Baroreflex modulation of sympathetic nerve activity to muscle in heat-stressed humans

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Baroreflex modulation of sympathetic nerve activity to muscle in heat-stressed humans. Am J Physiol Regulatory Integrative Comp Physiol 282: R252–R258, 2002; 10.1152/ajpregu.00337.2001.—To identify whether whole body heating alters arterial baroreflex control of muscle sympathetic nerve activity (MSNA), MSNA and beat-by-beat arterial blood pressure were recorded in seven healthy subjects during acute hypertensive and hypertensive stimuli in both normothermic and heat stress conditions. Whole body heating significantly increased sublingual temperature (P < 0.01), MSNA (P < 0.01), heart rate (P < 0.01), and skin blood flow (P < 0.001), whereas mean arterial blood pressure did not change significantly (P > 0.05). During both normothermic and heat stress conditions, MSNA increased and then decreased significantly when blood pressure was lowered and then raised via intravenous bolus infusions of sodium nitroprusside and phenylephrine HCl, respectively. The slope of the relationship between MSNA and diastolic blood pressure during heat stress (−128.3 ± 13.9 U·beats⁻¹·mmHg⁻¹) was similar (P = 0.31) with normothermia (−140.6 ± 21.1 U·beats⁻¹·mmHg⁻¹). Moreover, no significant change in the slope of the relationship between heart rate and systolic blood pressure was observed. These data suggest that arterial baroreflex modulation of MSNA and heart rate are not altered by whole body heating, with the exception of an upward shift of these baroreflex curves to accommodate changes in these variables that occur with whole body heating.

baroreflex sensitivity; muscle sympathetic nerve activity; heart rate; heat stress

DURING EXPOSURE TO INCREASED thermal load, humans become more susceptible to syncope during orthostatic or gravitational acceleration (1, 15). Baroreflexes are important in the regulation of blood pressure, but relatively little is known about the effects of whole body heating on baroreflex regulation. Previous studies revealed that electrical stimulation of the hypothalamus, which is the central neural structure governing thermoregulation (9, 20), modifies the baroreceptor reflex. Therefore, it is possible that whole body heating impairs baroreflex control of blood pressure, thereby contributing to the reduction in orthostatic tolerance during exposure to hyperthermic environmental conditions.

Although the effects of whole body heating on baroreflex function have not been fully identified, during heat stress, baroreflexes remain capable of modulating different sympathetic nerve activity as evidenced by the observation that increases in blood pressure via phenylephrine HCl infusion decrease muscle sympathetic nerve activity (MSNA) in heat-stressed humans (3). Similar changes in splanchic sympathetic nerve activity have been reported during blood pressure perturbations in hyperthermic rats (18).

Previously, studies investigated the effects of heat stress on baroreflex function in rats (18, 22) and baboons (10). With the use of different techniques, these investigators concluded that hyperthermic exposure either increased (22), decreased (18), or did not change (10) the gain expressing baroreflex control of heart rate. Moreover, in hyperthermic rats, the gain expressing the relationship between splanchic sympathetic nerve activity and blood pressure was unaltered relative to normothermia (18). In humans, we and others have shown that whole body heating does not alter the maximal gain of baroreflex control of heart rate during carotid baroreceptor perturbations (2, 26), it decreases the maximal gain of carotid-vasomotor responses (2), whereas the gain expressing changes in heart rate relative to spontaneous changes in blood pressure was either unchanged (27) or was reduced by heat stress (5).

Although previous studies investigating the effects of whole body heating on baroreflex responsiveness in humans are informative, the aforementioned studies limited their findings to either changes in heart rate that occurred during relatively small spontaneous changes in blood pressure or focused solely on the carotid baroreflex. Thus the effect of heat stress on arterial baroreflex control of sympathetic nerve activity in humans remains unknown. Measurement of MSNA simultaneously with pharmacological manipulation of arterial blood pressure would be beneficial in understanding the effects of whole body heating on baroreflex function.
arterial baroreflex regulation of blood pressure independent of possible heat-induced alterations of α-adrenergic receptor-mediated vasoconstrictor responsiveness observed in rats (14, 17) and in humans (unpublished observation). Therefore, the objective of this study was to test the hypothesis that whole body heating alters arterial baroreflex control of MSNA in response to acute changes in arterial blood pressure in humans.

METHODS

Subjects. Seven subjects (4 men and 3 women) participated in this study. The subjects’ average age was 29.7 ± 2.4 yr, and all were of normal height (176.0 ± 2.7 cm), weight (67.9 ± 4.0 kg), and health. A written informed consent from each subject was obtained before participation in this institutionally approved study.

Instrumentation. Each subject was instrumented for measurement of sublingual temperature (Tsl) with a thermistor placed under the tongue. Mean skin temperature (Tsk) was obtained from the electrical average of six thermocouples attached to the skin. The subject was then dressed in a tube-lined suit that permitted control of Tsk by changing the temperature of the water perfusing the suit. Arterial blood pressure was continuously recorded noninvasively from a finger (Finapres, Ohmeda, Louvsville, CO). Resting blood pressure obtained from the Finapres was verified during the experiment by auscultation of the brachial artery. Beat-by-beat heart rate was obtained from the electrocardiogram signal interfaced with a cardiotachometer (CWE, Ardmore, PA). Forearm skin blood flow was indexed by laser Doppler flowmetry (Perimed, North Royalton, OH). Respiratory excursions were monitored from a piezoelectric respiration transducer (Pneumotrace, Morro Bay, CA). Postganglionic MSNA was recorded from the peroneal nerve. This was accomplished by inserting a tungsten microelectrode with an uninsulated tapered tip of 1–5 μm (FHC, Bowdoinham, ME) into the peroneal nerve dorsal to the fibular head. A second uninsulated reference electrode was positioned within 3 cm of the recording electrode. The signal was amplified 30,000–70,000 times and passed through a band-pass filter (700–2,000 Hz) (Iowa Bioengineering, Iowa City, IA). The filtered neurogram was rectified and integrated (0.1-s time constant) and displayed on an oscilloscope with the blood pressure. This signal was also routed to an audio amplifier. Minor adjustments were made to the microelectrode until an acceptable MSNA signal was obtained. Verification of MSNA relative to skin sympathetic nerve activity was performed as previously reported (24).

Protocol. To assess arterial baroreflex sensitivity, changes in arterial blood pressure were induced via bolus injections of sodium nitroprusside and phenylephrine HCl (8, 11) during both normothermic and heat stress conditions with the subject in the supine position. These drugs were administered via a catheter placed in an antecubital vein. After a 10-min baseline period, 100 μg of sodium nitroprusside were administered, followed ~60 s later by 150 μg of phenylephrine HCl (21). These doses decreased arterial pressure 10–15 mmHg below baseline levels and then increased blood pressure 5–10 mmHg above the baseline levels, respectively. During normothermic conditions, three of these challenges were performed separated by a minimum of 15 min. This duration was sufficient for arterial blood pressure, heart rate, and MSNA to return to predrug levels. Results from these three trials were averaged and are reported as the normothermic trial.

After data collection for the normothermic trial, Tsk was increased to ~38°C by perfusing the tube-lined suit with 46°C water. After Tsl increased 0.5–1.0°C, the temperature of the water was reduced to 44–45°C in an attempt to reduce the rate of rise of internal temperature during the ensuing procedures. Bolus infusions of sodium nitroprusside and phenylephrine HCl were then administered using the same time course and doses as used in the normothermic trials. After a rest period of ~15 min to allow arterial pressure, heart rate, and MSNA to return to predrug levels, the pharmacological challenge was repeated during the heat stress condition. Results from these trials were averaged and are reported as the whole body heating trial.

Data analysis. Data were sampled at 200 Hz through a data-acquisition system (Biopac System, Santa Barbara, CA) and analyzed using LabView software (National Instruments, Austin, TX). The integrated neurogram was normalized by assigning the largest amplitude of a sympathetic burst during the first minute before the administration of drugs to a value of 100. All bursts for that trial were then normalized relative to that value (11). MSNA bursts were identified by manual inspection of the neurogram. Burst area of the integrated neurogram, instantaneous heart rate, and systolic and diastolic blood pressures were measured simultaneously on a beat-by-beat basis. Total MSNA of each burst was defined as the burst area of the rectified and integrated neurogram.

The sensitivity of arterial baroreflex control of MSNA was identified from the linear relationship between MSNA and diastolic pressure during pharmacologically induced changes in blood pressure (11, 21). Diastolic pressure was used because MSNA correlates closely with diastolic pressure but not with systolic pressure (23). To perform a linear regression between nerve activity and blood pressure, values for MSNA were averaged over 3-mmHg diastolic blood pressure ranges. This pooling procedure reduces the statistical impact of inherent beat-by-beat variability in nerve activity due to non-baroreflex influences (e.g., respiration) (11). Baroreflex modulation of heart rate was identified from the relationship between beat-by-beat heart rate and systolic blood pressure during pharmacologically induced changes in blood pressure. Beat-by-beat heart rates were also pooled over 3-mmHg systolic blood pressure ranges, followed by linear regression analysis between heart rate and systolic blood pressure.

Statistical analyses were performed using commercially available software (StatView 5.0). Differences in hemodynamic responses within normothermic trials or heat stress trials were evaluated using repeated-measures one-way ANOVA. Differences between pharmacologically induced responses during normothermic and heat stress trials were evaluated with repeated-measures two-way ANOVA. The effects of heat stress on baroreflex gains were compared with the normothermic trials via paired t-tests. All values are reported as means ± SE. P values of <0.05 were considered statistically significant.

RESULTS

After whole body heating, Tsl increased approximately 0.6°C. Heart rate and skin blood flow increased significantly (Table 1). Mean blood pressure did not change due to whole body heating. MSNA expressed as bursts per minute or bursts per 100 heart beats during heat stress increased significantly compared with that during normothermia. Recordings of integrated MSNA, blood pressure, instantaneous heart rate, res-
Table 1. Temperature and hemodynamic responses to heat stress

<table>
<thead>
<tr>
<th></th>
<th>Normothermia</th>
<th>Heat Stress</th>
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<tbody>
<tr>
<td>Tsl, °C</td>
<td>36.6 ± 0.1</td>
<td>37.2 ± 0.1*</td>
</tr>
<tr>
<td>Tsk, °C</td>
<td>34.5 ± 0.3</td>
<td>37.9 ± 0.4*</td>
</tr>
<tr>
<td>MAP, mmHg</td>
<td>80 ± 2</td>
<td>78 ± 2</td>
</tr>
<tr>
<td>HR, beats/min</td>
<td>50 ± 2</td>
<td>65 ± 2*</td>
</tr>
<tr>
<td>MSNA, bursts/min</td>
<td>10 ± 1</td>
<td>16 ± 2*</td>
</tr>
<tr>
<td>MSNA, bursts/100 heartbeats</td>
<td>19 ± 2</td>
<td>25 ± 3*</td>
</tr>
<tr>
<td>SBF, flowmetry U</td>
<td>20 ± 4</td>
<td>84 ± 9*</td>
</tr>
</tbody>
</table>

Values are means ± SE. Reported blood pressures were measured by auscultation of the brachial artery. Main blood pressure (MAP) was calculated as diastolic blood pressure plus one-third pulse pressure. *Significantly different from normothermia stage (P < 0.05).

Table 2 shows the individual and mean baroreflex relationships between MSNA and diastolic blood pressure during normothermia and heat stress conditions. The slope of the change in MSNA relative to the change in diastolic blood pressure increased in three subjects, decreased in three subjects, and did not change in one subject as a result of heating. The average slope of the relationship between blood pressure and MSNA during heat stress was not different from that during normothermia (-128.3 ± 13.9 U·beats⁻¹·mmHg⁻¹) was not different from that during normothermia (-140.6 ± 21.1 U·beats⁻¹·mmHg⁻¹, P = 0.31). These findings indicate that the heat stress did not alter arterial baroreflex regulation of MSNA due to ±10-15 mmHg changes in blood pressure around the operating point. However, the baroreflex curve was shifted upward before drug delivery by the heat stress as evidenced by a significant increase in MSNA without a change in blood pressure (Table 1).

A strong relationship between heart rate and systolic blood pressure was also found in each subject in both thermal conditions (mean r² = 0.72 ± 0.07 for normothermia, mean r² = 0.81 ± 0.04 for heat stress). Figure 2 shows the individual and mean baroreflex relationships between heart rate and systolic blood pressure during normothermia and heat stress conditions. The slope of the change in heart rate relative to the change in systolic blood pressure increased in three subjects, decreased in two subjects, and did not change in two subjects during heating. The slope of this relationship during heat stress (-1.07 ± 0.124 beats·min⁻¹·mmHg⁻¹) was not significantly different from that during normothermia (-1.10 ± 0.140 beats·min⁻¹·mmHg⁻¹, P = 0.42). The curve expressing this relationship before drug delivery was shifted upward during heat stress as evidenced by an increase in heart rate without a change in blood pressure (Table 2).
finding suggests that whole body heating does not alter the gain of arterial baroreflex regulation of heart rate due to ±10- to 15-mmHg changes in systolic blood pressure around the operating point. However, heat stress shifted baroreflex regulation of heart rate upward to accommodate the elevation in heart rate that accompanies whole body heating.

**DISCUSSION**

In the present study, arterial baroreflex modulation of MSNA and heart rate was assessed during blood pressure changes induced by sequential bolus infusions of sodium nitroprusside and phenylephrine HCl. The results show that unloading arterial baroreceptors via nitroprusside administration significantly increased MSNA and heart rate, whereas loading arterial baroreceptors via phenylephrine HCl administration significantly decreased MSNA and heart rate during both thermal conditions. The sensitivity of arterial baroreflex control of MSNA and heart rate was not changed in the heat stress condition relative to normothermia. This conclusion was based on the observation that the slope of the relationship between MSNA and diastolic blood pressure and the slope of the relationship between heart rate and systolic blood pressure were similar to that during normothermia. Taken together, these data indicate that arterial baroreflex modulation of MSNA and heart rate are preserved during acute changes in blood pressure in association with whole body heating.

Similar to previous studies (3, 19), in the present study, MSNA increased significantly during heat stress. This observation, coupled with a lack of change in the gain of the relationship between diastolic blood pressure and MSNA, suggests whole body heating caused an upward shift of the baroreflex curve governing MSNA (Table 1).

Previous studies investigating the effects of whole body heating on baroreflex functions have produced mixed results. With respect to nonhuman studies, depending on the technique used to assess the baroreflex and/or the animal model, heat stress increased (22), decreased (18), or did not change (10) the baroreflex gain expressing the relationship between heart rate and blood pressure. Moreover, the baroreflex gain expressing the relationship between splanchnic sympathetic nerve activity and blood pressure was unaffected.

### Table 2. Hemodynamic and neural responses to changes in blood pressure in both thermal conditions

<table>
<thead>
<tr>
<th></th>
<th>Normothermia</th>
<th>Heat Stress</th>
</tr>
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<tbody>
<tr>
<td></td>
<td>Baseline</td>
<td>Nitroprusside</td>
</tr>
<tr>
<td><strong>SBP, mmHg</strong></td>
<td>121 ± 3</td>
<td>105 ± 3*</td>
</tr>
<tr>
<td><strong>DBP, mmHg</strong></td>
<td>59 ± 2</td>
<td>50 ± 3*</td>
</tr>
<tr>
<td><strong>HR, beats/min</strong></td>
<td>50 ± 2</td>
<td>70 ± 5*</td>
</tr>
<tr>
<td><strong>MSNA, U × 10⁹/min</strong></td>
<td>64 ± 13</td>
<td>186 ± 13*</td>
</tr>
</tbody>
</table>

Data for baseline are mean values of −1 min before the infusion of nitroprusside; data for nitroprusside are mean values of −15 s during lowest blood pressure induced by nitroprusside; and data for phenylephrine are mean values of −15 s during highest blood pressure induced by phenylephrine. Reported blood pressures were measured with the Finapres. Reported MSNA values are normalized values. *Significantly different from baseline (P < 0.05). SBP, systolic blood pressure; DBP, diastolic blood pressure HR.
in hyperthermic rats (18). In humans, we (2) and others (26) reported that the gain of the carotid-cardiac baroreflex was not significantly affected by whole body heating, whereas the gain of the carotid-vasomotor baroreflex was significantly attenuated in this environment (2). Furthermore, we reported that the transfer function gain of the relationship between spontaneous fluctuations in blood pressure and heart rate was significantly attenuated within the high-frequency range (5). In contrast, Yamazaki and Sone (27) did not find a change in the gain of the response when assessing periods of spontaneously occurring sequences in which both arterial blood pressure and interbeat interval simultaneously increased or decreased. Taken together, differences in these findings likely relate to the animal model, as well as methodology, used to assess baroreflex responsiveness. Nevertheless, before the present study, nothing was known regarding the effects of whole body heating on arterial baroreflex regulation of MSNA in humans.

In the present study, the sensitivity of arterial baroreflex modulation of MSNA was calculated from the slope of total activity of MSNA to diastolic blood pressure during blood pressure changes induced by sequential bolus injections of nitroprusside and phenylephrine HCl. The relationship between MSNA and diastolic blood pressure during pharmacologically induced changes in arterial blood pressure has been used extensively to probe the role of the arterial baroreflex in humans (8, 11, 21). Bolus administration of sodium nitroprusside and phenylephrine HCl unloads and loads, respectively, both the aortic and carotid baroreceptors without causing measurable changes in central venous pressure (7). The present data suggest that the sensitivity of arterial baroreflex control of MSNA in humans is not changed during whole body heating. As previously mentioned, we reported that the gain of the carotid-vasomotor baroreflex in humans was significantly attenuated during heat stress (2). There are some important differences between these studies that likely explain the apparent discrepancies between the prior findings (2) and findings from the present study. In the prior study, only the carotid baroreceptors were stimulated, whereas both carotid and aortic baroreceptors were challenged in the present study. Moreover, changes in blood pressure due to carotid baroreceptor perturbation in the previous study were due to changes in cardiac output and total peripheral resistance, whereas MSNA solely reflects sympathetic outflow to the muscle vascular bed and not the end-organ response. Thus it is possible that differences between these findings may be related to the baroreceptor populations perturbed, differential baroreflex modulation of skin blood flow/skin sympathetic nerve activity relative to baroreflex modulation of MSNA (2, 4, 25), and/or the effects of whole body heating in altering postsynaptic responses (14, 17).

The present results show that whole body heating shifts the curve expressing arterial baroreflex control of heart rate upward without changing the slope of this relationship (Table 2). A lack of change in baroreflex gain is consistent with prior findings (2, 26, 27), but it is in contrast to one study (5). Conflicting findings between these studies are likely due to the methodology of assessing baroreflex regulation. For example, in the contrary study (5), baroreflex regulation of heart rate during spontaneous fluctuations in blood pressure was assessed using transfer function estimates of baroreflex gain within the low (0.03–0.15 Hz) and high-frequency ranges (0.2–0.3 Hz). It was only in the high-frequency region (i.e., primarily vagal modulation of heart rate) that baroreflex modulation of heart rate was attenuated during whole body heating.

Present and previous studies (3, 19) found an increase in MSNA burst rate due to heating. It is unlikely that the elevation in MSNA during whole body heating was mediated by arterial baroreceptors, because arterial blood pressure did not change significantly during the heat stress before drug administration. Previously, we reported that, unlike in normothermia, cardiopulmonary baroreceptor loading in heat-stressed individuals does not significantly alter MSNA (3). We suggested that one possible explanation for this finding is an uncoupling of baroreflex modulation of MSNA by the cardiopulmonary baroreceptors during whole body heating. In contrast to that hypothesis, data from the present experiment clearly indicate that arterial baroreflex modulation of MSNA is preserved during both hypotensive and hypertensive challenges in heat-stressed individuals. This is evidenced by similar and appropriate changes in MSNA during pharmacologically induced changes in blood pressure between thermal conditions.

The present study demonstrates that the MSNA-blood pressure curve is shifted upward in response to an elevation in internal temperature without a change in the slope. The elevation in MSNA occurred despite an absence of a decrease in arterial blood pressure during heating (Table 1). This finding, coupled with our prior findings that cardiopulmonary baroreceptor unloading is not responsible for the elevation in MSNA during heating (3), suggests that the increase in MSNA during heat stress is likely independent of baroreflex mechanisms. This conclusion is consistent with the observation that increases in splanchnic sympathetic nerve activity in hyperthermic rats were similar in baroreceptor-innervated and -denervated conditions (12). Thus the present finding supports the hypothesis that elevations in MSNA during heat stress may occur through central activation of the sympathetic nervous system and are independent of baroreflex control of MSNA (13).

Limitations to interpretation of the data. Internal temperature in the present experiment increased only moderately (i.e., ~0.6°C). However, this relatively small increase in internal temperature coupled with an elevation in Tsk to ~38°C resulted in a greater than fourfold increase in skin blood flow as well as a pronounced increase in heart rate. These findings indicate a significant thermal challenge due to this moderate heat stress. We are unaware of data that suggest heat-induced alterations in human baroreflex function.
are related to the severity of heat stress. Thus we cannot exclude the possibility that had more pronounced heat stress been applied, significant alterations in baroreflex control of MSNA and heart rate would have occurred.

In the present study, the sensitivity of arterial baroreflex control of MSNA was estimated from the linear slope of the relationship between MSNA and diastolic blood pressure. The relationship of MSNA and diastolic blood pressure is likely sigmoidal across a wide range of blood pressures. In the present study, relatively small changes in diastolic blood pressure occurred during the pharmacological intervention. Moreover, we only used the linear portion of the data to identify the slope of the relationship between MSNA and diastolic blood pressure (Fig. 2). Thus we do not know whether whole body heating alters the maximal gain of arterial baroreflex control of MSNA. We can, however, conclude that factors associated with whole body heating do not alter arterial baroreflex control of MSNA within a diastolic pressure range of ~20–25 mmHg around the operating point. Similar limitations should be recognized for the interpretation of the data identifying the effects of heat stress on baroreflex control of heart rate.

Significant variation in baroreflex slopes was observed between subjects. For example, the slope of the relationship between MSNA and blood pressure increased in three subjects, decreased in three subjects, and did not change in one subject during heating (Fig. 2). Similar variability was observed in identifying the effects of heat stress on baroreflex control of heart rate (Fig. 3). The number of subjects chosen to participate in this experiment was derived from previously obtained data in which similar analyses were conducted and assuming a similar change in baroreflex gain relative to that previously observed with metaboreceptor stimulation (6). We recognize that the relatively large degree of variability between subjects increases the likelihood of committing a beta error. However, given the present variability, an inordinate number of subjects (i.e., 50–70) would be required to confirm that heat stress does not alter baroreflex regulation of MSNA and heart rate. Thus, although we believe our interpretation of the data represents the overall effects of heat stress on baroreflex regulation of MSNA and heart rate, we recognize the potential of committing a beta error with respect to the interpretation of the data.

Perspectives

Previous studies showed that humans become more susceptible to fainting during orthostatic stress and gravitation acceleration when combined with heat stress (1, 15). Lind et al. (15) suggested that reductions in orthostatic tolerance in heated humans were not a result of vascular pooling. Thus a reasonable hypothesis leading to reduced tolerance to orthostatic stress would be heat-induced alterations in baroreflex control of blood pressure and heart rate. In contrast to this hypothesis, the present results demonstrate that baroreceptors remain capable of modulating MSNA and heart rate in supine humans, and they further demonstrate that the slope of these baroreflex responses, around the operating point, is not changed by moderate heat stress. However, the present data do not address the potential effects of heat stress-induced alterations of postsynaptic events. For example, rat mesentery arteries have reduced vascular reactivity during exposure to high local temperatures both in vivo (14, 17) and in isolated vessel preparations (16). Moreover, we recently reported that carotid baroreflex regulation of arterial blood pressure is impaired following whole body heating (2). Thus it remains a possibility that although baroreflex control of MSNA is not affected by elevations in internal temperature, altered postsynaptic responses may contribute to reduced tolerance to orthostatic stress observed in heat-stressed humans (1, 15).

In conclusion, results from this study suggest that whole body heating does not alter arterial baroreflex modulation of MSNA or heart rate. However, heat stress caused an upward shift of the baroreflex curves expressing the MSNA-diastolic blood pressure and heart rate-systolic blood pressure relationships.

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