The role of baseline in the cutaneous vasoconstrictor responses during combined local and whole body cooling in humans

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Hodges GJ, Kosiba WA, Zhao K, Alvarez GE, Johnson JM. The role of baseline in the cutaneous vasoconstrictor responses during combined local and whole body cooling in humans. Am J Physiol Heart Circ Physiol 293: H3187–H3192, 2007. First published September 28, 2007; doi:10.1152/ajpheart.00815.2007.—Previous work showed that local cooling (LC) attenuates the vasoconstrictor response to whole body cooling (WBC). We tested the extent to which this attenuation was due to the decreased baseline skin blood flow following LC. In eight subjects, skin blood flow was assessed using laser-Doppler flowmetry (LDF). Cutaneous vascular conductance (CVC) was expressed as LDF divided by blood pressure. Subjects were prepared with microdialysis fibers, local heating/cooling probe holders, and laser-Doppler probes. Three sites were locally cooled from 34 to 28°C, reducing CVC to 45.9 ± 3.9, 42 ± 3.9, and 44.5 ± 4.8% of baseline (P < 0.05 vs. baseline; P > 0.05 among sites). At two sites, CVC was restored to precooling baseline levels with sodium nitroprusside (SNP) or isoproterenol (Iso), increasing CVC to 106.4 ± 12.4 and 98.9 ± 10.1% of baseline, respectively (P > 0.05 vs. precooling). Whole body skin temperature, apart from the area of blood flow measurement, was reduced from 34 to 31°C. Relative to the original baseline, CVC decreased (P < 0.05) by 44.9 ± 2.8 (control), 11.3 ± 2.4 (LC only), 29 ± 3.7 (SNP), and 45.8 ± 8.7% (Iso). The reductions at LC only and SNP sites were less than at control or Iso sites (P < 0.05); the responses at those latter sites were not different (P > 0.05), suggesting that the baseline change in CVC with LC is important in the attenuation of reflex vasoconstrictor responses to WBC.

THERMAL HOMEOSTASIS IS MAINTAINED in part by active regulation of the cutaneous circulation, which has a wide range of adjustment. At rest, skin blood flow is ~5% of cardiac output (~0.25 l/min), whereas during hyperthermia skin blood flow can be as high as 6–8 l/min, accounting for ~60% of cardiac output. By contrast, during extreme hypothermia skin blood flow can be almost negligible (18, 30).

There have been many studies designed to characterize the cutaneous vasoconstrictor responses to direct local cooling (13, 16, 19, 24, 28, 29, 35, 36, 38) and to whole body cooling (28, 29, 33). It has been established that whole body cooling elicits a sympathetic vasoconstrictor reflex (18, 33) that can be abolished with presynaptic sympathetic vasoconstrictor nerve inhibition (20). Local skin cooling has a sympathetic vasoconstrictor component (3, 13, 16, 19, 24, 29, 38). The vasoconstriction induced by local cooling also has a component that is nonneural in nature, which has recently been shown to be due to effects of local temperature on both basal nitric oxide synthase function and other processes downstream of the nitric oxide synthase enzymes in that pathway (16, 38).

Despite the large volume of work assessing the cutaneous vascular responses to independent local and whole body cooling, there has been little investigation into their combined effects, as would occur under environmental cooling. Alvarez et al. (1) investigated the separate and combined effects of local and whole body cooling, finding that local cooling causes an attenuation of the reflex response to whole body cooling. In that study, Alvarez et al. showed that the response to combined local and whole body cooling was significantly less than the arithmetic sum of the responses when local and whole body cooling were performed individually. They further showed a significant attenuation of the reflex response in the presence of local cooling. It was this finding that prompted us to test the extent to which this decreased reflex response was due to the effects of local cooling on the reduced baseline blood flow. It has long been accepted that the measurement of autonomic responses frequently varies with the values extant before the stimulation (5, 23, 37). One way to establish the effects of cooling, per se, on the reflex response, distinct from the effects via the altered baseline, would be to restore CVC levels to the baseline that existed before local cooling. Therefore, to determine the effects of local cooling on the reflex response without a change in baseline blood flow, we locally cooled the skin, pharmacologically restored skin blood flow to precooling levels while maintaining the reduced local temperature, and then applied whole body cooling. We tested the hypothesis that restoration of skin blood flow to baseline from the reduced levels associated with local cooling would remove the apparent local cooling-induced attenuation of the reflex vasoconstrictor response. We also tested the hypothesis that such restoration with a nitric oxide donor would not as fully restore reflex vasoconstriction as would restoration with a β-adrenergic agonist. This latter hypothesis arose from the observation that nitric oxide has an inhibitory effect on adrenergic vasoconstrictor function in the skin (12), whereas presynaptic stimulation of β-adrenergic receptors enhances norepinephrine release (6, 32).

METHODS

Subjects. The local Institutional Review Board approved this study, which conformed to the standards set by the Declaration of Helsinki, and all subjects were fully informed of the methods and risks before written informed consent was obtained. All eight subjects who participated (6 men and 2 women; age 30 ± 2 yr), were healthy nonsmokers, nonobese (body mass index 25 ± 1.5), were not taking...
any medications, and refrained from alcoholic and caffeinated beverages for at least 12 h before the study. For the female subjects, the phase of the menstrual cycle, although recorded, was not considered in these experiments, since previous studies have reported that the vasoconstrictor responses to local cooling are unaffected by female reproductive hormones (7). Furthermore, the data from the female subjects displayed no differences from those of the males, so the data were combined.

*Instrumentation.* Subjects had four microdialysis probes placed intradermally on the ventral aspect of the left forearm. As described previously (11, 21), these custom-built probes consisted of 1 cm of microdialysis tubing (inner diameter 200 μm, 18-kDa nominal molecular mass cutoff) attached at each end to polyimide tubing. Before implantation, the area of forearm skin was temporarily anesthetized by the application of an ice pack for 5 min. A 25-gauge needle was introduced aseptically for ~2.5 cm into the dermis before exiting. The microdialysis probe (1 cm in length) and the connecting tubing were introduced into the skin via the lumen of the needle. The needle was then removed, leaving the probe in place. All probes were placed in this manner, and ~1.5 h were allowed for the effects of the insertion trauma to subside (2). The different probes were placed 3–5 cm apart.

*Measurements.* All measurements were performed with the subjects resting in the supine posture. Skin blood flow was measured from the ventral aspect of the forearm by laser-Doppler flowmetry (Moor Instruments, Axminster, UK) and expressed as laser-Doppler blood flow (17, 25). Laser-Doppler blood flow measurements are exclusive to the skin and are not influenced by underlying skeletal muscle blood flow (31). Local temperature control at the sites of blood flow measurement was achieved with custom-designed metal Peltier cooling/heating probe holders (1, 16, 19, 38). These controlled surface temperature within 0.1°C over an area of 6.3 cm² with the exception of a small aperture (0.28 cm²) in the center of the holder to enable placement of the laser-Doppler probe. A thermocouple placed between the skin surface and the probe holder enabled local skin temperature assessment and feedback control. Blood pressure was recorded noninvasively and continuously by using the Penaz method (27) from the left middle finger (Finapres; Ohmeda, Madison, WI). Mean arterial pressure was obtained from the electrical integration of the continuous blood pressure signal. Cutaneous vascular conductance (CVC) was calculated as the ratio of laser-Doppler flow to mean arterial pressure. Sodium nitroprusside and isoproterenol were administered at separate sites, via microdialysis, in concentrations aimed to restore skin blood flow to the values seen before local cooling. After skin blood flow had been restored to precooling levels, whole body cooling was applied in a 15-min ramp, reducing skin temperature from 34 to 31°C during this period to assess the reflex response to whole body skin cooling.

*Data analysis.* Data for CVC were analyzed from the final 5 min of each section (see Fig. 1). CVC was expressed relative to the initial baseline values for each site. Absolute values for CVC were used to determine whether sites were at unusually high or low values as a result of the pharmacological background or the preparatory procedures; however, there were no such cases in this study. Changes in CVC, expressed as a percentage of the original baseline CVC, were used to evaluate the vasoconstrictor response to each stage of the study including, importantly, the reduction in CVC in response to whole body cooling. Analysis was performed by using paired statistics or, when appropriate, repeated-measures ANOVA. Statistical significance was assumed when \( P < 0.05 \). Power analysis indicated that a minimum of seven subjects would be required for a \( P < 0.05 \) with 95% power.

**RESULTS**

Results from a complete study for the local cooling, baseline restoration, and reflex responses to whole body cooling are shown for a representative subject in Fig. 2. Results from the responses to local cooling, averaged from all eight subjects,
are shown in Fig. 3. Local cooling, applied to sites 2, 3, and 4 before the baseline restoration phase, caused significant vasoconstriction, which did not differ significantly among sites ($P > 0.05$). CVC fell to $45.9 \pm 3.9$, $42 \pm 3.9$, and $44.5 \pm 4.8\%$ of baseline at those sites ($P < 0.05$ relative to precooling; Fig. 3). We were successful in restoring baseline CVC at site 3 with sodium nitroprusside and at site 4 with isoproterenol. CVC at those sites rose to $106.4 \pm 12.4$ and $98.9 \pm 10.1\%$ of baseline, respectively, which did not differ significantly from the original baseline values ($P > 0.05$; Fig. 4).

Reflex responses were assessed from the changes in CVC accompanying whole body cooling (Fig. 5). There was a

Fig. 2. A representative tracing of CVC, normalized to baseline, from 1 subject for the protocol outlined in Fig. 1. Local cooling to 28°C began at 10 min at sites 2–4 (first vertical line) and was held at this level throughout the remainder of the study. Sites 3 and 4 had baseline CVC restored with SNP and Iso, respectively, at the 50-min mark, and these infusions continued throughout the rest of the study. Compare the large decrease at the control site to the small decrease at the LC site in response to WBC. Furthermore, note how, at the site with baseline restoration with Iso, the complete reflex response to WBC is shown, unlike that at the site with baseline CVC restored with SNP.

Fig. 3. Response to local cooling. Measured before pharmacological intervention for baseline restoration, CVC is expressed as a percentage of baseline at all 4 sites during local cooling of sites 2–4 ($n = 8$). Note the similar significant decreases at all 3 locally cooled sites. *$P < 0.05$ compared with baseline.

Fig. 4. Baseline restoration. Sites 3 and 4 were treated with SNP and Iso, respectively, to restore CVC to the original baseline. The restoration of baseline at sites 3 and 4 was successful, with CVC values not significantly different (n.s.) from the original baseline values ($n = 8$). *$P < 0.05$.
significant reduction in CVC at all sites in response to the reduction in whole body skin temperature (Fig. 5; *P < 0.05); nevertheless, the degree of vasoconstriction differed according to the status of the site. When the fall in CVC is expressed relative to the original baseline (BØ) control sites (no local cooling; site 1), the fall in CVC (−44.9 ± 2.8% BØ) significantly exceeded that at the locally cooled sites (site 2, −11.3 ± 2.4% BØ; *P < 0.05) in accordance with earlier findings (1). The reflex reduction in CVC at the locally cooled site with baseline restored with isoproterenol (site 4, −45.8 ± 8.7% BØ) did not differ from that at the control site (*P > 0.05; Fig. 5). At locally cooled sites with baseline restoration with sodium nitroprusside (site 3, −23 ± 3.7% BØ), there was an intermediate response such that the reduction in CVC at those sites significantly exceeded that at the locally cooled sites without baseline restoration (site 2; *P < 0.05) but was significantly less than that at either the control sites or at the sites with baseline restoration with isoproterenol (Fig. 5; *P < 0.05).

DISCUSSION

The major finding of this investigation is that the attenuation by local skin cooling of the reflex cutaneous vasoconstrictor response to whole body cooling is no longer observed when CVC is restored to precooling baseline levels (with isoproterenol), indicating an important role for the initial baseline in the magnitude of the reflex response to whole body cooling; i.e., a lower baseline reduced the subsequent response. In addition, we confirmed that exogenously administered nitric oxide (via sodium nitroprusside infusion) attenuates the reflex cutaneous vasoconstrictor response to whole body cooling, in agreement with earlier studies (12).

There has been a considerable amount of work investigating the effects of local or whole body cooling on the cutaneous circulation. When performed independently, each stimulus can elicit a pronounced vasoconstriction. Only recently, however, has there been any assessment of the interaction of the two stimuli, as would occur with environmental cooling. In a recent study (1), it was reported that when local and whole body cooling are performed, local cooling attenuates the reflex reduction in CVC to whole body cooling, suggesting an interaction between the stimuli. The same result could be anticipated from the results of Pérgola et al. (28), who examined the combined and separate effects of whole body and local cooling on the vasodilator responses to rising internal temperature. We tested to what extent this smaller reflex response was a function of the reduced baseline (lower CVC levels) following the application of local cooling (i.e., the response being a function of the initial levels) or whether this attenuation was because the lower temperature from local cooling inhibited the mechanisms that induce reflex vasoconstriction. We found the reduced baseline to explain much of the reduced sensitivity, but the extent depended on the method by which baseline CVC was restored. Restoration of CVC with isoproterenol to the level before local cooling essentially restored the reflex response, whereas restoration of CVC to the original baseline with sodium nitroprusside restored ~50% of the reflex response.

The possibility of baseline playing an important role in the above interaction was highlighted by the findings of Alvarez et al. (1). There were similar percent reflex reductions in CVC in response to whole body cooling with and without local cooling, and it was suggested that, at the levels of local cooling used, the influence of baseline could have been more important relative to the reduced vasoconstrictor response than the effects of local cooling on adrenergic vasoconstrictor mechanisms. The reflex vasoconstrictor response to whole body cooling is a relatively straightforward engagement of sympathetic vasoconstrictor nerve activity (18, 28, 33), whereas local cooling is a complex combination of the local stimulation of vasoconstrictor nerves, alterations in norepinephrine release, synthesis, and reuptake, adjustment of α-adrenergic receptor expression, and an inhibition of nitric oxide synthase and downstream nitric oxide-dependent processes (3, 4, 9, 13, 11, 19, 24, 29, 36, 38). As such, local cooling has multiple sites at which it could affect noradrenergic responses. For example, in aged skin, adrenergic responses are greatly reduced (35). Nevertheless, we found that with equipotent local and whole body cooling, the attenuation of the reflex response in CVC in the presence of local cooling was no longer present following the restoration of baseline CVC with isoproterenol. Whole body cooling following restoration of CVC to baseline with isoproterenol led to a complete restoration of the reflex response in CVC (control −44.9 ± 2.8% vs. isoproterenol −45.8 ± 8.7% BØ; Fig. 5). That is, the reflex activation of vasoconstrictor nerves had the same effect despite a difference in tissue temperature of 6°C. This finding confirmed our hypothesis that the reduced reflex response was due, at least in part, to the lowered baseline in the presence of local cooling. Although it might seem obvious, it is interesting to note that despite the change in response to whole body cooling at the locally cooled site being small, the absolute CVC levels at this site are the lowest. This suggests that despite pronounced cold-induced vasoconstriction already occurring, further constriction is possible.

The importance of baseline, or initial values, in determining the magnitude of reflex responses was noted over 50 years ago by Wilder (37), who termed the “Law of Initial Values” to describe this influence on experimental outcome. In addition, O’Leary (26) noted that the vascular response to a given stimulus varied with baseline and that whether a response was enhanced or reduced depended on initial values, which led to the enigmatic observation that the use of vascular resistance or conductance as a measure of response determined whether the
response to a second stimulus was enhanced or reduced by, for example, background thermal status. This problem would exist in the current study, as well, but we believed by restoring CVC (and vascular resistance), this problem was overcome by restoring baseline.

Therefore, these data suggest that the initial baseline is an important factor in the modification by local cooling of the magnitude of the reflex response to whole body cooling. Thus it might, initially at least, appear that the known effects of local cooling on norepinephrine synthesis, release, and reuptake (36), causing a translocation of \( \alpha_2 \)-receptors (3, 4, 9), and the inhibitory effect on nitric oxide synthase and downstream nitric oxide-dependent processes (16, 38) are not important. However, we do not believe this to be the case. As stated, once baseline CVC was restored, a complete reflex vasoconstriction occurred in response to the whole body cooling; this was despite the 6°C reduction in local temperature. With the cold-induced modifications mentioned above, however, it might be expected that the response to whole body cooling would have been enhanced due to the greater number of \( \alpha_2 \)-receptors and the decreased nitric oxide synthase activity (3, 16, 38). Furthermore, isoproterenol is known to enhance norepinephrine release through stimulation of presynaptic \( \beta \)-receptors (6, 32). Yet, it is known that reducing local temperatures also inhibits norepinephrine synthesis, release, and reuptake (36). Thus, an alternate possibility, which deserves strong consideration, is that the net vasoconstriction produced remained the same as the baseline vasoconstrictor function (12). A strength of the current investigation was that by using both of these, independently, to restore baseline CVC, we could estimate that the baseline effect accounts for between 50% (nitroprusside) and 100% (isoproterenol) of the reduction in reflex responsiveness, which is unlikely to be at either extreme. The effects of cooling on the vascular smooth muscle-vasoconstrictor nerve unit would account for the remainder of the reduction in response. Furthermore, the degree of local cooling used may have also influenced the results. With more aggressive local cooling (e.g., local temperature <26°C, whole body <29°C), roles for receptor translocation, beyond baseline influences, could become more prominent. This was our original aim, but we found that at lower local temperatures, isoproterenol was ineffective in restoring baseline CVC (unpublished data).

In summary, the present study suggests that the attenuation of the reflex cutaneous vasoconstrictor response to whole body cooling in the presence of local cooling is due largely to the reduced baseline. We also found further evidence that nitric oxide attenuates adrenergic vasoconstrictor responses in the cutaneous circulation.

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GRANTS

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REFERENCES


