Cutaneous vasoconstrictor response to whole body skin cooling is altered by time of day

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Submit 28 August 2002; accepted in final form 14 November 2002

Aoki, Ken, Dan P. Stephens, Adham R. Saad, and John M. Johnson. Cutaneous vasoconstrictor response to whole body skin cooling is altered by time of day. J Appl Physiol 94: 930–934, 2003. First published November 15, 2002; 10.1152/japplphysiol.00792.2002.—To test for a diurnal difference in the vasoconstrictor control of the cutaneous circulation, we performed whole body skin cooling (water-perfused suits) at 0600 (AM) and 1600 (PM). After whole body skin temperature (Tsk) was controlled at 35°C for 10 min, it was progressively lowered to 32°C over 18–20 min. Skin blood flow (SkBF) was monitored by laser-Doppler flowmetry at three control sites and at a site that had been pretreated with bretylium to block noradrenergic vasoconstriction. After whole body skin cooling, maximal cutaneous vascular conductance (CVC) was measured by locally warming the sites of SkBF measurement to 42°C for 30 min. Before whole body skin cooling, sublingual temperature (Tm) in the PM was significantly higher than that in the AM (P < 0.05), but CVC, expressed as a percentage of maximal CVC (%CVCmax), was not statistically different between AM and PM. During whole body skin cooling, %CVCmax levels at bretylium-treated sites in AM or PM were not significantly reduced from baseline. In the PM, %CVCmax at control sites fell significantly at Tsk of 34.3 ± 0.1°C and lower (P < 0.05). In contrast, in the AM %CVCmax at control sites was not significantly reduced from baseline until Tsk reached 32.3 ± 0.1°C and lower (P < 0.05). Furthermore, the decrease in %CVCmax in the PM was significantly greater than that in AM at Tsk of 33.3 ± 0.1°C and lower (P < 0.05). Integrative analysis of the CVC response with respect to both Tm and Tsk showed that the cutaneous vasoconstrictor response was shifted to higher internal temperatures in the PM. These findings suggest that during whole body skin cooling the reflex control of the cutaneous vasoconstrictor system is shifted to a higher internal temperature in the PM. Furthermore, the slope of the relationship between CVC and Tsk is steeper in the PM compared with that in the AM.

Diurnal changes in effector responses to hypothermic challenges have also been investigated (6, 14, 21). Under conditions of constant whole body skin temperature (Tsk), the internal temperature threshold for vasoconstriction of the fingertip during central venous infusion of cold Ringer solution showed a statistically identifiable shift between 3 AM and other times of the day (21). It was not clear whether there were any diurnal effects on the degree of vasoconstriction relative to the thermal challenge or the extent to which these findings apply as well to nonglabrous skin. Also, Castellani and co-workers (6) found evidence based on heat flow measurements and plasma norepinephrine levels that the vasoconstrictor response during acute whole body immersion in cold water did not differ between the morning and evening. These authors mentioned the possibility that the acute cold exposure may central warm receptors and the cutaneous active vasodilator system are the predominant sensory and effector mechanisms, whereas surface and/or internal cold receptors and the cutaneous vasoconstrictor system are predominant during periods of a hypothermic challenge. The vasoconstrictor system also mediates the subtle reflex changes in SkBF that occur during periods of normothermia (5). The circadian rhythm in resting internal temperature in humans shows a nadir in the early morning and a peak in the evening (4, 13). Thermoregulatory control of the cutaneous vasculature is also subject to a diurnal shift in response to hypothermic challenges. For example, the internal temperature threshold for cutaneous vasodilation in hyperthermia is shifted in parallel to the diurnal variation in resting internal temperature (1–3, 20, 21, 24).

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Efferent neural control of skin blood flow (SkBF) is accomplished through two sympathetic pathways: a noradrenergic vasoconstrictor system and a nonadrenergic active vasodilator system (11). During the hyperthermia of whole body heating or dynamic exercise, central warm receptors and the cutaneous active vasodilator system are the predominant sensory and effector mechanisms, whereas surface and/or internal cold receptors and the cutaneous vasoconstrictor system are predominant during periods of a hypothermic challenge. The vasoconstrictor system also mediates the subtle reflex changes in SkBF that occur during periods of normothermia (5). The circadian rhythm in resting internal temperature in humans shows a nadir in the early morning and a peak in the evening (4, 13). Thermoregulatory control of the cutaneous vasculature is also subject to a diurnal shift in response to hypothermic challenges. For example, the internal temperature threshold for cutaneous vasodilation in hyperthermia is shifted in parallel to the diurnal variation in resting internal temperature (1–3, 20, 21, 24). We recently showed that this diurnal shift in threshold depends on the active vasodilator system (3). However, the slope of cutaneous vasodilation relative to internal temperature is lower in the early morning and is dependent on a diurnal variation in sympathetic noradrenergic vasoconstrictor function (3). These results indicate diurnal changes in the thermoregulatory control of cutaneous vasodilator and vasoconstrictor systems, at least in the responses to hyperthermia.

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have elicited maximal responses, potentially masking time-of-day differences in thermoregulatory reflex control (6). Furthermore, the vascular responses included both the effects of direct local cooling as well as the reflex effects of whole body skin cooling. Also, because heat flow was affected by the acute change in thermal gradient, its use as an index of cutaneous circulatory function may have missed the effects of more subtle modulating influences such as circadian rhythms. It is not clear at present whether, with less severe skin cooling and the elimination of the effects of local cooling, a diurnal variation in reflex cutaneous vasconstrictor control might be revealed.

The purpose of this study was to discover the role of time of day in the control of the cutaneous vasconstrictor system during cold stress. To this end, we performed slow, graded decreases in $T_{sk}$ (7, 18, 19) in the early morning and in the evening. We separated reflex from local effects by keeping the temperature at the sites of blood flow measurement constant. Furthermore, to rule out a role for any tonic cutaneous active vasodilator system activity or its removal in response to decreasing $T_{sk}$, we measured cutaneous vascular responses at bretylium-treated sites (12) as well as at control sites.

MATERIALS AND METHODS

The protocol for this study was approved by the Institutional Review Board of the University of Texas Health Science Center at San Antonio. Eight male subjects (age 18–32 yr) were recruited for this study and provided written informed consent before participation. All subjects were non-smokers and in good health. They did not consume caffeine within 12 h before the beginning of any experiment or take any medication, including pain medication, within 24 h of the experiments. Each subject was tested on two occasions, beginning at 0600 (AM) and at 1600 (PM). The order of the two experiments was randomized, and at least a week elapsed between them.

Measurements. Sublingual temperature ($T_{or}$) as an index of internal temperature, $T_{sk}$, SkBF from four sites of the dorsal forearm (1 bretylium-treated site and 3 control sites), mean arterial pressure (MAP), and heart rate (HR) were monitored continuously throughout each study. $T_{sk}$ was controlled by a water-perfused suit that covered the entire body with the exception of the head, the feet, and the arm where SkBF was measured. $T_{sk}$ was assessed as the weighted average from six sites: chest, upper back, lower back, abdomen, thigh, and calf (22). SkBF was measured by laser-Doppler flowmetry (Moor MBF3D or Vasamedics LaserFlo) on the dorsal forearm (3, 18, 19). Combination probe holder-local heaters allowed control of the local temperature of the 12 cm² surrounding the site of blood flow measurement. The laser light shone through a 3-mm² aperture in the center of this metal holder. Local temperature was monitored with a thermocouple placed between the holder and the skin within 7 mm of the aperture. Arterial blood pressure was measured continuously at the finger by photoplethysmography using the Penaz principle (Finnapres, Ohmeda) (8, 16). MAP was assessed from the electrical integration of the pulsatile signal. HR was also obtained from the blood pressure signal. We calculated cutaneous vascular conductance (CVC) as SkBF divided by MAP, normalized to the maximal value for each site (see Data processing and statistical analysis).

Protocol. The same protocol was followed at each session. First, bretylium was applied by iontophoresis to a 0.6-cm² area on the dorsal forearm by a weak current (240 $\mu$A; 400 $\mu$A/cm²) for 10 min to block all neurotransmission from sympathetic noradrenergic nerve terminals (9, 12, 17). Approximately 30–40 min after the application of bretylium, the subject dressed in a water-perfused suit (22). The subject then rested in the supine position for 20–30 min during instrumentation before the beginning of the cooling protocol. Initial $T_{sk}$ was held at 35°C for baseline measurements. The local temperatures at the sites of SkBF measurement were maintained at 34°C throughout the baseline and cooling portions of the protocol. After a 10- to 15-min baseline period, $T_{sk}$ was slowly lowered from 35 to 32°C over 18–20 min. After whole body skin cooling, $T_{sk}$ was restored to 35°C, and maximal CVC (CVCmax) was measured by locally warming the sites of SkBF measurement to 42°C for 30–40 min (10, 22). For each site, CVC was expressed as a percentage of CVCmax (%CVCmax). This method of normalization was followed to reduce the effects of site-to-site differences in the absolute values of CVC and to refer values to the common characteristic of maximal vasodilation (22) optimizing the ability to make day-to-day comparisons of response.

Data processing and statistical analysis. All measurements were recorded once per second (LabView, National Instruments) and averaged into 20-s periods by a laboratory computer. Data for CVC from bretylium-treated and control sites were further compiled into 1-min averages and analyzed relative to $T_{sk}$ during the whole body skin cooling period (18, 19). Before statistical analysis, the CVC data from the three control sites were averaged for each subject. To detect a reduction in CVC from the precooling baseline, data were analyzed independently by one-way analysis of variance with repeated measures. A Dunnett’s multiple-comparison test was applied when a significant difference was detected. To test whether the responses in CVC differed according to the time of day, values for CVC were analyzed by two-way analysis of variance with repeated measures and a Bonferroni post hoc test when a significant difference was identified. $T_{or}$, MAP, and HR from the precooling baseline and from the end of whole body skin cooling were each compared between AM and PM by paired t-test. Also, a paired t-test was used to test for a time-of-day effect for the change in $T_{or}$ during whole body skin cooling. The relationship between CVC and $T_{sk}$ was assessed by regression analysis for each experiment by using data from whole body skin cooling. The slope of the first-order regression of the CVC-$T_{sk}$ relationship was taken as the sensitivity of the cutaneous vascular response to whole body skin cooling. The slopes of CVC with respect to $T_{sk}$ were analyzed by two-way analysis of variance with repeated measures (time of day and bretylium treatment) and a Bonferroni post hoc test when a significant difference was identified. The level of significance was set at $P < 0.05$. All data are expressed as means ± SE.

To assess the integrated thermoregulatory control of the cutaneous vasconstrictor system, a three-dimensional graphical analysis was performed (7). This graph consisted of the independent variables, $T_{or}$ and $T_{sk}$, with CVC responses on the y-axis such that its response to the separate influences of $T_{or}$ and $T_{sk}$ could be viewed simultaneously.

RESULTS

$T_{or}$ at baseline ($T_{sk} = 35°C$) before whole body cold stress was significantly lower in the AM than in the PM ($P < 0.01$). No significant differences in HR or MAP at baseline were found between AM and PM (Table 1).
Table 1. Sublingual temperature, heart rate, and mean arterial pressure at baseline and at the end of whole body skin cooling in the early morning and the evening

<table>
<thead>
<tr>
<th></th>
<th>Tsk = 35°C (Baseline)</th>
<th>Tsk = 32°C (End of Cooling)</th>
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<tr>
<td></td>
<td>AM</td>
<td>PM</td>
</tr>
<tr>
<td>T&lt;sub&gt;a&lt;/sub&gt; °C</td>
<td>36.18 ± 0.06</td>
<td>36.70 ± 0.08*</td>
</tr>
<tr>
<td>HR, beats/min</td>
<td>57.0 ± 2.9</td>
<td>60.6 ± 6.1</td>
</tr>
<tr>
<td>MAP, mmHg</td>
<td>83.7 ± 3.6</td>
<td>84.7 ± 1.9</td>
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Values are means ± SE from 8 subjects. AM, morning; PM, evening; T<sub>a</sub>, sublingual temperature; HR, heart rate; MAP, mean arterial pressure; Tsk, whole body skin temperature. *Significantly different from AM for the same state, P < 0.01.

Figure 1 shows responses in Tsk, T<sub>a</sub>, HR, and %CVC<sub>max</sub> (control and bretylium-treated sites) during whole body skin cooling in the AM for one individual. Whole body skin cooling was associated with elevations in T<sub>a</sub> and MAP (Fig. 1, Table 1). T<sub>a</sub> at the end of whole body skin cooling was significantly lower in the AM than in the PM (P < 0.01), but the changes in T<sub>a</sub> during whole body skin cooling were not statistically different between AM and PM [0.07 ± 0.03 (AM) and 0.07 ± 0.02°C (PM)]. HR and MAP at the end of whole body skin cooling also did not differ significantly between AM and PM (Table 1).

Figure 2 shows the average responses in %CVC<sub>max</sub> from control and bretylium-treated sites for AM and PM studies during whole body skin cooling as a function of Tsk. We found no statistically significant effect of time of day on baseline CVC (Tsk = 35°C) at either site. During whole body skin cooling, Tsk was reduced from 35.0 ± 0.01 to 32.0 ± 0.01°C. CVC at bretylium-treated sites did not significantly decrease during whole body skin cooling (Fig. 2A). This result indicated that withdrawal of active vasodilator system activity was not involved in cutaneous vasoconstrictor responses during whole body skin cooling at either time of day. Whole body skin cooling caused a progressive decrease in CVC at control sites. In the PM, %CVC<sub>max</sub> at control sites fell significantly at Tsk of 34.0 ± 0.01°C and lower (P < 0.05), but in the AM %CVC<sub>max</sub> at these sites was not significantly reduced from baseline until Tsk reached 32.3 ± 0.01°C and lower (P < 0.05) (Fig. 2B). The slopes of the CVC-Tsk regressions from control sites were significantly steeper in the PM than in the AM [0.98 ± 0.28 (AM) vs. 2.33 ± 0.52%CVC<sub>max</sub>/°C (PM); P < 0.05] (Fig. 3). The slopes at the bretylium-treated sites were not significantly different from zero at either time of day. The decrease in %CVC<sub>max</sub> in the PM was significantly greater than that in the AM at Tsk of 33.3 ± 0.01°C and lower (P < 0.05) (Fig. 4).

Figure 5 illustrates the integrated analysis of the thermoregulatory control of cutaneous vasoconstriction for the two times of day. The CVC response was shifted with respect to T<sub>a</sub> such that, in the PM, the cutaneous vasoconstrictor response was centered around a higher T<sub>a</sub>. On the other hand, the CVC response with respect to Tsk was steeper in the PM compared with the AM.

**DISCUSSION**

The major findings from this study are that, relative to the AM, the reflex cutaneous vasoconstrictor response to gradual whole body skin cooling is shifted to higher internal temperatures in the PM and that the vasoconstriction in the PM is more pronounced relative to Tsk. In the present study, we confirmed the CVC response during whole body skin cooling from a Tsk of 35°C depended specifically on vasoconstrictor system function, because at bretylium-treated sites there was no statistically significant response to whole body skin cooling at either time of day (see Fig. 2). This is similar to our laboratory’s earlier observations of responses in CVC in men (18) and women (7, 19) to whole body skin cooling. Altogether, these results show that the active vasodilator system is not involved in the control of SkBF in resting conditions at Tsk values up to 37°C (17).
and therefore does not contribute to the diurnal differences seen here.

In the present study, the cutaneous vasoconstrictor response to decreasing $T_{sk}$ was shifted to higher $T_{or}$ in the PM (see Fig. 5). This finding in nonglabrous (forearm) skin is similar to the findings for glabrous skin by Tayefeh et al. (21), who infused cold Ringer solution and saw a lower internal temperature threshold for fingertip vasoconstriction at 3 AM than later in the day. The net change in the relationship of $SkBF$ to internal temperature in that study, without skin cooling, was similar to that seen in the present study, with skin cooling. This shift in the cutaneous vasoconstrictor response with respect to internal temperature in the evening is consistent with a shift in the regulation of internal temperature to higher levels (1–3, 6, 20, 21, 24).

A unique finding of the present study was that the reflex vasoconstriction during whole body skin cooling in the PM was not only shifted relative to internal temperature but was also significantly steeper relative to $T_{sk}$ than in the AM. This finding is not consistent with that from an earlier study by Castellani et al. (6), who reported no diurnal effect on cold-induced vasoconstriction during cold-water immersion (20°C) between the morning and evening. However, we agree with those authors that diurnal differences in thermoregulatory responses could have been masked by the

**Fig. 2.** Relationship between CVC (expressed as CVC$_{max}$) and $T_{sk}$ from the AM and evening (PM) studies. A: responses at bretylium-treated sites. B: responses at control sites. Protocol began with $T_{sk} = 35^\circ C$, which was progressively lowered over a 20-min period. (Note that the order of the $x$-axis is reversed.) Values are 1-min averages ± SE. %CVC$_{max}$ levels at bretylium-treated sites in AM or PM were not significantly reduced from baseline. At control sites, %CVC$_{max}$ in PM was reduced at higher levels of $T_{sk}$ than that in AM. Significantly different from precooling baseline (by 1-way repeated-measures ANOVA and Dunnett’s multiple-comparison test): *$P < 0.05$, †$P < 0.01$.

**Fig. 3.** Slopes of the relationship of CVC (expressed as %CVC$_{max}$) to $T_{sk}$ at control sites from AM and PM studies. Values are means ± SE from all 8 subjects. At bretylium-treated sites, the slopes from both AM and PM sessions were not significantly different from zero. *AM significantly different from PM (by 2-way repeated-measures ANOVA and Bonferroni post hoc test), $P < 0.05$.

**Fig. 4.** Relationship between the decrease in CVC (expressed relative to changes in %CVC$_{max}$ (%CVC$_{max}$)) and $T_{sk}$ during whole body skin cooling from the AM and PM studies. Values are 1-min averages ± SE. Significantly different from precooling baseline (by 1-way repeated-measures ANOVA and Dunnett’s multiple-comparison test): *$P < 0.05$, †$P < 0.01$. *Significantly different from AM for same levels of $T_{sk}$ (by 2-way repeated-measures ANOVA and Bonferroni post hoc test), $P < 0.05$.

**Fig. 5.** Average changes in CVC (expressed as Δ%CVC$_{max}$) during whole body skin cooling with respect to both $T_{or}$ and $T_{sk}$ for both the AM and PM studies. Values are 1-min averages ± SE. Note that there is a shift in the reflex thermoregulatory control of reflex vasoconstriction system to higher $T_{or}$ in the PM and that this vasoconstriction during whole body skin cooling is initiated with less cooling in PM compared with AM.
severity of the cold stress and the attendant responses. This includes the effects of direct local cooling combined with the reflex effects of general skin cooling. In the present study, Tsk was gradually decreased at a constant local skin temperature and was mild enough not to cause shivering. The results from this more moderate cooling uniquely show that the reflex component of the cutaneous vasoconstriction in the PM has a steeper relationship to Tsk than in the AM. There are several sites in the overall reflex control scheme for SkBF by the vasoconstrictor system that might provide the mechanism for the observed diurnal effects. Generally increased sympathetic vasoconstrictor activity in the PM seems unlikely. Panza et al. (15) suggest tonic sympathetic activity in normothermic conditions to be greater in the AM. Our earlier findings in hyperthermia that the lower CVC/internal temperature slope in the AM could be elevated to PM levels by the antidiurenergic drug bretylum are consistent with that conclusion (3). Nevertheless, those observations do not rule out the possibility of a greater sympathoexcitation for a given reduction in Tsk. Available data do not speak to this issue. It is also not known whether transmitter release from sympathetic terminals is affected by the time of day, so that cannot be ruled out as a possible site for the diurnal effects. Indeed, it is even possible that the balance of norepinephrine and co-transmitter release might be affected in a circadian pattern, as it is between phases of the menstrual cycle (19). There are observations suggestive of a diurnal effect on ß-adrenoceptor function such that the pressor response to the adrenergic agonist phenylephrine was significantly greater in the afternoon and evening than in the morning (23). If true for the cutaneous vasculature, equal sympathoexcitation from cooling in the PM would lead to a greater vasoconstrictor response.

In summary, we found an enhanced reflex cutaneous vasoconstriction relative to lowering Tsk and that vasoconstrictor system control is shifted to higher internal temperatures in the evening compared with the morning. This diurnal shift of the function of the vasoconstrictor system is analogous to the time-of-day effect on the cutaneous vasodilator response to hyperthermia (1–3, 20, 21, 24) and suggests controls of both the cutaneous vasoconstrictor and active vasodilator systems are affected by the time of day.

We sincerely thank Wojciech Kosiba for expert technical support. We are grateful to all volunteer subjects who participated in the study.

This work was supported in part by a Grant-in-Aid for the Encouragement of Young Scientists from the Ministry of Education, Culture, Sports, Science and Technology of Japan (Grant 13780028) and by National Heart, Lung, and Blood Institute Grant HL-59166.

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