Diurnal variation in cutaneous vasodilator and vasoconstrictor systems during heat stress

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Aoki, Ken, Dan P. Stephens, and John M. Johnson. Diurnal variation in cutaneous vasodilator and vasoconstrictor systems during heat stress. Am J Physiol Regulatory Integrative Comp Physiol 281: R591–R595, 2001.—It is not clear whether the diurnal variation in the cutaneous circulatory response to heat stress is via the noradrenergic vasoconstrictor system or the nonadrenergic active vasodilator system. We conducted whole body heating experiments in eight male subjects at 0630 (AM) and 1630 (PM). Skin blood flow was monitored by laser-Doppler flowmetry at control sites and at sites pretreated with bretylium (BT) to block noradrenergic vasoconstriction. Noninvasive blood pressure was used to calculate cutaneous vascular conductance. The sublingual temperature (Tsub) threshold for cutaneous vasodilation was significantly higher in PM at control and at BT-treated sites (both P < 0.01), suggesting the diurnal shift in threshold depends on the active vasodilator system. The slope of cutaneous vascular conductance as a percentage of its maximum with respect to Tsub was significantly lower in AM at control sites only. Also, in the AM, the slope at control sites was significantly lower than that at BT-treated sites (P < 0.05), suggesting that the diurnal change in the sensitivity of cutaneous vasodilation depends on vasoconstrictor system function. Overall, the diurnal variation in the reflex control of skin blood flow during heat stress involves both vasoconstrictor and active vasodilator systems.

human; circadian rhythm; skin blood flow; vasodilation; vasoconstriction; thermoregulation; sweating; laser-Doppler flowmetry; bretylium

IT IS WELL RECOGNIZED that the circadian rhythm in resting internal temperature in humans shows a nadir in the early morning and a peak in the evening. This rhythm in internal temperature results from an endogenous circadian rhythm in heat production and heat dissipation (4, 13). In resting conditions, skin blood flow (SkBF), which has an important role in thermoregulatory heat dissipation, has a circadian rhythm that leads that of internal temperature (20). Effector responses to thermoregulatory challenges also show a diurnal rhythm. For example, during heat stress (passive heat stress or dynamic exercise) with changing internal and skin temperatures, cutaneous vascular control is subject to a circadian rhythm in the internal temperature threshold for the cutaneous vasodilator response to hyperthermia (1, 3, 21, 22, 28). During both passive heat stress (1) and dynamic exercise (3, 21, 22), these thresholds were shown to be shifted to higher internal temperatures in the evening compared with thresholds observed in the early morning. Furthermore, the sensitivity of cutaneous vasodilation relative to internal temperature during passive heat stress was significantly lower in the morning than in the evening (1).

Efferent neural control of SkBF is accomplished through the sympathetic noradrenergic vasoconstrictor system and the nonadrenergic active vasodilator system (10). How these separate pathways contribute to the diurnal pattern of control of SkBF is not entirely clear. Panza et al. (16) noted forearm blood flow, which includes changes in both skin and muscle, of a resting normothermic subject to be lower in the morning and suggested this effect to be at least partly due to increased α-sympathetic vasoconstrictor activity. The contribution by SkBF to these changes in forearm blood flow is not known. It is also unknown whether the diurnal rhythms in the threshold and sensitivity of the SkBF response to hyperthermia, mentioned above, are due to the sympathetic adrenergic vasoconstrictor system alone or if the active vasodilator system has an important role (10, 18). The active vasodilator system can be studied in the absence of a functional vasoconstrictor system by iontophoretically applying bretylium to an area of skin (11). Bretylium acts to block transmitter release from noradrenergic terminals (8). By using this method, Kellogg et al. (12) documented that the increased internal temperature threshold for cutaneous vasodilation caused by dynamic exercise is due to modulation of the activation of the nonadrenergic active vasodilator system (12). Similarly, in female subjects taking oral contraceptive hormones, it was found that the nonadrenergic active vasodilator system mediates the shift in the internal temperature threshold for cutaneous vasodilation accompanying elevated progesterone and estrogen (5). However, it is not clear...
at present whether the diurnal shift in the threshold for cutaneous vasodilation is also due to a shift in the control of the active vasodilator system or is, instead, due to the noradrenergic vasoconstrictor system. Similarly, the roles of these systems in the diurnal changes in the sensitivity of the vasodilator response to hyperthermia are not clear.

The purpose of this study was to discover the roles of the cutaneous vasodilator and vasoconstrictor systems in the diurnal changes in the control of SkBF during heat stress. To this end, we measured responses relative to internal temperature at bretylium-treated sites and at control sites during whole body heating in the early morning and in the evening.

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 in good health were recruited for this study and provided written informed consent before participation. Each subject was tested on two occasions, beginning at 0500 (AM) and at 1500 (PM). The two experiments were performed in random order, and at least 1 wk elapsed between experiments. Subjects were nonsmokers and did not consume caffeine within 12 h of any experiments, take any pain medication, or perform heavy exercise within 24 h of the experiments. Furthermore, subjects were prohibited from taking food from 3 h before each session. Subjects occasionally dozed during the study, but were typically awake during the period of heat stress near and following thresholds. There was no apparent difference in this pattern between AM and PM experiments.

The same protocol was followed at each session. All experiments were conducted in a laboratory at a constant ambient temperature and light intensity. On arriving at the laboratory at 0500 or 1500, bretylium was applied by iontophoresis to two 0.6-cm² spots on the dorsal forearm of subjects by a weak current (250 μA; 400 μA/cm²) for 10 min (11). The vasoconstrictor nerve function is selectively abolished by local application of bretylium (8, 11). Approximately 1 h after the application of bretylium, the subject dressed in a water-perfused suit designed to control whole body skin temperature (Tsk) (6, 11, 17). The suit covered the entire body with the exception of the head, feet, and arm where SkBF was measured. The subject then rested in the supine position for 20–30 min during instrumentation before the beginning of the protocol. Initial Tsk was held at 35°C for baseline measurements. In addition, the local skin temperature at each of the sites of SkBF measurement was set to 32.5°C. After a baseline period, whole body cold stress was performed for 2–3 min by decreasing Tsk to test for the adequacy of the blockade of the vasoconstrictor system. If the blockade at a site was incomplete, the data from that site were excluded.

For whole body heating, Tsk was elevated to 38.0°C for 30–50 min. After whole body heating, Tsk was decreased to initial levels, and maximal cutaneous vascular conductance (CVCmax) was measured by locally warming the sites of SkBF measurement to 42°C for 30–40 min (9, 25).

Measurements. Subjects were instrumented for the measurement of sublingual temperature (Tsub) as an index of internal temperature. Mean Tsk was measured from the weighted average of six thermocouples (upper back, lower back, abdomen, upper chest, thigh, and calf) (24). SkBF was measured from three sites of the dorsal forearm (2 bretylium-treated sites and 1 control site) by laser-Doppler flowmetry (Moor, MBF3D or Vasamedics, LaserFlo). Metal holders for the flow probes allowed control of the local temperature of the 10-cm² area surrounding the site of blood flow measurement. The laser light shone through a 3-mm² aperture in the center of this holder. Local temperature was monitored with a thermocouple placed between the holder and the skin within 7 mm of the aperture. Mean arterial pressure (MAP) and heart rate (HR) were measured noninvasively from the middle finger of the left hand using the Penaz principle (Finapres) (7, 15). We calculated cutaneous vascular conductance (CVC) from the SkBF value divided by MAP. For each site, CVC was expressed as a percentage of CVCmax (%CVCmax). Sweating rate (SR) was monitored from a 0.45-cm² area using a ventilated capsule surrounding the laser-Doppler flow probe. The effluent air from the capsule was led to a highly sensitive capacitance hygrometer to measure relative humidity, which was used to calculate local SR.

Data processing and statistical analysis. All measurements were recorded every second and averaged into 20-s periods by a laboratory computer. For the determination of thresholds for the onset of cutaneous vasodilation or sweating, unlabeled plots of CVC or SR as functions of time were given to an investigator blinded as to the variable, time, and subject. The threshold for vasodilation was defined as the Tor at which CVC began to increase steadily. The threshold for sweating was identified as the Tor at which active sweating began. CVC, SR, and HR were plotted as functions of Tor for analysis of the sensitivity of their responses during whole body heating. The regressions between CVC or SR and Tor for each experiment were calculated using data from the beginning of vasodilation or onset of sweating up to the time of achieving a steady state. The data from the two bretylium-treated sites were averaged when there was adequate blockade of the vasoconstrictor system at both sites. Linear regression analysis was also conducted for the HR-Tor relationship for each subject and time of day. In addition, Tor for a standard HR of 75 beats/min during whole body heating was calculated from the regression analysis. The threshold for cutaneous vasodilation and the sensitivity of CVC with respect to Tor were analyzed by two-way analysis of variance with repeated measures (time of day and bretylium treatment). Furthermore, these variables were compared between AM and PM or control and bretylium-treated sites by planned contrasts, when main effects (time of day or bretylium treatment) were significant. Tor, MAP, and HR at rest were compared between AM and PM by paired t-test as were the results for SR and HR. The level of significance was set at P < 0.05. All data are expressed as means ± SE.

RESULTS

Preheating data. Tsk at rest before whole body cold stress was significantly lower in AM than in PM [36.11 ± 0.08 (AM) vs. 36.62 ± 0.06°C (PM), P < 0.01]. Also, MAP at rest was significantly lower in AM than in PM [76.1 ± 1.5 (AM) vs. 80.1 ± 1.5 mmHg (PM), P < 0.05]. No significant differences in HR [56.8 ± 2.1 (AM) vs. 58.8 ± 2.6 beats/min (PM), P > 0.1] at rest were found between AM and PM. Whole body cold stress induced significant vasoconstriction at control sites (30.7 ± 4.8% decrease in CVC, P < 0.01), but bretylium-treated sites did not significantly change by this test (7.2 ± 2.1% decrease in CVC, P > 0.1). This result indicated that the noradrenergic vasoconstrictor function at bretylium-treated sites was effectively abolished (5, 11, 12, 18, 19).
Physiological responses during whole body heating.

Figure 1 shows responses in $T_{or}$, HR, $\%CVC_{max}$ (control and bretylium-treated sites), and SR during whole body heating for one individual. Whole body heating caused marked cutaneous vasodilation and sweating in each subject. The $T_{or}$ thresholds for cutaneous vasodilation from both sites and the slopes of the $\%CVC_{max}$-$T_{or}$ regressions are shown for an individual in Fig. 2 and for the whole group in Table 1. The thresholds for vasodilation at both control and bretylium-treated sites were significantly higher in the PM than in the AM ($P < 0.01$). However, we did not detect a statistically significant difference in this shift between control (0.48 ± 0.08°C) and bretylium-treated (0.45 ± 0.08°C) sites ($P > 0.1$). As shown by individual data in Fig. 3 and by the whole group data in Table 1, at control sites, the sensitivity of the cutaneous vasodilator response with respect to $T_{or}$ in the AM was significantly lower than that in the PM ($P < 0.02$), whereas at bretylium-treated sites the sensitivity was not significantly affected by the time of day ($P > 0.1$). Also, in the AM, the sensitivity at control sites was significantly lower than that at bretylium-treated sites ($P < 0.05$). Although the $T_{or}$ threshold for sweating in the PM was significantly higher than that in the AM [36.31 ± 0.06 (AM) vs. 36.77 ± 0.06 (PM)°C, $P < 0.01$], the sensitivity of the sweating response to $T_{or}$ did not show a statistically identifiable time of day effect [1.15 ± 0.22 (AM) vs. 1.55 ± 0.16 (PM) mg·cm$^{-2}$·min$^{-1}$·°C$^{-1}$, $P > 0.1$].

Figure 4 shows the relationship of HR to $T_{or}$ in the AM and PM for one individual. The $T_{or}$ corresponding to a standard HR of 75 beats/min was significantly higher in the evening than the morning [36.29 ± 0.07 (AM) vs. 36.75 ± 0.06 (PM)°C, $P < 0.01$]. On the other hand, the slope of this relationship was not signifi-

Table 1. Internal temperature thresholds for the onset of vasodilation and the sensitivity of the vasodilator response to internal temperature for the morning and evening studies at both untreated control sites and at BT-treated sites

<table>
<thead>
<tr>
<th>Threshold, °C</th>
<th>Sensitivity, $%CVC_{max}$°C</th>
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<tbody>
<tr>
<td><strong>AM</strong></td>
<td><strong>PM</strong></td>
</tr>
<tr>
<td>Control sites</td>
<td></td>
</tr>
<tr>
<td>36.32 ± 0.07</td>
<td>36.79 ± 0.06†</td>
</tr>
<tr>
<td>74.72 ± 12.46</td>
<td>143.30 ± 17.27*</td>
</tr>
<tr>
<td>BT sites</td>
<td></td>
</tr>
<tr>
<td>36.43 ± 0.07</td>
<td>36.88 ± 0.06†</td>
</tr>
<tr>
<td>128.10 ± 35.87§</td>
<td>162.53 ± 23.31†</td>
</tr>
</tbody>
</table>

Data are means ± SE from 7 subjects. BT, bretylium; CVC$_{max}$, maximum cutaneous vascular conductance. Significantly different from AM for same treatment group (†$P < 0.01$; *$P < 0.05$). §Significantly different from control sites for same time of day ($P < 0.05$).
The unique findings of this study were the diurnal changes of control of the cutaneous circulatory systems: the vasoconstrictor and active vasodilator systems in the diurnal shift of the internal temperature threshold for cutaneous vasodilation. These changes were significant between times of day [54.81 ± 11.17 (AM) vs. 62.77 ± 12.03 (PM) beats·min⁻¹·°C⁻¹, P > 0.1].

**DISCUSSION**

The major purpose of this study was to find the role of the vasoconstrictor and active vasodilator systems in the diurnal changes of control of the cutaneous circulation. The unique findings of this study were: 1) the mechanism for the diurnal shift in the internal temperature threshold for cutaneous vasodilation was through a similar shift in the function of the cutaneous active vasodilator system and 2) the significant decrease in the sensitivity of the cutaneous vasodilator response with respect to internal temperature in the early morning was due, at least in part, to a diurnal effect on the function of the sympathetic noradrenergic vasoconstrictor system.

The internal temperature threshold for cutaneous vasodilation during heat stress (passive heat stress or dynamic exercise) shows a circadian variation (1, 3, 21, 22, 28). In the present study, a shift of the internal temperature threshold for cutaneous vasodilation at control sites was consistent with these previous results. Furthermore, the threshold for cutaneous vasodilation at the bretylium-treated sites also had a similar diurnal shift. This indicates that the diurnal shift of the internal temperature threshold for the onset of cutaneous vasodilation was caused by a similar change in the function of the nonadrenergic cutaneous active vasodilator system. In previous studies, our laboratory used the technique of selective blockade of the vasoconstrictor system to show that the shifts in threshold for vasodilation produced by exercise (12), whole body skin cooling (17), or oral contraceptives (5, 6) are also due to altered noradrenergic vasoconstrictor function. Panza et al. (16) found that resting forearm blood flow (plethysmography) and forearm vascular resistance had a circadian rhythm and that the vasodilator effect of the α-adrenergic antagonist phentolamine was significantly greater in the morning than at other times of day. These authors suggested that this difference is due to a diurnal rhythm in vasoconstrictor activity (16). By contrast, α-adrenoceptor-mediated vascular responses to the same doses of phenylephrine (an α-adrenergic agonist) in the morning were significantly lower than in the evening or night (26). In addition, plasma epinephrine and norepinephrine in humans peak in the early morning (14). Taken together, these findings indicate that the lower sensitivity of the cutaneous vasodilator response to increased internal temperature in the early morning is due to a higher activity of the sympathetic noradrenergic vasoconstrictor system, rather than increased sensitivity of postsynaptic α-receptors.

The internal temperature threshold for the onset of sweating showed a shift similar to that for cutaneous vasodilation. These results were consistent with previous studies (2, 3, 21–23, 27, 28). On the other hand, the sensitivity of sweating to internal temperature did not significantly alter between times of day [54.81 ± 11.17 (AM) vs. 62.77 ± 12.03 (PM) beats·min⁻¹·°C⁻¹, P > 0.1].

Interestingly, Pérgola et al. (17) found that the sensitivity of CVC relative to internal temperature was reduced by local cooling of the skin (27°C), an effect that was reversed by local treatment with bretylium, suggesting this effect on sensitivity by local cooling also is partly or completely due to altered noradrenergic vasoconstrictor function. Panza et al. (16) found that resting forearm blood flow (plethysmography) and forearm vascular resistance had a circadian rhythm and that the vasodilator effect of the α-adrenergic antagonist phentolamine was significantly greater in the morning than at other times of day. These authors suggested that this difference is due to a diurnal rhythm in vasoconstrictor activity (16). By contrast, α-adrenoceptor-mediated vascular responses to the same doses of phenylephrine (an α-adrenergic agonist) in the morning were significantly lower than in the evening or night (26). In addition, plasma epinephrine and norepinephrine in humans peak in the early morning (14). Taken together, these findings indicate that the lower sensitivity of the cutaneous vasodilator response to increased internal temperature in the early morning is due to a higher activity of the sympathetic noradrenergic vasoconstrictor system, rather than increased sensitivity of postsynaptic α-receptors.

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**Fig. 3.** Individual responses in the slopes of the %CVC_max-Tor regressions during whole body heating at different times of day (left: control sites; right: BT-treated sites). At control sites, the sensitivity was consistently lower in the AM than in the PM, but this was not the case at BT-treated sites. See Table 1 for average results.
change between the early morning and evening, also in keeping with earlier reports (2, 3, 21, 22, 27, 28). Similarly, the sensitivity of the control of HR relative to internal temperature did not significantly differ between AM and PM. Such was also the case for the control of HR between phases of the menstrual cycle (5).

In conclusion, the results from this study indicate that the diurnal shift in the internal temperature threshold for cutaneous vasodilation is caused by a similar shift in the nonadrenergic active vasodilator system and that the diurnal shift in the sensitivity of cutaneous vasodilation with respect to internal temperature is due largely or entirely to a diurnal variation in the effects of the sympathetic noradrenergic vasoconstrictor system. The thermoregulatory control of HR and SR also showed shifts between morning and evening, but these did not exhibit diurnal changes in sensitivity.

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