36.5 ± 0.4 °C; EMLA = 38.4 ± 0.5 °C; both $P < 0.05$). At both CTRL and EMLA, T_{loc} onset was higher in the leg compared to the forearm (both $P < 0.05$). In the forearm, median effective temperature to elicit 50% vasodilatation (ET_{50}) was similar between sites (CTRL = 39.7 ± 0.3 °C; EMLA = 40.2 ± 0.4 °C; $P = 0.09$); however, in the leg, EMLA significantly increased ET_{50} (CTRL = 40.2 ± 0.3 °C; EMLA = 41.0 ± 0.3 °C)($P < 0.05$). At CTRL sites, no limb difference was observed for ET_{50} ($P = 0.06$); however, with EMLA, ET_{50} was significantly higher in the leg ($P < 0.05$). EMLA significantly increased the gain of the slope at the forearm, (CTRL = 0.31 ± 0.01%CVC_{max}·°C^{-1}; EMLA = 0.45 ± 0.07%CVC_{max}·°C^{-1}), and leg (CTRL = 0.37 ± 0.05%CVC_{max}·°C^{-1}; EMLA = 0.54 ± 0.04%CVC_{max}·°C^{-1})(both $P < 0.05$). At CTRL sites, the gain was significantly higher in the leg ($P < 0.05$); however, for EMLA, no significant limb difference existed ($P = 0.10$). These data indicate that the onset of vasodilatation occurs at a lower temperature in the forearm than the legs, and sensory nerves play an important role in both limbs.

Introduction

The cutaneous vascular response to a local skin heating, without a change in core temperature, is not dependent on reflex innervation (Pergola et al., 1993), and appears to be an entirely local phenomenon (Johnson et al., 2014). Localized heating of the skin elicits a biphasic vasodilatory response. There is an early, rapid vasodilatation during the first 5 min of heating ('initial peak') that is dependent on local sensory nerve function (Carter and Hodges, 2011; Hodges et al., 2015; Minson et al., 2001; Strom et al., 2010; Tew et al., 2011). This is succeeded by a secondary, gradual and prolonged vasodilatation (plateau phase) that is dependent on endothelial nitric oxide (eNOS) (Hodges and Sparks, 2013; Kellogg et al., 2008, 2009) and endothelial derived hyperpolarizing factor (Brunt and Minson, 2012).

We have previously observed marked differences between the thermal vasodilatory responses in the skin of the forearm and leg. In response to local skin heating, the magnitude of the initial peak is greater in the forearm compared to the leg. Sensory nerve blockade abolishes the difference in the initial peak response between the forearm and leg (Hodges et al., 2015). During prolonged skin heating, the leg presents with higher vasodilatation compared to the arm, which appears to be due to increased endothelial function (Hodges and Del Pozzi, 2014), a difference that is abolished with NOS inhibition (Del Pozzi et al., 2013). It is clear that there are differences in cutaneous vasodilatation to local skin heating between the limbs.

There are also clear temporal differences in the onset of vasodilatation in the skin of the forearm (~20 s) and leg (~50 s) (Del Pozzi and Hodges, 2015a; Hodges et al., 2015) in response to a standard local skin heating protocol (+3 °C·min^{-1}). We observed that blockade of sensory nerve function (with topical EMLA cream) delayed the onset of vasodilatation, but the difference in the magnitude of the sustained vasodilatory response between limbs persisted (Hodges et al., 2015); however, sensory nerve blockade with EMLA abolished this difference

Abbreviations: CTRL, untreated control; CVC, cutaneous vascular conductance; ET_{50}, median (or half-maximal) temperature; LDF, laser-Doppler fluxmetry; MAP, mean arterial pressure; NOS, nitric oxide synthase; Thr_{on}, onset threshold; T_{loc}, local skin temperature; T_{sk}, mean whole body skin temperature.

* Corresponding author at: Department of Kinesiology, Brock University, St. Catharines, ON L2S 3A1, Canada.
E-mail address: scheung@brocku.ca (S.S. Cheung).

[1] G.J. Hodges and G.W. McGarr contributed equally to this work.

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between limbs and greatly increased time to onset of vasodilatation (both ~150 s) (Hodges et al., 2015). Additionally, these temporal differences between regions and with sensory nerve blockade occurred for all phases of the local heating response. While the duration for onset of vasodilatation was clearly longer in the leg compared to the arm, the rapid heating rate ($+3$ °C·min^{-1}) did not allow for a determination of whether this difference was simply due to the duration of local heating *per se*, or if a greater local temperature (higher temperature onset threshold) was required to elicit vasodilatation in the legs compared to the forearms.

Therefore, the aim of the present study was to examine the temperature onset threshold for cutaneous vasodilatation in the skin of the forearm and leg, with and without sensory nerve blockade, in response to gradual, step increases in local skin temperature ($+1$ °C·10 min^{-1}); to achieve this, four skin sites were examined, two on each of the forearm and leg. On each limb, one site was treated with EMLA to block sensory nerve function while the other remained untreated. Laser-Doppler probes with integrated skin heaters were placed at the predetermined sites and local skin temperature was maintained at 32 °C for basal measures and progressively increased to 44 °C.

Material and methods

Participants

All participants were fully informed of the experimental methods and risks prior to volunteering and each provided verbal and written informed consent. The study was approved by the Bioscience Research Ethics Board at Brock University (REB 14-112). All experimental protocols conformed to the guidelines set forth by the Declaration of Helsinki.

Nine healthy, active participants (5 men and 4 women, 25 ± 1 years, 170 ± 6 cm, 70 ± 3 kg) volunteered for this study and attended the laboratory twice. Participants were not diagnosed with any metabolic or cardiovascular disease, were nonsmokers and not taking any medication other than prescription birth control. Participants were instructed to abstain from caffeine for 12 h and alcohol for 24 h prior to testing. Additionally, the participants were instructed not to eat for 1 h prior to the testing session, but were encouraged to drink water *ad libitum*. As in our laboratory's previous studies female participants were currently in the low hormone phase of their birth control routine as verified through self-report (Del Pozzi and Hodges, 2015a, 2015b, 2015c; Hodges et al., 2015).

Instrumentation and experimental procedures

All procedures were performed with the participants resting and supine. Laboratory ambient temperature was 22 ± 1 °C and the relative humidity was 33 ± 4% during experimental sessions. The two experimental sessions were identical in protocol, except that one session tested the forearm and one session the leg; the order of presentation was counterbalanced among participants, and sessions were performed during the same time of day and no more than two days apart. Two skin sites on both the forearm and leg were examined. Specifically, the dorsal aspect of the forearm equivalent to one-third of the way from the antecubitis and the anterolateral aspect of the calf, equivalent to one-third from the popliteus. One site was randomly chosen to receive topical anesthetic (EMLA) cream treatment (AstraZeneca, Wilmington DE, USA; 2.5% lidocaine and 2.5% prilocaine) while the other site remained untreated (CTRL). As per our previous experiments blocking cutaneous sensory function with EMLA cream (Carter and Hodges, 2011; Hodges et al., 2007, 2009a, 2015; Tew et al., 2011), approximately 2.5 g of the EMLA cream was applied on the forearm or leg to an area of skin roughly equal to 4 cm^2. An occlusive dressing (Tegaderm, 3M, London, ON, Canada) was placed over the applied EMLA cream and left for 1 h. The EMLA cream was then wiped clear and a second application was applied for an additional hour. After the second application of EMLA was removed from the site and following the completion of the study, sensory nerve blockade over the area of interest was verified by alternating between brushing, pricking, and pinching with a set of pointed tweezers (Carter and Hodges, 2011; Hodges et al., 2007, 2015; Lorenzo and Minson, 2007; Minson et al., 2001; Tew et al., 2011). Previous work with this approach of two, 1 h applications of EMLA generally blocks sensory nerve function for >3.5 h. A combined skin heater and temperature monitor and controller (5010 LDPM, Perimed, Järfälla, Sweden) was used to adjust and control local skin temperature (T_{loc}) at the skin sites. Red blood cell flux was measured via laser-Doppler fluxmetry (PF5000, Perimed, Järfälla, Sweden) and was used to provide an index of skin blood flow (Johnson, 1990; Öberg, 1990). Integrated laser-Doppler flux (LDF) and skin heater probes (Small angled thermostatic probe 457, Perimed, Järfälla, Sweden) were used to monitor red blood cell flux and monitor and control T_{loc}. The LDF probes were placed on CTRL and EMLA treated sites and T_{loc} was set at 32 °C. Mean whole body skin temperature ($\overline{T}_{sk}$) was obtained from four thermocouples (PVC-T-24-190, Omega Environmental Inc., Laval, QC, CAN) that were taped (Transpore™, 3M, St. Paul, MIN, USA) to the chest, thigh, arm, and calf. $\overline{T}_{skin}$ was calculated using the Ramanathan equation (Ramanathan, 1964):

$$0.3(\text{thigh}) + 0.3(\text{chest}) + 0.2(\text{calf}) + 0.2(\text{arm})$$

After confirming that LDF values were stable, baseline measurements were recorded for 10 min. Following baseline, local skin heating was performed by increasing T_{loc} by $+1$ °C·10 min^{-1} to 44 °C.

Blood pressure

Blood pressure from the left arm was measured by auscultation every 20 min throughout the duration of the experiment. Direct (Park and Guntheroth, 1970) and indirect (Sareen et al., 2012) methods demonstrate that supine mean arterial pressure (MAP) does not differ between the arm and the leg. MAP was calculated as:

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

Data collection and analysis

All continuous data were collected at 40 Hz (PowerLab, ADInstruments, Colorado Springs CO, USA) and stored on a personal computer to be analyzed offline using signal-processing software (Lab Chart v8, ADInstruments, Colorado Springs CO, USA). Laser-Doppler fluxmetry data were converted to cutaneous vascular conductance (CVC) by dividing LDF (mV) by the calculated MAP (mm Hg). Data were normalized to maximal vasodilatation and expressed as a percentage of the maximum CVC (%CVC$_{max}$) for each site. $\overline{T}_{sk}$ was collected and expressed in °C.

The final 2 min during each local skin temperature manipulation period was used for subsequent analyses. Consistent with the analytical approach previously described by Wenner et al. (2011), the CVC data were fitted using a four-parameter logistic equation with a variable slope. The curves were further normalized, with constraints set for the bottom (0%CVC$_{max}$; T_{loc} = 32 °C) and top (100%CVC$_{max}$; T_{loc} = 44 °C) of each curve to allow for direct comparisons across multiple skin sites. The effective T_{loc} that elicits 50% of the maximum skin blood flow response (ET$_{50}$) and gain of the slope from each stimulus–response curve were determined by non-linear regression curve fitting according to the following equation:

$$Y = 100/(1 + 10\ \hat{}\ ((\log ET_{50} - X) * \text{slope}))$$

where Y is the %CVC$_{max}$, and X is the local skin temperature (°C) applied. The slope here defines the sensitivity (gain) of the skin blood flow