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 tested at control or Iso 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 the 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.

nitrergic tone, nitric oxide; cutaneous circulation; isoproterenol

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 nitrergic 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 extent 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

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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 measures 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 probes (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 measured 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 (in arbitrary units). Whole body skin temperature was the weighted mean from six thermocouples placed on the body surface and was controlled by the use of water-perfused suits (34). The suit covered the entire body surface apart from the head, hands, feet, and the forearm used for the blood flow measurements. This arrangement allowed independent control of local skin temperature and whole body skin temperature. All variables were collected at 1-s intervals and stored as 20-s averages.

Sodium nitroprusside and isoproterenol (Sigma Chemical, St. Louis, MO) were used to reverse the vasoconstrictor effects of local cooling and were administered via microdialysis. They were prepared in sterile saline at concentrations of 20 or 25 μM sodium nitroprusside and 300 or 350 μM isoproterenol; solutions were perfused at 4 μl/min for 20 min. Different concentrations were used because we were aiming to restore CVC as close to baseline values as possible, and the dose required varied slightly among subjects.

**Protocols.** Protocols were designed to test whether restoration of baseline CVC in the presence of local cooling reversed the attenuation of the reflex response to whole body cooling; i.e., what is the role of lower temperature per se and what is the role of a lower initial baseline CVC? In each of eight subjects, four forearm sites were prepared with microdialysis fibers, Peltier probe holders, and laser-Doppler probes. Figure 1 shows the protocol. After 10–15 min of baseline measurements, during which whole body skin temperature and all local temperatures were maintained at 34°C, we applied slow local cooling (~0.33°C/min) to three of the four sites, maintaining the remaining site at 34°C. Local temperature at those three sites was cooled to 28°C and was held at that level for the remainder of the study. Once stabilization of CVC had occurred (~20 min), 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 ± 3.9, 42 ± 3.9, and 44.5 ± 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 ± 12.4 and 98.9 ± 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
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 preclothing 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 inter-

**DISCUSSION**

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**Fig. 5.** Response to whole body cooling. CVC is expressed as the percent change from original baseline levels (n = 8). Note the small decreases at the LC sites and the intermediate responses at the SNP-treated sites. Importantly, observe the fully restored responses at the Iso-treated sites and how they are similar to those at the control sites. *P < 0.05 compared with control and Iso-treated sites. †P < 0.05 compared with SNP-treated sites.

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 (BO) control sites (no local cooling; site 1), the fall in CVC (−44.9 ± 2.8% BO) significantly exceeded that at the locally cooled sites (site 2, −11.3 ± 2.4% BO; 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% BO) 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% BO), 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).
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 α2C-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 α2C-receptors and the decreased nitric oxide synthase activity (3, 16, 38). Furthermore, isoproterenol is known to enhance norepinephrine release through stimulation of presynaptic β-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 norepinephrine synthesis, release, and reuptake (36). Thus an alternate possibility, which deserves strong consideration, is that the net vasoconstriction produced remained the same as a result of the increased number of postjunctural α2C-receptors and stimulatory effects of isoproterenol balanced by cold-induced reduction of norepinephrine synthesis, release, and reuptake.

Importantly, the degree of restoration of the reflex response depends on the vasodilator used to restore CVC to original baseline levels. Whole body cooling following restoration of CVC to original baseline levels with isoproterenol led to a complete restoration of the reflex vasoconstriction in CVC (Fig. 5). In contrast, baseline restoration with sodium nitroprusside led to only a partial restoration of the reflex response in CVC (Fig. 5). This is probably due the effects of the vasodilators used. As noted above, β-adrenergic stimulation facilitates the release of norepinephrine (6, 32), whereas nitric oxide has an inhibitory effect on cutaneous reflex adrenergic vasoconstrictor function (12). For example, Durand et al. (12) reported that the reduction in CVC in response to a 3-min whole body cooling stress test was less when performed in the presence of sodium nitroprusside than in the presence of isoproterenol. Their data suggested that in skin, as in other vascular beds (8, 10, 14, 15, 22, 39), nitric oxide is capable of inhibiting sympathetically mediated vasoconstriction. Hence, the smaller reflex response at the site treated with sodium nitroprusside is not likely to be due only to the lower local temperature but also to the inhibitory effects of sodium nitroprusside on the vasoconstrictor effects of released norepinephrine.

A limitation to this study is that any vasodilator used to restore baseline will potentially enhance or inhibit some element within the reflex so that restoration of the baseline may bring about an altered reflex in some part of the synthesis, release, and/or postsynaptic effects of norepinephrine. As pointed out earlier, isoproterenol enhances norepinephrine release (6, 32), and nitroprusside inhibits adrenergic 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

H3192  SKIN COOLING AND SKIN BLOOD FLOW