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

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Shibasaki M, Aoki K, Morimoto K, Johnson JM, Takamata A. Plasma hyperosmolality elevates the internal temperature threshold for active thermoregulatory vasodilation during heat stress in humans. Am J Physiol Regul Integr Comp Physiol 297: R1706–R1712, 2009. First published October 7, 2009; doi:10.1152/ajpregu.00242.2009.—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 to 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 (Tes) 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 Tes threshold for cutaneous vasodilation was observed between osmotic conditions at the BT-treated sites (37.74 ± 0.18°C 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. Plasma hyperosmolality elevates the internal temperature threshold for cutaneous vasodilation during heat stress in humans. The elevation of the Tes 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 iontophoretically 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 Tes 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 Tes, and the shifted Tes threshold for cutaneous vasodilation occurs in parallel to that altered baseline Tes; the rise in Tes required to elicit cutaneous vasodilation is apparently not altered by these factors (1, 8). In contrast, plasma hyperosmolality does not affect baseline Tes, but nevertheless increases the threshold Tes required to initiate cutaneous vasodilation (32, 34). Moreover, either dehydration or hyperosmotic saline infusion can acutely increase P(osmol). Thus the mechanism for the elevation of the Tes 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 Tes threshold for cutaneous vasodilation through an effect on active vasodilator system function (16). The increased P(osmol) produced by exercise-induced fluid shifts is thought to be the origin of the increase in the Tes threshold for cutaneous vasodilation (21, 33). Based on these findings, the elevated Tes 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 Tes 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 Tes 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 vasoconstrictor function of the increase in plasma osmolality (P(osmol)). Thus the elevated P(osmol) during dehydration modifies thermoregulation.
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 iontophoresetically 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 vasconstrictor 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 vasconstrictor 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 Tₑₑ. Tₑₑ was measured at seven sites with thermocouples, and mean Tₑₑ was calculated according to the method of Hardy and DuBois (11). Systolic and diastolic blood pressures were recorded every minute by electrosphygmomanometry 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 Tₛ was at the blood flow measurement sites was raised to 43°C by a 3-cm-diameter heating device that housed the laser-Doppler flow probe. Local Tₛ was held at this level for 40 min to elicit maximal cutaneous vasodilation (14, 36). An index of cutaneous vascular conductance (CVC) was calculated from the ratio of laser Doppler flux to MAP. CVC was then expressed as a percent of maximal CVC as determined from local heating.

Sweat rate was measured using the ventilated capsule method with nitrogen as the carrier gas delivered at a rate of 1,000 ml/min. The capsule (surface area = 2.01 cm²) was placed adjacent to the local heating devices. Absolute humidity was calculated from the RH and the temperature of the gas exiting the chambers, with the detector (HMP 233, Vaisala) positioned 1 m from the capsule on the skin. Sweat rate was calculated as:

\[
\text{Sweat rate} = \frac{\text{absolute humidity} \times \text{gas flow}}{\text{capsule surface area}} \quad (1)
\]

Blood samples were taken without stasis just before heating began and every 20 min during body heating. An aliquot for the measurement of Pₑₑ was immediately transferred to a heparinized tube and centrifuged. The separated plasma was stored frozen at −20°C until measurement. Blood for the determination of hematocrit and hemoglobin concentration was immediately processed. Pₑₑ was determined by freezing point depression (Vogel OM 801 Osmometer), hemoglobin concentration by the cyanomethemoglobin method (Wako Hemoglobin Kit), and hematocrit by capillary centrifugation (Kubota 3220).

![Graph](http://ajpregu.physiology.org/DownloadedFrom/10.22033.1)
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:

$$\Delta PV (%) = 100 \times \frac{(Hb_B/Hb_A) \times [(1 - (Hct_B/100)]}{[1 - (Hct_A/100))] - 100}$$

where $\Delta PV$ 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/
The increase in sweat rate during passive heating were smaller and did not change \( \Delta P_{\text{osmol}} \) (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.

\( T_{\text{sk}} \) 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 \( T_{\text{es}} \) 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, \( P_{\text{osmol}} \) 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).

\( T_{\text{es}}, T_{\text{sk}}, \) and heart rate rose during passive heating (Fig. 2). The increase in \( T_{\text{es}} \) was significantly greater in HOSM than in IOSM \( (1.56 ± 0.16 \) vs. \( 0.84 ± 0.10°C \) \( P < 0.001) \), whereas the increase in \( T_{\text{sk}} \) was similar between the osmotic conditions \( (2.98 ± 0.34 \) vs. \( 2.99 ± 0.24°C \) \( P < 0.047) \). Heat rate and MAP at the end of heating were greater in HOSM relative to IOSM \( (92.6 ± 4.3 \) vs. \( 81.5 ± 3.5 \) beats/min and \( 86.1 ± 2.6 \) vs. \( 78.2 ± 2.3 \) mmHg, both \( P < 0.05) \) although MAP in both conditions did not change significantly during passive heating.

The increases in CVC at BT-treated and untreated sites and the increased in sweat rate during passive heating were smaller in HOSM than in IOSM. Figure 3, top, shows the relationship between CVC at the untreated control site and \( \Delta T_{\text{es}} \) from a representative subject during passive heating. The osmotically induced rightward shift of the \( \Delta T_{\text{es}} \) threshold for the onset of cutaneous vasodilation shown in Fig. 3 occurred in all subjects. At the untreated site, the \( T_{\text{es}} \) threshold for the onset of cutaneous vasodilation was significantly higher in HOSM relative to IOSM, and the rise in \( T_{\text{es}} \) from preheating to the onset of cutaneous vasodilation was also significantly greater in HOSM (Table 1). A similar rightward shift was observed at the BT-treated sites (Fig. 3, middle, and Table 1). Importantly, the osmotically induced rightward shift of the \( \Delta T_{\text{es}} \) threshold for cutaneous vasodilation was similar between the BT-treated and untreated sites \( (0.86 ± 0.16°C \) vs. \( 0.88 ± 0.08°C \) \( P = 0.84) \). The increase in CVC for a given increase in \( T_{\text{es}} \) above the \( T_{\text{es}} \) threshold (slope) was not significantly affected by the osmotic condition or by BT treatment \( (P > 0.05) \). Similarly, the \( T_{\text{es}} \) threshold for sweating was significantly higher in HOSM relative to IOSM, whereas the sensitivity of sweating relative to \( T_{\text{es}} \) was not different between osmotic conditions (Fig. 3, bottom, and Table 1).

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

**DISCUSSION**

The primary finding of this study is that the hyperosmotic elevation of the \( T_{\text{es}} \) 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 \( T_{\text{es}} \) thresholds for cutaneous vasodilation at untreated sites and for sweating were significantly elevated in HOSM relative to IOSM and \( \Delta T_{\text{es}} \) 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 \( T_{\text{es}} \) threshold for cutaneous vasodilation and the slope of the relationship between cutaneous vasodilation and \( T_{\text{es}} \) 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 \( T_{\text{es}} \) 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

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<th>Hyperosmotic Condition</th>
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<td></td>
<td>Threshold</td>
<td>Slope</td>
<td>( \Delta T_{\text{es}} )</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>
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<tr>
<td>BT treated sites</td>
<td>36.67±0.07</td>
<td>115.3±28.0</td>
<td>0.30±0.08</td>
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<td>Sweating</td>
<td>36.70±0.10</td>
<td>1.14±0.15</td>
<td>0.34±0.09</td>
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<td></td>
<td>37.73±0.11*</td>
<td>87.7±16.5</td>
<td>1.15±0.14*</td>
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<td>37.74±0.18*</td>
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<td>0.85±0.21</td>
<td>1.05±0.21*</td>
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Values are °C for threshold and \( \Delta T_{\text{es}} \), and °C/°C for slope. *Significant difference from isosmotic condition, \( P < 0.05 \).

vasodilation during passive heat stress (18). Acetylcholine released from cholinergic nerves causes vasodilation mediated through a nitric oxide mechanism, but the role of acetylcholine is apparently less when internal temperature is substantially elevated (28). There may also be a presynaptic source of the nitric oxide (19). Recently, vasoactive intestinal polypeptide, which is one of the cotransmitters 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 ($\Delta T_{es}$ required to elicit a response) was unchanged. In contrast, infusion of hypertonic NaCl solution acutely increased $P_{osmol}$, but $T_{es}$ throughout infusion was not significantly changed. Nevertheless, the $T_{es}$ 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 $T_{es}$ 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 $T_{es}$ 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 $\Delta T_{es}$ threshold for cutaneous vasodilation was elevated, regardless of BT treatment. As in our previous studies (32, 33), the shifted $T_{es}$ 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 internal temperature during body heating. cutaneous vasodilation and in sweating onset due to increased subjects for patient participation.

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