Plasma hyperosmolality elevates the internal temperature threshold for active thermoregulatory vasodilation during heat stress in humans

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Submitted 1 May 2009; accepted in final form 4 October 2009


Plasma hyperosmolality delays the response in skin blood flow to heat stress by elevating the internal temperature threshold for cutaneous vasodilation. This elevation could be because of a delayed onset of cutaneous active vasodilation and/or persistent cutaneous active vasoconstriction. Seven healthy men were infused with either hypertonic (3% NaCl) or isotonic (0.9% NaCl) saline and passively heated by immersing their lower legs in 42°C water for 60 min (room temperature, 28°C; relative humidity, 40%). Skin blood flow was monitored via laser-Doppler flowmetry at sites pretreated with bretylium tosylate (BT) to block sympathetic vasoconstriction selectively and at adjacent control sites. Plasma osmolality was increased by ~13 mosmol/kgH2O following hypertonic saline infusion and was unchanged following isotonic saline infusion. The esophageal temperature (Tc) threshold for cutaneous vasodilation at untreated sites was significantly elevated in the hyperosmotic state (37.73 ± 0.11°C) relative to the isosmotic state (36.63 ± 0.12°C, P < 0.001). A similar elevation of the Tc threshold for cutaneous vasodilation was observed between osmotic conditions at the BT-treated sites (37.74 ± 0.18 vs. 36.67 ± 0.07°C, P < 0.001) as well as sweating. These results suggest that the hyperosmotically induced elevation of the internal temperature threshold for cutaneous vasodilation is due primarily to an elevation in the internal temperature threshold for the onset of active vasodilation, and not to an enhancement of vasoconstrictor activity.

osmoregulation; body fluid; thermoregulation; skin blood flow; sweating

HEAT STRESS ELEVATES INTERNAL and skin temperatures (Tsk), and, subsequently, skin blood flow and sweat rate increase as thermoregulatory responses. Other factors related to exercise or body fluid regulation are capable of modulating these responses, independent of thermal changes (27). It is well known that dehydration (hypovolemic hyperosmolality) reduces thermoregulatory cutaneous vasodilation and sweating (23, 26, 31). Nadel et al. (24) demonstrated that thermoregulatory cutaneous vasodilation was reduced during isotonic hypohydration relative to euhydration but was not changed by hyperhydration. Takamata et al. (32, 34) found that plasma hyperosmolality delays thermoregulatory cutaneous vasodilation and sweating by elevating the body core temperature (Tc) thresholds for the initiation of these responses as a linear function of the increase in plasma osmolality (Posmol). Thus the elevated Posmol during dehydration modifies thermoregulation.

Cutaneous blood flow is controlled by a sympathetic active vasoconstrictor system and a separate active vasodilator system (15). The elevation of the Tc threshold for cutaneous vasodilation by plasma hyperosmolality might be attributed to a stimulation of active vasoconstrictor system activity and/or delay of active vasodilator system activity. The contribution of the active vasodilator system can be examined by eliminating vasoconstrictor system function with iontophoresically applied bretylium (BT), which blocks the release of neurotransmitters from vasoconstrictor nerve endings (17). By means of this method, investigators have shown that the shifts in the Tc threshold for cutaneous vasodilation during the menstrual cycle and by circadian rhythms during passive heating are due to the modulation of active vasodilator function (1, 8). Both circadian rhythms and the menstrual cycle modify baseline Tc, and the shifted Tc threshold for cutaneous vasodilation occurs in parallel to that altered baseline Tc; the rise in Tc required to elicit cutaneous vasodilation is apparently not altered by these factors (1, 8). In contrast, plasma hyperosmolality does not affect baseline Tc, but nevertheless increases the threshold Tc required to initiate cutaneous vasodilation (32, 34). Moreover, either dehydration or hyperosmotic saline infusion can acutely increase Posmol. Thus the mechanism for the elevation of the Tc threshold for cutaneous vasodilation by plasma hyperosmolality might differ from that for altered control of cutaneous vasodilation by circadian rhythms or by the menstrual cycle. Exercise is another factor known to increase the Tc threshold for cutaneous vasodilation through an effect on active vasodilator system function (16). The increased Posmol produced by exercise-induced fluid shifts is thought to be the origin of the increase in the Tc threshold for cutaneous vasodilation (21, 33). Based on these findings, the elevated Tc threshold for cutaneous vasodilation induced by plasma hyperosmolality might result from a delay in the onset of active vasodilator system activity. However, no study has directly examined the contribution of either the vasoconstrictor system or the vasodilator system to the osmotically induced elevations in the Tc threshold for cutaneous vasodilation. Thus it remains unknown whether the osmotic effect on the onset of cutaneous vasodilation during passive heating is due to increased or persistent vasoconstrictor activity or to a delay of active vasodilator function. Thus the purpose of the present study was to elucidate the mechanism for the hyperosmotic elevation of the Tc threshold for cutaneous vasodilation. We used the local application of BT to test the hypothesis that the osmotic elevation in vasodilator threshold was dependent on an intact vasoconstric-
tor system function. If that is not the case, a role for modified active vasodilator system function is implied.

METHODS

Seven young men (22 ± 1 yr) participated in this study. They were relatively active but were not participants in an intense exercise-training program. Subjects were of normal weight (66.0 ± 2.0 kg) and height (172.7 ± 2.4 cm). They were informed of the purpose and risks of this study before providing their written consent. The study procedures were approved by the Ethics Committee of Nara Women’s University, and all experiments were performed in accordance with the Declaration of Helsinki. All were healthy nonsmokers, free of any known cardiovascular, renal, neurologic, or metabolic diseases. Subjects refrained from alcohol, caffeine, and salty food for 24 h before testing.

Experimental protocol. Each subject participated in two experiments, held on separate days. In these tests, subjects were infused with either an isotonic (0.9%; IOSM) or a hypertonic (3%; HOSM) NaCl solution. The order of experimental conditions was randomized, and the tests were separated by at least 3 days.

Subjects reported to the laboratory at 0900 having already ingested 500 ml water. They were provided a light breakfast (400 kcal; Calorie Mate, Otsuka Pharmaceutical). After entering a chamber controlled at 28°C and 40% relative humidity (RH), subjects swallowed a probe for esophageal temperature (Tes) measurement. A venous catheter was placed in an antecubital vein. These procedures required ~30 min. After that, subjects sat on a chair for at least 30 min, after which a blood sample was drawn without stasis.

Subjects were infused with either 0.9 or 3% NaCl solution for 90 min. The infusion rate was 0.2 and 0.125 ml·min⁻¹·kg⁻¹ body wt for 0.9 and 3% NaCl solution, respectively. These infusion rates were chosen to expand plasma volume (PV) by similar amounts in the two conditions (32).

During the infusion period, BT was iontophoretically applied to two 0.6-cm² spots on the dorsal right forearm by a weak current (250 μA; 400 μA/cm²) for 10 min (17). This application has been shown to provide a complete local blockade of sympathetic vasoconstrictor function, lasting several hours (17).

After the infusion period, subjects dressed in a water-perfused suit. The suit covered the upper body except for the head, hands, and right forearm where skin blood flow was measured. After a 10-min equilibration period, cold stress was applied by circulating cold water through the tubing of the suit for 3–5 min to test the adequacy of the blockade of the vasoconstrictor system at the BT-treated sites. If the blockade at a site was incomplete, the data from that site were excluded. Subjects then took off the water-perfused suit and were seated for 20 min to recover from the cold stress. After a 5-min baseline data collection period, subjects immersed their lower legs in a circulating water bath controlled at 42°C for 60 min, following a previously used protocol (30, 32, 34).

Although a second cold stress at the end of the study would confirm the efficacy of the BT blockade of vasoconstrictor system function, we elected not to perform that second cold stress because the subjects had been seated ~3 h by the end of the heat stress and because such blockade with BT lasts well past that period of time (17).

Measurements. A thermistor probe (Technol Seven) was swallowed to the level of the left atrium, estimated as one-fourth of the subject’s height from the nostril for the measurement Tc. Tsk was measured at seven sites with thermocouples, and mean Tsk was calculated according to the method of Hardy and DuBois (11). Systolic and diastolic blood pressures were recorded every minute by electroxymyomonometer applied to the left upper arm, and heart rate was monitored from the electrocardiogram (STBP780; Colin). Mean arterial pressure (MAP) was calculated as diastolic pressure plus one-third pulse pressure.

Skin blood flows at BT-treated and untreated sites were monitored with integrated laser-Doppler flowmetry probes (Moor Instruments). Following the heat stress protocol, local Tsk at the blood flow measure-

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Data analyses. All measurements were averaged into 1-min periods and were expressed as means ± SE for the seven subjects. CVC and sweat rate were plotted as functions of T_{es} for analyses of the threshold and sensitivity of the responses during heat stress (35). Data from the intervals before and after a marked change in the rate of rise of CVC or sweat rate were fitted by separate linear regression equations. The intersection of these regression lines was taken to represent the threshold for vasodilation or sweating, and the slope of second component as the sensitivity. Percent change in PV was calculated from the hematocrit and hemoglobin concentration using the equation:

\[
\Delta PV (\%) = 100 \times \left( \frac{[1 - (Hct_B/100)]}{[1 - (Hct_A/100)]} \right) - 100
\]

where ∆PV is the percent change in PV, Hb is the hemoglobin concentration, and Hct is the hematocrit. Subscript B indicates data from before (control), and subscript A indicates values at the 20-min intervals. Data from rest and from the averages over the last 5 min of heating were compared between HOSM and IOSM by paired t-test. Data from the periods of heating were analyzed via a two-way repeated-measures ANOVA with main factors of plasma osmotic condition and time. The thresholds for cutaneous vasodilation and the sensitivities of CVC with respect to T_{es} were analyzed by two-way repeated-measures ANOVA with main factors of plasma osmotic condition and BT treatment. Statistical significance was set at an alpha level of 0.05. All data are presented as means ± SE.

RESULTS

Preheating condition. Hypertonic saline infusion significantly increased P_{osmol} (293.3 ± 1.8 to 307.0 ± 1.5 mosmol/...
kgH2O, P < 0.005), whereas isotonic saline infusion did not change Posmol (292.4 ± 1.8 to 293.6 ± 1.9 mosmol/kgH2O). ΔPV after infusion and before the beginning of passive heating was 6.5 ± 0.7% (isotonic saline infusion) and 10.1 ± 0.7% (hypertonic saline infusion), which differed significantly between conditions (P < 0.05).

Cold stress caused significant vasoconstriction at untreated sites (13.1 ± 2.3% decrease in CVC, P < 0.001), but not at the BT-treated site (0.2 ± 2.3% increase in CVC, P > 0.05), indicating that vasoconstrictor function was selectively abolished at the BT-treated sites. CVC returned to the precooling levels before passive heating began.

Ta and heart rate before passive heating were similar between HOSM and IOSM (32.2 ± 0.3 vs. 32.2 ± 0.2°C and 61.8 ± 3.9 vs. 62.9 ± 4.6 beats/min). The baseline Tsk before passive heating tended to be higher in HOSM than in IOSM (36.63 ± 0.07 vs. 36.47 ± 0.02°C), but this difference did not reach statistically significant levels (P = 0.09).

Passive heating test. During passive heating, Posmol in IOSM increased slightly but remained significantly lower when compared with HOSM. During passive heating, ΔPV in both conditions gradually decreased from the initially elevated values, such that the change in ΔPV during passive heating did not differ between HOSM and IOSM (Fig. 1).

Tsk and heart rate before passive heating were similar between conditions (13.1 ± 2.3 vs. 13.1 ± 2.3°C, P = 0.07) although MAP in both conditions increased slightly but remained significantly lower when compared with HOSM during passive heating (2.3 mmHg, both P < 0.05). The baseline Tes at the untreated control site and between CVC at the untreated control site and treated sites (13.1 ± 2.3 mosmol/kgH2O, which differed significantly between conditions (P < 0.05).

The increase in CVC at BT-treated and untreated sites and the increase in sweat rate during passive heating were smaller and did not change significantly during passive heating.

Figure 3 shows ΔTsk thresholds for CVC at BT-treated and untreated sites, ΔTes thresholds for sweating, and ΔTes at steady state 10 min before the end of heating, all as functions of Posmol. These variables showed high linear correlations with Posmol. The relationship between the ΔTes threshold for cutaneous vasodilation and Posmol was not affected by BT treatment (Fig. 4). Regression analysis indicated that the increase in ΔTes thresholds for CVC at untreated sites per unit rise in Posmol was 0.05°C, for the BT-treated sites 0.059°C, and for sweating 0.047°C. The increase in ΔTes at steady state per unit increase in Posmol was 0.048°C.

DISCUSSION

The primary finding of this study is that the hyperosmotic elevation of the Tes threshold for the onset of cutaneous vasodilation is not influenced by eliminating vasoconstrictor function, suggesting that the osmotic delay of the onset of cutaneous vasodilation is caused primarily by an inhibition of active vasodilator system function and not by enhanced vasoconstrictor activity. The Tes thresholds for cutaneous vasodilation at untreated sites and for sweating were significantly higher in HOSM relative to IOSM and ΔTes at the end of the experiment was significantly higher in HOSM than IOSM. These observations are consistent with findings from previous studies (10, 32, 34). The osmotically induced increase in the Tes threshold for cutaneous vasodilation and the slope of the relationship between cutaneous vasodilation and Tes were not affected by BT treatment. The cold stress test before passive heating confirmed that vasoconstrictor function was effectively abolished at BT-treated sites. These results, coupled with the observation that the vasoconstrictor response to cold stress did not differ between IOSM and HOSM (13.1 ± 3.7 vs. 13.3 ± 3.2%, P = 0.99), indicate that plasma hyperosmolality specifically inhibits activation of the cutaneous active vasodilator system, causing the elevation in the Tes threshold for cutaneous vasodilation.

The cutaneous active vasodilator system is known to be mediated by cholinergic nerves, since presynaptic blockade of cholinergic nerves with botulinum toxin abolishes cutaneous

### Table 1. Tes threshold for cutaneous vasodilation with and without bretylium (BT) treatment, sweating, the sensitivities of cutaneous vasodilator response and sweating with respect to Tes (slope), and the increase in Tes (∆Tes) from preheating to the onset of cutaneous vasodilation and sweating in isosmotic and hyperosmotic conditions

<table>
<thead>
<tr>
<th>Conditions</th>
<th>Threshold</th>
<th>Slope</th>
<th>∆Tes</th>
<th>Threshold</th>
<th>Slope</th>
<th>∆Tes</th>
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<tr>
<td></td>
<td>Isosmotic Condition</td>
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<td>Hyperosmotic Condition</td>
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<tr>
<td>Cutaneous vasodilation</td>
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<td></td>
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<tr>
<td>BT-untreated sites</td>
<td>36.63±0.12</td>
<td>91.8±9.3</td>
<td>0.27±0.11</td>
<td>37.73±0.11</td>
<td>87.7±16.5</td>
<td>1.15±0.14*</td>
</tr>
<tr>
<td>BT treated sites</td>
<td>36.67±0.07</td>
<td>115.5±28.0</td>
<td>0.30±0.08</td>
<td>37.74±0.18*</td>
<td>117.4±20.1</td>
<td>1.16±0.21*</td>
</tr>
<tr>
<td>Sweating</td>
<td>36.70±0.10</td>
<td>1.14±0.15</td>
<td>0.34±0.09</td>
<td>37.63±0.18*</td>
<td>0.85±0.21</td>
<td>1.05±0.21*</td>
</tr>
</tbody>
</table>

Values are °C for threshold and ∆Tes, and %CVCmax/°C for slope. *Significant difference from isosmotic condition, P < 0.05.
vasodilation during passive heat stress (18). Acetylcholine released from cholinergic nerve endings, has been suggested to play an important role as a cholinergic cotransmitter for cutaneous vasodilation during passive heating (3). Other transmitters postulated to be involved in cutaneous active vasodilation include histamine (38), prostanoids (20), and substance P (37). It is not clear that hyperosmolality acts directly on the secretion or action of any of these proposed transmitters or cotransmitters. Rather, it is more likely that the effect is in the control of active vasodilator outflow. Support for this notion comes, in part, from the observation that hypertonic saline infusion also delays the sweating response to passive heat stress through an elevation in the threshold internal temperature for sweating onset (30, 34), which was also seen in the present study (Table 1 and Fig. 3, bottom). This suggests an effect of osmolality on central control centers, which is in keeping with previous in vitro findings (4).

That the increase in the internal temperature threshold for cutaneous vasodilation with plasma hyperosmolality is due to a delay of the onset of active vasodilation rather than enhanced vasoconstrictor tone is similar to earlier findings. The variation in the internal temperature threshold for cutaneous vasodilation observed during the menstrual cycle (6, 8) and the diurnal variation in the threshold (1, 2) are both due to similar shifts in the activation of the active vasodilator system during heat stress, i.e., are unaffected by BT pretreatment. In those studies, baseline internal temperature was already elevated in the evening or by oral contraceptives. In those cases, the shifted internal temperature threshold for cutaneous vasodilation and sweating could be due to an alternation in the hypothalamic thermoregulatory set-point temperature, whereas the differential from baseline to the threshold (ΔT<sub>es</sub> required to elicit a response) was unchanged. In contrast, infusion of hypertonic NaCl solution acutely increased P<sub>osmol</sub>, but T<sub>es</sub> throughout infusion was not significantly changed. Nevertheless, the T<sub>es</sub> threshold for cutaneous vasodilation was elevated by plasma hyperosmolality. In that respect, the present data indicate that the central mechanism for the osmotically induced elevation of T<sub>es</sub> threshold for cutaneous vasodilation during heat stress differs from that of the menstrual cycle or circadian rhythms, although all inhibit active vasodilator system function. A reasonable speculation for the diurnal and menstrual cycle effects is that, in addition to inhibition of active vasodilator system function, there is also some increased vasoconstrictor system activity in normothermic conditions. Even a very small increase, when sustained, would cause the observed elevation in baseline internal temperature (5, 7, 13). The absence of a significant change in baseline temperature with hyperosmotic saline infusion is in keeping with the lack of a significant effect on vasoconstrictor system activity. It also must be recalled that the menstrual cycle and the diurnal effects are more slowly developing and in place longer than are the osmotic changes induced here. Whether that difference in timing contributes to the above differences is not known.

The rise in T<sub>es</sub> following the onset of cutaneous vasodilation was significantly greater in HOSM than in IOSM (Fig. 2, top, P < 0.05). Also, the osmotically induced rightward shift of the ΔT<sub>es</sub> threshold for cutaneous vasodilation was elevated, regardless of BT treatment. As in our previous studies (32, 33), the shifted T<sub>es</sub> threshold for cutaneous vasodilation and the
elevation of $T_{es}$ during heating were both correlated with the change in $P_{osmol}$ (Fig. 4). The delays in the onset of active cutaneous vasodilation and in sweating onset due to increased $P_{osmol}$ are the most likely causes of the observed elevation of internal temperature during body heating.

PV was significantly increased by the infusion in both IOSM and HOSM. We calculated an expected increase in PV for the end of each infusion rate. To do so, we assumed baseline $P_{osmol}$ and HOSM. We calculated an expected increase in PV for the internal temperature during body heating.

Cutaneous vasodilation and in sweating onset due to increased change in $P_{osmol}$ (Fig. 4). The delays in the onset of active subjects for patient participation.

Threat of hyperthermia as a consequence.

Reduced and/or delayed active vasodilator system activity and the delay in the onset of active vasodilation by dynamic exercise. In this scenario, the pathway for that suppression would be worse during exercise in the heat, when hyperosmolality may well further compromise the ability to manage the osmolal state being the source of the differences in response between HOSM and IOSM. If the difference in volume had any role, it would be to minimize the change in threshold between trials, and therefore we may have underestimated the effect of plasma hyperosmolality on the CVC response.

Perspective and Significance

Osmoregulation interacts strongly with thermoregulation centrally, since hyperosmolality has been shown to inhibit hypothalamic thermosensitive neurons (4, 25, 29). The findings from the current study lead us to speculate that such an integration of osmotic and thermal regulatory systems finds the cutaneous circulation, in particular the powerful active vasoconstrictor system, as a shared point of control.

An implication of this is that the combined presence of hyperosmotic conditions and heat stress degrade the ability of the body to respond adequately to thermal challenge. Such a problem would be worse during exercise in the heat, when hyperosmolality may well further compromise the ability to regulate body temperature. The value of such a shift in control may not be so much in the compromise in the control of skin blood flow and the active vasodilator system, but in the suppression of sweating, limiting further increases in osmolality. In this scenario, the pathway for that suppression would be through altered thermoregulatory control generally, with reduced and/or delayed active vasodilator system activity and the threat of hyperthermia as a consequence.

ACKNOWLEDGMENTS

We thank Mayumi Oda and Mieko Sakai for technical support and the subjects for patient participation.

GRANTS

This research project was funded in part by a Grant-in-Aid for the Encouragement of Young Scientists (JSPS 14704020 and JSPS15650151; to M. Shibasaki) and Grant-in-Aid for Scientific Research (JSPS 14570684; to A. Takamata).

DISCLOSURES

No conflicts of interest are declared by the authors.

REFERENCES