Local ascorbate administration inhibits the adrenergic vasoconstrictor response to local cooling in the human skin

Fumio Yamazaki

Laboratory for Human Physiology, School of Health Sciences, University of Occupational and Environmental Health, Kitakyushu, Japan

Submitted 27 July 2009; accepted in final form 7 December 2009

Yamazaki F. Local ascorbate administration inhibits the adrenergic vasoconstrictor response to local cooling in the human skin. J Appl Physiol 108: 328–333, 2010. First published December 10, 2009; doi:10.1152/japplphysiol.00814.2009.—Local cooling (LC) of nonglabrous skin causes vasoconstriction via the adrenergic and removal of nitric oxide (NO) systems. Since cooling increases reactive oxygen species in smooth muscle cells and induces increased sensitivity of \( \alpha \)-adrenergic receptors, antioxidant supplementation may attenuate the vasoconstrictor response to skin LC via adrenergic and/or NO systems. To test this hypothesis, we examined the effects of acute \( \alpha \)-ascorbate (Asc, 10 mM) supplementation on the adrenergic component in the vasoconstrictor responses to LC in skin with and without NO synthase (NOS) inhibition or adrenergic receptor blockade. In a three-part study, forearm sites were instrumented with microdialysis fibers, local coolers, and laser-Doppler flow (LDF) probes in healthy volunteers. Sites were cooled from 34 to 24°C at \(-1°C/min\) and maintained at 24°C for 20 min (parts 1 and 2) or 30 min (part 3). During the last 10 min of LC in parts 1 and 2, whole body cooling was performed to increase sympathetic vasoconstrictor activity. Cutaneous vascular conductance (CVC) was calculated as the ratio of LDF to blood pressure and expressed relative to the baseline value before cooling. Treatment in each part were as follows: part 1 untreated, Asc; part 2 \( N^\varphi \)-nitro-\( l \)-arginine methyl ester (l-NNAME) to inhibit NOS, combined l-NNAME + Asc; part 3 yohimbine (YOH) + propranolol (PRO) to antagonize \( \alpha \)- and \( \beta \)-adrenergic receptors and combined YOH + PRO + Asc. CVC reduction during LC was smaller \((P < 0.001)\) at Asc sites \((-31 \pm 4%)\) than at untreated sites \((-56 \pm 5%)\). LC-induced reduction in CVC was smaller \((P < 0.05)\) at l-NNAME + Asc sites \((-23 \pm 8%)\) than at l-NNAME sites \((-43 \pm 7%)\). LC-induced reduction in CVC did not differ between at PRO + YOH sites \((-56 \pm 3%)\) and at PRO + YOH + Asc sites \((-50 \pm 3%)\). These findings suggest that antioxidant supplementation inhibits the vasoconstrictor response to direct cooling through an adrenoceptor-dependent mechanism in human skin.

skin blood flow; microdialysis

CUTANEOUS VASOCONSTRICTION is a physiological response for preventing hypothermia during exposure to a cold environment. The vasoconstrictor response in human skin includes both reflexes and local factors. In nonglabrous skin, the reflex vasoconstriction that occurs during whole body cooling is known to occur with the release of norepinephrine (NE) and cotransmitters from sympathetic adrenergic nerve terminals (17, 20, 27, 28). On the other hand, localized cooling of the cutaneous blood vessels evokes vasoconstriction through both adrenergic and nonadrenergic mechanisms. With adrenergic participation, the affinity of postsynaptic \( \alpha_2 \)-adrenergic recep-

Address for reprint requests and other correspondence: F. Yamazaki, Laboratory for Human Physiology, School of Health Sciences, Univ. of Occupational and Environmental Health (UOEH), 1-1 Iseigaoka, Yahatanishi-ku, 807-8555 Kitakyushu, Japan (e-mail: yamazaki@health.uoeh-u.ac.jp).
METHODS

Subjects. A total of 16 subjects (12 men and 4 women; age range 19–28 yr old; part 1, 4 men and 4 women; part 2, all who participated in part 1; part 3, 8 men) participated in the experiments. Their average age was 21 ± 1 (SE) yr, average weight was 57 ± 3 kg, and average height was 165 ± 3 cm. All subjects were healthy nonsmokers with no history of cardiovascular disease. The menstrual status of the female subjects was recorded, but their responses did not differ perceptibly from those of the male subjects, and their results were combined for analysis. Written informed consent was obtained after a thorough explanation of the present study, including its purpose and risks. The experiments were approved by the Ethics Committee of Medical Care and Research of the University of Occupational and Environmental Health.

Instrumentation. Each subject had two microdialysis probes placed intradermally on the ventral aspect of the forearm. These probes consisted of 1 cm of microdialysis tubing (regenerated cellulose, inner diameter 200 μm, 18-kDa nominal molecular mass cutoff) attached at each end to polyamide tubing. Before implantation, the area of skin was temporarily anesthetized by the application of a cold pack for 5 min. Needles (25 g) were inserted intradermally into the arm ~2.5 cm. The probes were then fed through the lumen of the needle. Probes were aligned such that the microdialysis membranes were centered within the dermis. The needles were then removed, leaving the probes in place. To allow for the effects of the insertion trauma, we waited 1.5 h before any protocols began.

Measurements. All measurements were performed with the subjects resting in the supine position. Skin blood flow (SBKBF) was monitored continuously with laser-Doppler flowmeters (ALF21, Advance, Tokyo). The blood flow measurements are specific to the skin and are not influenced by blood flow to underlying skeletal muscle (26). Local temperature (Tloc) of the 6.3-cm² area surrounding the site of SBKBF measurement was controlled by a custom-built metal sleeve for the flow probe that had a Peltier element. A thermocouple between the skin surface and the sleeve served for measurement and feedback control. Tloc can be precisely maintained within 0.1°C with this controller. Mean arterial pressure (MAP) was measured continuously from a cuff on the middle finger (Finapres, Ohmeda, Madison, WI). The blood pressure values by Finapres during baseline control were verified by a Dymap automatic oscilometric blood pressure device (model 8100, Critikon, Tampa, FL). Cutaneous vascular conductance (CVC) was calculated from the ratio of SBKBF to MAP. Oral temperature was measured with a polyethylene-sealed thermocouple placed in the sublingual sulcus, and subjects did not open their mouths during protocols. Whole body skin temperature (Tsk) was measured using copper-constantan thermocouples on the chest, upper back, lower back, abdomen, thigh, and calf, and mean Tsk was calculated (29). Mean Tsk was controlled by a water-perfused suit. The measured variables were recorded by a data logger (DE1200 universal, NEC Sanei, Tokyo, Japan).

Agents delivered by microdialysis were filter-sterilized with 0.2-μm micro pore syringe filters (AGC techno glass, Funabashi, 0.2-μm micropore syringe filters (AGC techno glass, Funabashi, 0.2-μm micropore syringe filters (AGC techno glass, Funabashi, 0.2-μm micropore syringe filters (AGC techno glass, Funabashi, 0.2-μm micropore syringe filters (AGC techno glass, Funabashi, 0.2-μm micropore syringe filters (AGC techno glass, Funabashi). A solution at a concentration of 10 mM of Asc in sterile saline was perfused at 4 μl/min. L-NAME has been shown to produce complete NOS inhibition after 50 min under resting conditions (34).

Experimental protocols. The experiments consisted of three different parts, each conducted under constant environmental conditions (ambient temperature 28 ± 0.5°C; relative humidity 50 ± 5%). In each part of the experiments, two sites on the ventral aspect of the left forearm were prepared with microdialysis fibers, SBKBF probes, and micropore syringe filters (AGC techno glass, Funabashi, 0.2-μm micropore syringe filters (AGC techno glass, Funabashi, 0.2-μm micropore syringe filters (AGC techno glass, Funabashi, 0.2-μm micropore syringe filters (AGC techno glass, Funabashi, 0.2-μm micropore syringe filters (AGC techno glass, Funabashi, 0.2-μm micropore syringe filters (AGC techno glass, Funabashi). To attain maximal SkBF levels, Tloc was increased to 42°C (29), and sodium nitroprusside (SNP, Sigma), dissolved in sterile saline at a concentration of 56 mM, was administered via microdialysis at a rate of 4 μl/min, which has previously been shown to elicit maximal SkBF (19, 21).

Fig. 1. Design for protocols in parts 1 and 2 (top) and in part 3 (bottom). Mean skin temperature (mean Tsk) was controlled by using a water perfused suit. Local skin temperature (Tloc) was decreased at the rate of −1°C/min in the initial phase of local cooling in all protocols. In part 1, saline was delivered by microdialysis to two sites, followed by L-ascorbate (Asc) to one site. In part 2, saline was infused at one site for 40–50 min to decrease the oxidant stress in part 1. To minimize tonic vasoconstrictor activity before and during local cooling, mean Tsk values were kept at 35–36°C (11). Tloc at the two sites was then decreased to 24°C over a 10-min period (−1.0°C/min) and maintained at 24°C for 20 min. For the last 10 min of local cooling, mean Tsk was decreased to 33°C over a 10-min period. Mild cooling of the body surface was applied to increase sympathetic vasconstrictor activity in the skin and to examine the effect of Asc on the vasoconstrictor response to increased sympathetic activity at locally cooled sites. In part 2, L-NAME plus Asc at the other site was delivered by microdialysis after 10 min in the Tloc of 34°C (Fig. 1). Following this, Asc was infused at one site for 40–50 min to decrease the oxidant stress in part 1. To minimize tonic vasoconstrictor activity before and during local cooling, mean Tsk values were kept at 35–36°C (11). Tloc at the two sites was then decreased to 24°C over a 10-min period (−1.0°C/min) and maintained at 24°C for 20 min. For the last 10 min of local cooling, mean Tsk was decreased to 33°C over a 10-min period. Mild cooling of the body surface was applied to increase sympathetic vasconstrictor activity in the skin and to examine the effect of Asc on the vasoconstrictor response to increased sympathetic activity at locally cooled sites. It was hypothesized that an expected inhibition of adrenoceptor function by Asc supplementation during local cooling addition to α-adrenergic receptors was achieved by applying a combination of PRO and YOH to antagonize those receptors. The antagonists were applied continuously by microdialysis in a sterile saline solution of YOH (5 mM) and PRO (1 mM) at 4 μl/min. This combination and level of adrenergic antagonists was shown to be effective in inhibiting the cutaneous vascular responses to exogenous NE (27), suggesting that all α- and β-adrenergic receptors are blocked.

To attain maximal SkBF levels, Tloc was increased to 42°C (29), and sodium nitroprusside (SNP, Sigma), dissolved in sterile saline at a concentration of 56 mM, was administered via microdialysis at a rate of 4 μl/min, which has previously been shown to elicit maximal SkBF (19, 21).
may act to reduce cutaneous vasoconstrictor responses to whole body cooling. $T_{\text{loc}}$ at the two sites and mean Tsk were then returned to initial baseline levels, and followed by a recovery period of 15 min.

In part 2, to inhibit NO production by NOS, L-NAME was infused at two sites with and without Asc supplementation for 50 min. $T_{\text{loc}}$ and mean Tsk were adjusted to be the same as in the protocol for part 1.

In part 3, YOH and PRO were infused at two sites for 40–50 min. $T_{\text{loc}}$ at the two sites was decreased from 34°C to 24°C over a 10-min period ($-1.0°C/\text{min}$) and maintained at 24°C for 30 min. $T_{\text{loc}}$ was then returned to 34°C, followed by a recovery period of 15 min. After the recovery period in each part of the experiments, $T_{\text{loc}}$ at the two sites was heated to 42°C, and 56 mM of SNP was infused to obtain maximal CVC values (Fig. 1). Mean Tsk was maintained at 35–36°C throughout the experiments.

Data processing and statistical analysis. The measured variables were sampled every 5 s and averaged over 1-min intervals. The data for the precooling baseline were averaged over the 5-min period before local cooling. The changes in CVC were expressed as percentage changes from the precooling baseline values (%baseline) after drug treatment. The CVC values were also expressed as percentages of maximal values (%max) to determine changes in the baseline values by Asc supplementation. Effects of Asc and cooling on changes in CVC were evaluated using two-way repeated-measures ANOVA. When significant F ratios were defined, Fisher’s protected least significant difference between the means was calculated. Effects of Asc on CVC values during the precooling baseline and last minute of local and whole body cooling were evaluated using the Student’s paired t-test. $P < 0.05$ was considered significant. All data are expressed as means ± SE.

RESULTS

Part 1. Supplementation of Asc increased ($P < 0.05$) the baseline level of CVC in %max (Table 1). The increased levels of CVC at the Asc sites were maintained during local cooling and combination of local and whole body cooling. Figure 2 shows changes in CVC from the precooling baseline in response to cooling stimuli in part 1. Local cooling reduced ($P = 0.001$) CVC at the both saline control and Asc sites, and subsequent whole body cooling further reduced CVC at the two sites. The reduction in CVC at Asc sites was more slowly initiated than that at control sites during the cooling protocol. That is, a significant decrease in CVC from the baseline was obtained at 3 min of cooling ($T_{\text{loc}} = 31°C$) at saline sites but was not observed until 8 min ($T_{\text{loc}} = 26°C$) of cooling at Asc sites. As a result, CVC decreased less ($P < 0.05$) at Asc sites than at control sites for minutes 5–20 of local cooling (control, $-56 \pm 5%$/baseline; Asc, $-31 \pm 4%$/baseline at the end of local cooling, $P < 0.05$). When the changes in CVC by whole body cooling were expressed as percentage changes from the CVC values at the end of local cooling alone, whole body cooling similarly decreased CVC at the two sites (control, $-47 \pm 14%$; Asc, $-46 \pm 13%$, $P = 0.85$).

Part 2. The baseline level of CVC at sites treated with L-NAME + Asc did not differ from that at sites treated with L-NAME only (Table 1). Figure 3 shows changes in CVC from the precooling baseline in response to the cooling stimuli in part 2. Local cooling reduced ($P = 0.001$) CVC at both the L-NAME and L-NAME + Asc sites, and subsequent whole body cooling reduced CVC more at the two sites. The reduction in CVC at L-NAME + Asc sites was more slowly initiated than that at L-NAME sites during the cooling protocol. That is, a significant decrease in CVC from the baseline was observed at 6 min of cooling ($T_{\text{loc}} = 28°C$) at L-NAME sites but was not observed until 13 min of cooling at L-NAME + Asc sites. As a result, CVC decreased less ($P < 0.05$) at L-NAME + Asc sites than at L-NAME sites for minutes 3–20 of local cooling (L-NAME, $-43 \pm 7%$/baseline; L-NAME + Asc, $-23 \pm 8%$/baseline at the end of local cooling, $P < 0.05$). When the changes in CVC by whole body cooling were expressed as percentage changes from the CVC values at the end of local cooling alone, whole body cooling similarly decreased CVC at

Table 1. CVC levels during precooling baseline and local cooling and combined local cooling and whole body cooling

<table>
<thead>
<tr>
<th>Sites</th>
<th>Precooling Baseline</th>
<th>LC</th>
<th>LC + WBC</th>
</tr>
</thead>
<tbody>
<tr>
<td>Part 1</td>
<td></td>
<td></td>
<td></td>
</tr>
<tr>
<td>Control</td>
<td>20.0 ± 3.1</td>
<td>9.5 ± 2.2*</td>
<td>3.8 ± 0.8*†</td>
</tr>
<tr>
<td>Asc</td>
<td>33.9 ± 5.5†</td>
<td>24.3 ± 4.7*†</td>
<td>10.7 ± 1.7*†‡</td>
</tr>
<tr>
<td>Part 2</td>
<td></td>
<td></td>
<td></td>
</tr>
<tr>
<td>L-NAME</td>
<td>16.6 ± 2.3</td>
<td>9.8 ± 1.9*</td>
<td>4.2 ± 0.8*‡</td>
</tr>
<tr>
<td>L-NAME + Asc</td>
<td>14.5 ± 1.2</td>
<td>11.4 ± 1.6*</td>
<td>5.7 ± 0.7*‡</td>
</tr>
<tr>
<td>Part 3</td>
<td></td>
<td></td>
<td></td>
</tr>
<tr>
<td>YOH + PRO</td>
<td>25.2 ± 2.7</td>
<td>11.3 ± 1.6*</td>
<td></td>
</tr>
<tr>
<td>YOH + PRO + Asc</td>
<td>29.6 ± 4.5</td>
<td>14.8 ± 2.5*</td>
<td></td>
</tr>
</tbody>
</table>

Values are means ± SE expressed as a percentage of maximal cutaneous vascular conductance (CVC) for treatment with the ascorbic acid (Asc), with the nitrergic oxide synthase inhibitor Nω-nitro-L-arginine methyl ester (L-NAME), with α- and β-adrenergic antagonists [yohimbine (YOH) + propranolol (PRO)], or with the combinations [L-NAME + Asc] or (YOH + PRO + Asc)]. Data are from the period before local cooling (LC) and at last minute of LC alone and at last minute of combined applications of LC and whole body cooling (WBC). *$P < 0.05$ vs. precooling baseline, †$P < 0.05$ vs. control; ‡$P < 0.05$ vs. LC.
the two sites (L-NAME, \(-53 \pm 5\%\); L-NAME + Asc, \(-48 \pm 7\%\), \(P = 0.53\)).

Part 3. The baseline level of CVC at sites treated with YOH + PRO + Asc did not differ from that at sites treated with YOH + PRO (Table 1). Changes in CVC during local cooling in part 3 are shown in Fig. 4. Local cooling similarly decreased \((P = 0.001)\) CVC at both the YOH + PRO and YOH + PRO + Asc sites. There was no difference in the changes in CVC between the two sites throughout the cooling protocol (YOH + PRO, \(-56 \pm 3\%\) baseline; YOH + PRO + Asc, \(-50 \pm 3\%\) baseline at the end of cooling, \(P = 0.16\)). Oral temperature did not change during any of the cooling protocols.

**DISCUSSION**

There were three major findings from the present study. First, local Asc administration inhibited cutaneous vasoconstrictor responses to local cooling in intact skin (part 1). Second, the inhibitory effects of Asc on the local cooling-induced vasoconstriction persisted during L-NAME treatment (part 2). Finally, the YOH and PRO treatments completely blocked the inhibitory effect of Asc on the cutaneous vasoconstrictor response to local cooling (part 3). These findings suggest that the inhibitory effect of Asc on the vasoconstrictr response to direct skin cooling was mainly mediated through modification of an adrenergic receptor-dependent mechanism, but not in the NO system.

The cutaneous vasoconstrictor responses to local cooling occur through adrenergic and nonadrenergic mechanisms (18, 24, 25, 34). In the early phase of cooling (<10 min), locally evoked NE release is the major mechanism responsible for the immediate vasoconstrictor response to direct local cooling (18, 24, 25, 34). In previous reports, the initial vasoconstrictor response to local cooling was reversed to vasodilation by blockade of transmitter release from vasoconstrictor nerves, adrenergic receptor antagonism or by sensory nerve block (18, 24, 34). This vasodilation is not diminished by NOS inhibition but can be minimized by reducing the rate at which cooling is performed (34). Thus, when the inhibitory influence of Asc supplementation on adrenergic receptor function during local cooling is examined, it is important to consider the rate of cooling. In previous studies (33, 34), we observed that a cooling rate of \(-1^\circ\text{C/min}\) is the fastest speed that does not cause significant nonadrenergic vasodilation in the early phase of cooling, so slow cooling was used in this study.

The direct effect of cooling in the skin induces an augmentation of \(\alpha_2\)-adrenoceptor reactivity (5–7), with a translocation of \(\alpha_2\)-adrenoceptors from the Golgi apparatus to the vascular smooth muscle cell surface by Rho kinase (1, 16). Bailey et al. (2) suggested that the initial stimulus for cold-induced signaling in cutaneous arteries is an increase in ROS activity from smooth muscle mitochondria, which results in RhoA activation and the subsequent translocation of \(\alpha_2\)-adrenoceptors to the cell surface, enabling enhanced \(\alpha_2\)-adrenoceptor reactivity. Additionally, Rho activation increases calcium sensitivity of cutaneous vasoconstriction through inhibition of myosin light chain phosphatase (1). Thompson-Torgerson et al. (30) have confirmed the roles of Rho kinase in the local cooling-induced vasoconstriction in an in vivo human skin. Agreeing with the findings of an in vitro study (2), the results from parts 1 and 3...
in human skin in vivo suggest that antioxidant supplementation inhibits vasoconstrictor response by decreasing adrenoceptor reactivity.

β-Adrenergic receptors were antagonized to exclude the confusing effect of the vasodilator response to cooling-induced NE release on α-adrenoceptor mediated vasoconstriction. Although the influence of Asc on β-adrenoceptor reactivity is unknown, local administration of Asc may potentiate β-adrenoceptor reactivity and result in inhibiting vasoconstriction during local cooling. Thus a potential limitation of the present study is that it is unclear whether Asc influences activity of α-adrenergic receptors alone.

As direct cooling proceeds (>10 min), nonadrenergic vasoconstrictor mechanisms prevail in the local component of direct skin cooling (18, 25, 34). As the primary nonadrenergic mechanism, longer term cooling influences the NO system (12, 34). Whereas CVC expressed as %max was consistently higher at Asc sites than at control sites during the cooling protocol in parts 2 and 3 (Table 1), the changes in CVC from the baseline levels during local cooling were similar between at the two sites (control, 11%max; Asc, 10%max). Thus the fact that vasoconstriction was not increased when beginning from a higher baseline would suggest some inhibition of the local cooling response by Asc. Importantly, baseline CVC was similar between Asc- and non-Asc-treated sites in the experiments for parts 2 and 3. Therefore, these findings again suggest that antioxidant supplementation inhibits vasoconstriction via non-NOS mechanisms (i.e., adrenoceptor mechanisms) in the skin but not by the altered baseline level.

In parts 1 and 2, whole body skin cooling in addition to local skin cooling was performed to evaluate the vascular responses to increased adrenergic vasoconstrictor activity in intact and NOS-inhibited skin sites. The results from the part 1 and 2 protocols suggest that local administration of Asc does not alter the vasoconstrictor response during increasing sympathetic adrenergic activity by whole body cooling. The unaltered vasoconstrictor response during whole body cooling implies that adrenergic pathways are functional despite an expected inhibition of postsynaptic adrenoceptor function by antioxidant supplementation during local cooling. Therefore, Asc might affect the vasoconstrictor response through some mechanisms upstream from the adrenergic receptors and outside the adrenergic pathway. It has been known that Asc enhances the biological action of catecholamines by preventing the oxidation of catecholamines (22, 23). In addition, Asc at >15 μM enhances NE-induced contractions of rabbit aortic smooth muscle independent of the oxidation of NE (4). Thus effects of Asc on NE-induced vasoconstriction during increasing sympathetic adrenergic activity by whole body cooling might be counteracted with inhibition of adrenoceptor function by Asc administration during local cooling.

In conclusion, the findings of this study suggest that antioxidant supplementation inhibits the cutaneous vasoconstrictor response to local skin cooling through adrenoceptor mechanisms in humans.

ACKNOWLEDGMENTS

The author gratefully acknowledges the subjects who participated in this experiment.

GRANTS

This work was supported by a Grant-in-Aid for Scientific Research (C) (20500589).

DISCLOSURES

No conflict of interest was declared by the author.

REFERENCES


