Regulation of the sympathetic nerve discharge bursting pattern during heat stress

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Kenney, Michael J., Dale E. Claassen, Michelle R. Bishop, and Richard J. Fels. Regulation of the sympathetic nerve discharge bursting pattern during heat stress. Am. J. Physiol. 275 (Regulatory Integrative Comp. Physiol. 44): R1992–R2001, 1998.—Frequency-domain analyses were used to determine the effect of heat stress on the relationships between the discharge bursts of sympathetic nerve pairs and sympathetic and phrenic nerve pairs in chloralose-anesthetized rats. Sympathetic nerve discharge (SND) was recorded from the renal, splanchnic, splenic, and lumbar nerves during increases in core body temperature (Tc) from 38 to 41.4 ± 0.3°C. The following observations were made: 1) hyperthermia transformed the cardiac-related bursting pattern of SND to a pattern that contained low-frequency, non-cardiac-related bursts, 2) the pattern transformation was uniform in regionally selective sympathetic nerves, 3) hyperthermia enhanced the frequency-domain coupling between SND and phrenic nerve bursts, and 4) low-frequency SND bursts recorded during hyperthermia contained significantly more activity than cardiac-related bursts. We conclude that acute heat stress profoundly affects the organization of neural circuits responsible for the frequency components in sympathetic nerve activity and that SND pattern transformation provides an important strategy for increasing the level of activity in sympathetic nerves during increased Tc.

Increased internal body temperature (Tc) provides a potent stimulus to the sympathetic nervous system as acute heating significantly increases the activity in sympathetic nerves (renal, splanchnic, and lumbar) that innervate different regional arterial beds (9, 14). In addition, the pattern of sympathetic nerve discharge (SND) bursts is altered during elevations in Tc (14). Specifically, the cardiac-locked bursting pattern of renal SND can be transformed to a pattern that contains low-frequency, non-cardiac-related SND bursts at or near the frequency of ventilation during heating in chloralose-anesthetized rats (14). Although this hyperthermia-induced change in the pattern of SND bursts demonstrates plasticity in the neural circuits responsible for efferent sympathetic neural outflow, our understanding of the influence of acute heat stress on sympathetic neural regulatory mechanisms is limited by several important factors.

Because hyperthermia-induced SND pattern changes have been identified in experiments from which sympathetic activity was recorded from a single nerve, it is not known whether there is uniformity or nonuniformity in the SND bursting pattern in regionally selective sympathetic nerves during increases in Tc. Relative to this point, the sources of synchronized discharges in different sympathetic nerves uncouple (reduced coherence) in response to some types of acute stress (i.e., asphyxia and after sustained stimulation of baroreceptor afferents) despite the fact that these interventions produce directionally similar changes in the activity in different sympathetic nerves (5, 13). Uncoupling of the sources of synchronized discharges may be one strategy by which the central nervous system exerts selective control over the activity in different sympathetic nerves during interventions that produce similar changes in efferent sympathetic nerve outflow. Whether there is a transformation from a system of highly coupled circuits to one of multiple generators exerting selective control over efferent SND during hyperthermia in chloralose-anesthetized rats has not been established. One goal of this study was to determine the influence of acute heat stress on the frequency-domain relationships between the discharges in sympathetic nerve pairs (renal-splanchnic, renal-splenic, and renal-lumbar). Splanchnic SND was recorded because celiac ganglionectomy abolishes the increase in mesenteric resistance to hyperthermia (20) and loss of splanchic vasomotor function contributes to circulatory failure in heat stroke (23). Renal SND was recorded because the sympathetic neural innervation to the kidney affects renal blood flow, renal release, and salt and water retention by the renal tubules (6), physiological alterations that likely influence the cardiovascular responses to increased Tc. Because the spleen provides an important contribution to immunological defense mechanisms (26), splenic SND recordings provided information concerning the influence of hyperthermia on the sympathetic nerve responses to a visceral organ with a markedly different physiological function than the kidneys and abdominal organs. The recording of lumbar SND determined the influence of elevated Tc on the sympathetic innervation to the rat hindlimb skeletal muscle and skin vasculatures.

Although our recent work has focused on characterizing the influence of acute stress on the basic pattern of SND bursts, the functional role of SND pattern changes remains to be elucidated. Because increases in sympathetic nerve activity and changes in the SND bursting pattern occur progressively as Tc is elevated (14), we reasoned that SND pattern changes may provide an important central nervous system strategy for increasing the level of activity in sympathetic nerves in response to acute stress. A second goal was to determine if heating-induced changes in the pattern of SND

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bursts directly contribute to increasing efferent sympathetic nerve activity.

The presence of SND bursts with a slow periodicity at or near the frequency of ventilation suggests prominent coupling between central respiratory drive and efferent sympathetic outflow during heating (14); however, the lack of information from simultaneous phrenic nerve discharge (PND) and efferent SND recordings in chloralose-anesthetized rats limits this interpretation. A third goal was to examine the respiratory modulation of sympathetic nerve activity during acute heat stress by determining the frequency-domain relationships between PND and renal SND bursts during progressive increases in Tc.

Hyperthermia produced by acute heating in chloralose-anesthetized rats significantly increases arterial blood pressure (9, 14, 20, 23). A role for the sympathetic nervous system in the pressor response to acute heat stress in chloralose-anesthetized rats has been established, because surgical removal of the celiac ganglion attenuates the rise in arterial blood pressure to hyperthermia (20). In addition to activation of the sympathetic nervous system, acute heating produces a moderate increase in plasma arginine vasopressin levels in conscious rats (22). Interestingly, antagonism of central neural ANG II receptors attenuates hyperthermia-induced increases in arterial blood pressure and eliminates the splanchic SND and arginine vasopressin responses to heat stress, indicating that neuroendocrine interactions play an important role in arterial blood pressure regulation during heating (22). We reasoned that, regardless of the potentially complex central neural and humoral interactions that may contribute to heating-induced elevations in arterial blood pressure, the sympathetic nervous system is likely the most prominent effector, because hyperthermia increases the activity in sympathetic nerves that innervate regionally selective arterial beds (9, 14, 22). A fourth goal of this study was to determine the effect of ganglionic blockade on the level of arterial blood pressure before and after Tc had been elevated to 41.5°C.

**METHODS**

General procedures. The surgical procedures and experimental protocols used were approved by the Institutional Animal Use and Care Committee. Experiments were performed on male Sprague-Dawley rats (250–350 g). Anesthesia was initially induced with methohexital sodium (Brevital; 50–60 mg/kg ip). Two catheters (PE-10 and PE-50) were placed in the femoral vein. The PE-10 catheter was used during the surgical preparation for administration of maintenance doses of methohexital sodium (10–20 mg/kg) and during the experimental protocols for administration of drugs. The PE-50 catheter was used for the administration of an initial dose of α-chloralose (50 mg/kg) and for maintenance doses (35 mg·kg⁻¹·h⁻¹) throughout the surgical preparation and experiment. The trachea was cannulated with a PE-240 catheter, and rats were allowed either to breathe oxygen-enriched air spontaneously or were paralyzed with gallamine triethiodide (5–10 mg/kg iv initial dose) and artificially ventilated. Femoral arterial pressure and heart rate (HR) were recorded using standard procedures. Tc was measured with a thermistor probe inserted ~5–6 cm into the colon and was kept at 38.0°C during surgery by a temperature-controlled table.

Bilateral denervation of the aortic arch was completed by 1) cutting the superior laryngeal nerve near its junction with the vagus nerve and 2) removing the superior cervical ganglion (24). Bilateral carotid sinus denervation was completed by removing the adventitia from the area of the carotid sinus bifurcation and applying 10% phenol to this area (24). Bilateral cervical vagotomies were completed by sectioning the vagus nerves at the level of the carotid sinus bifurcation. Denervation was considered complete by 1) the loss of coherence between the arterial pulse and SND (12, 19) and/or 2) the absence of a reflex change in SND from control levels during increases in arterial pressure produced by the administration of phenylephrine hydrochloride (3–5 μg/kg iv) and during decreases in arterial pressure produced by the administration of sodium nitroprusside (3–5 μg/kg iv).

Neural recordings. Activity was recorded biphasically with a platinum bipolar electrode after capacity-coupled preamplification (band pass 30–3,000 Hz) from the central end of cut or distally crushed renal, splanchnic, splenic, and lumbar sympathetic nerves and the phrenic nerve. The left renal and splanchnic nerves were isolated either retroperitoneally or after a midline laparotomy. The left lumbar nerve was isolated from a midline approach. The left phrenic nerve was isolated in the cervical region. The nerve-electrode preparations were covered with a silicone gel to prevent exposure to room air. The sympathetic and phrenic nerve potentials were full-wave rectified and integrated (time constant 10 ms), which produced a smooth tracing of the synchronized discharges. Activity in SND and PND recordings was quantified as volts × seconds (V·s). The sympathetic nerve recordings were corrected for background noise after administration of the ganglionic blocker trimethaphan camsylate (10–15 mg/kg iv).

Experimental protocols. After surgery, the chloralose-anesthetized rats were allowed to stabilize for 30–60 min before initiation of the experimental protocols. At the end of this control period, Tc was increased at a rate of ~0.1°C/min from 38 ± 0.1 to 41.4 ± 0.3°C using a heat lamp positioned ~40 cm above the animal. Mean arterial pressure (MAP), HR, and sympathetic (SND and PND) bursts were recorded continuously during progressive increases in Tc. Four protocols were completed.

**Protocol I** determined the effect of increased Tc on the frequency-domain relationships (as determined by coherence analysis) between the simultaneously recorded discharges of sympathetic nerve pairs (renal-splanchnic, n = 8; renal-splenic, n = 5; renal-lumbar, n = 5) in baroreceptor-innervated rats. In addition, the change in the level of activity associated with SND pattern alterations was determined in each of these experiments. For this purpose, the amount of activity (V·s after integration) in cardiac-related SND bursts was compared with that in low-frequency SND bursts. Comparisons were made on temporally continuous data sequences. Experiments were also completed to determine if heating-induced SND pattern changes result primarily from the duration of the heat exposure or from the magnitude of the Tc increase. For this purpose, renal SND was recorded in baroreceptor-innervated rats (n = 4) during a heating protocol that increased Tc from 38 to 40.4 ± 0.2°C, followed by a maintenance phase in which Tc was kept at this elevated level for an additional 20 min. SND autospectra were constructed during control, immediately after Tc had been raised to 40.4 ± 0.2°C, and after maintaining Tc for 20 min at this elevated level. Protocol II determined the effect of increased Tc on the basic pattern of renal SND bursts in baroreceptor-denervated rats (n = 4). Protocol III determined the effect of progressive...
increases in Tc on the relationships between the simultaneously recorded discharges of SND (renal, n = 8; splanchic, n = 2) and PND bursts using the coherence function. Bilateral vagotomies were completed in six experiments, and end-tidal CO2 was kept between 4.5 and 5.0% by adjusting the frequency of respiration during hyperthermia. In four experiments, the vagus nerves were left intact and the animals were allowed to breathe room air spontaneously during the heating protocol. End-tidal CO2 remained between 4.5 and 5.0% during heating in these animals. Because there were no differences in the results from experiments using vagotomized or intact animals (control coherence: vagotomized, 0.56 ± 0.09; intact, 0.61 ± 0.04 and heating coherence: vagotomized, 0.84 ± 0.02; intact, 0.81 ± 0.03), the data were combined for presentation. Protocol IV determined the effect of ganglionic blockade (produced by administration of 10 mg/kg trimethaphan camsylate) on the level of arterial blood pressure before and after Tc was elevated to 41.5°C (n = 8).

Data analysis. Autospectra and coherence analyses of the arterial pulse, SND bursts, and PND bursts were computed using the methods and programs described earlier (13, 19). Fast Fourier transform was performed on 12–24 contiguous windows of data that were 5 s in duration. The signals were sampled at 200 Hz. Autospectra and coherence functions were computed over a frequency band of 0–15 Hz. The amplitude of the autospectra was autoscaled to the highest peak (19). The frequency resolution was 0.2 Hz/bin. SND autospectra were constructed every 0.5°C change in Tc during the initial stages of the heating protocol (Tc 38–39°C) and every 0.1–0.2°C change in Tc during the middle and later stages of heating (Tc 39–41.5°C). The significance of nonzero coherence was tested using the 95% confidence intervals of Benignus (3). The coherent frequency band was defined as the range of frequencies over which the coherence values were significantly different from 0.

Spectral analyses provide the following information (7, 8, 16, 19). The autospectrum of a signal shows the relative power present at each frequency. The coherence function (normalized cross spectrum) provides a measure of the strength of linear correlation of two signals as a function of frequency. The squared coherence value (referred to as coherence value) is 1.0 in the case of a linear system undisturbed by noise and 0 if the two signals are completely unrelated. The coherence value is significantly > 0 but < 1 when 1) the two signals arise from common and uncommon sources, 2) noise is present in the system, and/or 3) the system relating the two signals is nonlinear.

Control values of SND were taken as 100%. Values in the text and figures are means ± SE. Statistical analysis was performed using Student’s t-test for pairwise comparisons and repeated-measures ANOVA followed by Bonferroni post hoc tests. P < 0.05 indicated statistical significance.

RESULTS

Protocol I: Effects of hyperthermia on SND frequency components in baroreceptor-innervated rats. The effect of increases in Tc on MAP, HR, and the frequency-domain characteristics of efferent SND in baroreceptor-innervated rats were determined in 18 experiments. SND was recorded from sympathetic nerve pairs (renal-splanchic, n = 8; renal-splenic, n = 5; renal-lumbar, n = 5). Figure 1 shows traces (from 3 separate experiments) of simultaneously recorded sympathetic nerve slow waves (A, renal-splanchic; B, renal-splenic; C, renal-lumbar) and pulsatile arterial blood pressure traces during control (38°C; left) and after heating to 40.5°C (middle) and 41.5°C (right). MAP values recorded during each period are shown (below pulsatile arterial pressure traces; Fig. 1). During control, the majority of SND slow waves were coupled to the arterial pulse. After heating to 40.5°C, the sympathetic nerve signals contained both cardiac-locked and low-frequency bursts. At 41.5°C, activity in each of the regionally selective sympathetic nerves was dominated by the presence of low-frequency bursts. MAP was progressively increased from control values during heating in each experiment.

MAP, HR, and SND data were analyzed at three points: control before heating (Tc 38°C); during moderate increases in Tc to 40.3 ± 0.2°C when the SND bursting pattern was characterized by the presence of both cardiac-locked and low-frequency bursts (referred to as transition period); and after raising Tc to 41.4 ± 0.3°C when the SND pattern was dominated by the presence of low-frequency bursts (referred to as hyperthermia). Autospectral analysis was used to identify SND pattern changes at specific Tc values. Coherence analysis was used to determine the effect of hyperthermia on the frequency-domain relationships between simultaneously recorded discharges in sympathetic nerve pairs. MAP (control, 121 ± 5 mmHg; transition, 133 ± 6 mmHg; hyperthermia, 140 ± 4 mmHg; P <
During control, the autospectrum of each nerve (Fig. 2, 41.5°C from one experiment are shown in Fig. 2. renal and splanchnic SND recorded at 38, 40.2, and control) increased from control values during heating. hyperthermia values were significantly different from hyperthermia, 552 ± 6 0.05 compared with control values) and HR (control, 425 ± 13 beats/min; transition, 508 ± 15 beats/min; hyperthermia, 552 ± 10 beats/min; both transition and hyperthermia values were significantly different from control) increased from control values during heating.

The results of autospectral and coherence analyses of renal and splanchnic SND recorded at 38, 40.2, and 41.5°C from one experiment are shown in Fig. 2. During control, the autospectrum of each nerve (Fig. 2, A and B, left) contained a primary peak at the frequency of the HR (7.0 Hz). The coherence function (Fig. 2C, left) describing the relationship between the discharges in these nerves demonstrated a significant correlation that extended from 0 to 15 Hz, and the peak coherence value was 0.96 at 7.0 Hz. During heating at a Tc of 40.2°C (transition period), the primary peaks in the renal and splanchnic SND autospectra (Fig. 2, A and B, right) remained at 1.8 Hz, the peak coherence value was 0.97 at 1.8 Hz (frequency of cardiac cycle was 10.0 Hz), and the coherent frequency band extended from 0 to 15 Hz (Fig. 2C, right).

The peak coherence values relating the discharges in the renal-splanchnic, renal-splenic, and renal-lumbar nerve pairs remained unchanged from control levels during the transition and hyperthermia periods (Table 1). The coherent frequency band for each sympathetic nerve pair extended from 0 Hz to at least 12 Hz during the control, transition, and hyperthermia periods. Activity in the renal, splancnic, splenic, and lumbar nerves increased from control values during heating, with the most prominent sympathoexcitatory responses occurring during the hyperthermia period (Table 2). Heating-induced increases in SND recorded during the transition and hyperthermia periods were not significantly different between individual nerves in each pair (this included renal-lumbar nerve pair, although heating-induced sympathoexcitation tended (P < 0.10) to be higher in renal than in lumbar nerve).

The change in the level of activity associated with the SND pattern transformation was determined by comparing the amount of activity in cardiac-locked and low-frequency bursts during the transition periods. Comparisons were completed on temporally continuous data sequences at the same level of arterial pressure and for the same amount of time. Figure 3 shows continuous traces of renal and splanchnic SND slow waves recorded during heating from one representative experiment. The lines below the SND traces depict the time interval when the level of activity in cardiac-locked and low-frequency bursts were compared. Three points are worth noting. First, the SND tracings con-
tain both low-frequency and cardiac-locked bursts. Second, pulsatile arterial blood pressure remained essentially unchanged during the specified time intervals. Third, when the level of activity (V·s) in the specific bursts is compared for the same time interval, the amount of activity is higher during those intervals when low-frequency bursts are evident. An increased amount of activity was present during the low-frequency bursts because 1) activity remained elevated from baseline levels during the entire burst and/or 2) the amplitude of the sympathetic signal was increased during these bursts.

The level of activity present during cardiac-related and low-frequency bursts was compared for the same time interval (ranging between 320 and 480 ms) and level of MAP in each of the 18 experiments in which dual nerve recordings were completed. The amount of activity in the renal ($0.32 \pm 0.02$ to $0.50 \pm 0.04$ V·s, $P < 0.05$, 78 comparisons), splanchnic ($0.40 \pm 0.04$ to $0.64 \pm 0.05$ V·s, $P < 0.05$, 28 comparisons), splenic ($0.35 \pm 0.04$ to $0.53 \pm 0.05$ V·s, $P < 0.05$, 26 comparisons), and lumbar ($0.25 \pm 0.02$ to $0.47 \pm 0.04$ V·s, $P < 0.05$, 24 comparisons) nerves was increased during those time intervals in which low-frequency rather than pulse-synchronous bursts were present. The amount of activity was also compared during heating at $T_c$, when the sympathetic signal contained primarily cardiac-locked ($T_c$ generally between 39.5–40°C) or low-frequency ($T_c$ near 41.5°C) bursts. The level of activity was compared for the same number of cardiac cycles (30–35), not for the same amount of time, to control for heating-induced increases in HR. Figure 4 shows the results of one representative experiment. At 41°C, SND exhibited primarily cardiac-locked bursts with an integrated voltage of 2.99 V·s for 30 cardiac cycles (3.9 s of data). At 41.5°C, SND was characterized by the presence of low-frequency bursts and the level of activity was increased (4.46 V·s for 30 cardiac cycles), despite the fact that the interval of time (3.2 s of data) was reduced. The level of sympathetic nerve activity was increased (renal SND, 66%, $P < 0.05$; splanchnic SND, 65%, $P < 0.05$; splenic SND, 56%, $P < 0.05$; lumbar SND, 31%, $P < 0.07$) for the same number of cardiac cycles when the SND bursting pattern was transformed from cardiac-locked to low-frequency bursts during heating.

Because 30–35 min were required to increase $T_c$ from 38 to 41.5°C, it was unclear whether heating-induced SND pattern changes resulted primarily from the duration of the heat exposure or from the magnitude of the increase in $T_c$. To address this issue, we completed four experiments in which renal SND was recorded during a heating protocol that involved raising $T_c$ at a rate of 0.1°C/min from 38 to ~40.5°C ($T_c$), followed by a maintenance phase whereby $T_c$ was kept constant at this elevated level for an additional 20 min. These experiments were completed in baroreceptor-innervated rats. Figure 5 shows the results of a representative experiment. At 41°C, SND exhibited primarily cardiac-locked bursts with an integrated voltage of 2.99 V·s for 30 cardiac cycles (3.9 s of data). At 41.5°C, SND was characterized by the presence of low-frequency bursts and the level of activity was increased (4.46 V·s for 30 cardiac cycles), despite the fact that the interval of time (3.2 s of data) was reduced. The level of sympathetic nerve activity was increased (renal SND, 66%, $P < 0.05$; splanchnic SND, 65%, $P < 0.05$; splenic SND, 56%, $P < 0.05$; lumbar SND, 31%, $P < 0.07$) for the same number of cardiac cycles when the SND bursting pattern was transformed from cardiac-locked to low-frequency bursts during heating.

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signal was characterized by discharge bursts that were not related to pulsatile arterial pressure and the SND autospectra contained a primary peak between 0 and 3 Hz with a broad-band frequency distribution that extended from 0 to 15 Hz. There was an absence of a primary peak at the frequency of the cardiac cycle (7.8 Hz in this experiment). Importantly, in the absence of baroreceptor afferents, the sympathetic nerve signal during heating was dominated by the presence of low-frequency bursts, and the SND autospectrum exhibited a narrow band with a prominent peak at 1.4 Hz. Similar heating-induced changes in the renal SND bursting pattern were observed in four baroreceptor-denervated rats after elevation of Tc from 38 to 41 ± 0.4°C. MAP (38°C, 108 ± 6 mmHg; 41 ± 0.4°C, 138 ± 5 mmHg; P < 0.05 compared with control) and HR (38°C, 425 ± 36 beats/min; 41 ± 0.4°C, 573 ± 19 beats/min; P < 0.05 compared with control) increased from control values during heating in sinoaortic-denervated rats.

Protocol III: Effect of acute heat stress on frequency-domain relationships between renal and phrenic nerves. Figure 7 shows tracings of phrenic and renal nerve discharge bursts and pulsatile arterial pressure recorded during control at 38°C and after heating to 41.5°C. In addition to the prominent coupling of SND to the arterial pulse during control, periodic fluctuations in the amplitude of SND bursts were evident toward the end of each PND burst, demonstrating respiratory modulation of basal SND. The respiratory modulation of SND was more prominent during heating, because efferent SND exhibited low-frequency bursts that were prominently coupled to PND bursts. Specifically, SND remained elevated during the intervals between PND bursts and returned to baseline levels toward the end of each PND burst.

The influence of hyperthermia on the relationships between the discharge bursts in sympathetic and phrenic nerves was determined in 10 experiments. Figure 8, A and B, shows the results of one experiment in which renal SND and PND autospectra and the coherence function (Fig. 8C) describing the relationships between the activity in these nerves were constructed during control at 38.0°C (left) and after heating to 41.5°C (right). During control, the renal SND autospectrum contained a primary peak at 6.4 Hz, although there was also power at non-cardiac-related frequencies. Importantly, a small but distinct peak in the renal SND autospectrum was evident at the frequency of PND (1.0 Hz). The renal nerve and phrenic nerve coherence function exhibited a significant peak at 1.0 Hz.

Fig. 5. Autospectra of renal SND constructed in a baroreceptor-innervated rat during control (A), immediately after increasing Tc to 40.8°C (B), and after maintaining Tc at 40.8°C for 20 min (C). Frequency band is displayed from 0 to 15 Hz.

Fig. 6. Traces of integrated renal SND bursts and pulsatile AP (A) and autospectra of renal SND (B) during control (38°C) and after heating to 41°C in a baroreceptor-denervated rat. Frequency band is displayed from 0 to 15 Hz. Horizontal calibration bar is 500 ms.
(0.68) at 1.0 Hz. During heating, the primary peaks in the renal SND and PND autospectra were at 1.6 Hz and the peak coherence value relating the activity in these nerves was increased to 0.91. In 10 experiments, the peak coherence value relating PND and SND bursts was increased \( (P < 0.05) \) from 0.58 ± 0.06 during control to 0.83 ± 0.02 after elevation of \( T_c \) to between 41 and 41.5°C. As expected, the primary peak in the SND autospectra in these experiments (both vagotomized and intact) was shifted from the frequency of the cardiac cycle during control to the frequency of PND during heating. The frequency of PND bursts increased \( (P < 0.05) \) from 1.0 ± 0.1 Hz during control to 1.4 ± 0.08 Hz at 41.5°C. In addition, the amount of activity in the phrenic neurogram increased \( (P < 0.05) \) from 56 ± 12 V·s during control to 92 ± 23 V·s after elevating \( T_c \) to 41.5°C.

**Protocol IV: Effect of ganglionic blockade on arterial blood pressure maintenance during hyperthermia.** The effect of ganglionic blockade (produced by trimethaphan administration) on the level of arterial pressure before \( (T_c = 38°C) \) and after heating to 41.5°C was determined in eight experiments. Ganglionic blockade at 38°C reduced MAP from 148 ± 6 to 4 ± 2 mmHg. At 41.5°C, trimethaphan administration (same dose as used during control) decreased MAP from 177 ± 6 to 51 ± 3 mmHg. Importantly, the level of MAP recorded after trimethaphan administration at 41.5°C was significantly less than the lowest level recorded after ganglionic blockade during control \( (38°C, 64 ± 2 \text{ mmHg}; 41.5°C, 51 ± 3 \text{ mmHg}) \). This was evident despite the fact that the background level of activity that remained after ganglionic blockade at 41.5°C \( (10 ± 2 \text{ V·s for 60 s}) \) was higher than that recorded after ganglionic blockade at 38°C \( (4 ± 1 \text{ V·s for 60 s}) \), presumably because heating-induced increases in SND require a higher dose of trimethaphan to produce the same level of ganglionic blockade.

**DISCUSSION**

This study examined the effects of acute heat stress on the frequency-domain relationships between the discharge bursts of sympathetic nerve pairs and sympathetic and phrenic nerve pairs in baroreceptor-innervated, chloralose-anesthetized rats. Our results provide experimental support for three new findings that are central to understanding how periods of acute heat stress influence the organization of sympathetic neural circuits responsible for efferent nerve outflow. First, increased \( T_c \) produced similar changes in the discharge pattern of different sympathetic nerves, demonstrating a lack of regional selectivity in frequency-domain SND responses to hyperthermia. Second, hyperthermia enhanced the coupling between PND and SND bursts, demonstrating prominent respiratory modulation of efferent sympathetic nerve outflow during hyperthermia. Third, the low-frequency SND bursts recorded during heating contained more activity than cardiac-related bursts, demonstrating that SND pattern changes contribute significantly to increasing efferent sympathetic nerve activity during progressive increases in \( T_c \). In addition, our results show that ganglionic blockade during heat stress reduces arterial pressure to levels below those produced by ganglionic blockade at control, demonstrating an important role.
for efferent sympathetic nerve outflow in arterial blood pressure regulation during hyperthermia.

It is widely accepted that the sympathetic nervous system is capable of producing nonuniform changes in peripheral SND to selectively control different regional circulations (1, 2). At least two types of nonuniformity have been described. First, directionally opposite changes in the level of activity in regionally selective sympathetic nerves have been demonstrated during a variety of interventions (e.g., hemorrhagic hypotension, hypertonic saline infusion, activation of vagal nerve afferents, and nitric oxide synthase inhibition) (11, 12, 30, 31). Second, stress-induced changes in the frequency-domain relationships between the activity in sympathetic nerve pairs have been observed. Specifically, the sources of synchronized discharges in different sympathetic nerves uncouple (i.e., reduced coherence) during periods of asphyxia (13) and after sustained activation of arterial baroreceptor afferents (5) in chloralose-anesthetized rats. The current results demonstrate that acute heating produces similar changes in the pattern of SND bursts in regionally selective sympathetic nerves (i.e., renal, splanchnic, splenic, and lumbal) and is associated with strong coupling between the discharge bursts in selected sympathetic nerve pairs. Moreover, in the current study, the level of activity in each of the regionally selective sympathetic nerves was significantly increased from control values during heating. These observations, along with the previously documented heating-induced increases in renal, lumbar, and splanchnic SND (9, 13), indicate that there is little regional selectivity in the responses of sympathetic nerves innervating different target organs during hyperthermia. The lack of regional selectivity in the frequency-domain characteristics of SND suggests that acute heat stress is likely not associated with a transformation to a central system of multiple generators exerting selective control over SND.

Results from the current and other studies (13–15, 17, 18) demonstrate that experimental interventions that produce marked increases in sympathetic nerve activity are associated with changes in the frequency of the SND pattern. These observations support the concept that the neural circuits responsible for efferent SND are capable of generating different types of activity patterns, depending on the physiological state of the animal. The results of the current study extend these findings by demonstrating that hyperthermia-induced pattern alterations involve enhanced coupling between respiratory and sympathetic nerve-related central circuits. Relative to this point, the low-frequency SND bursts recorded during acute heat stress are prominently coupled to PND bursts as evidenced by significant increases from control levels in the peak coherence values relating the activity in these nerves. The prominent respiratory-related activity in efferent SND recordings during acute heating is not simply the result of entrainment of central neurons by inputs from pulmonary stretch receptors, because efferent SND bursts were locked to the central respiratory cycle in vagotomized and paralyzed animals. The enhanced respiratory modulation of SND bursts during heating did not result from changes in respiratory drive, because end-tidal CO2 levels remained constant during elevations in Tc.

Although acute stress can alter the pattern of efferent SND (13, 14, 17, 18), the functional significance of SND pattern changes is not well understood. Relative to this point, the results of the present study demonstrate that wide-band SND bursts recorded during hyperthermia contain significantly more activity (V·s) than cardiac-related bursts (when quantified for same time interval or same number of cardiac cycles), establishing pattern transformation as an important strategy for increasing the level of efferent sympathetic nerve activity during acute heat stress. Importantly, as demonstrated by the results of the trimethaphan experiments, the ability to generate an activated sympathetic state during heat stress is essential for the maintenance of arterial blood pressure and vital organ perfusion pressure. In this regard, the ability of sympathetic neural circuits to alter the pattern of efferent discharge bursts and thereby increase total activity is important for the physiological adjustments to heat stress. Although the current results do not establish the mechanism(s) involved in changing the pattern of efferent SND bursts during hyperthermia (it does appear that magnitude of increase in Tc is important), it is unlikely that cardiopulmonary and arterial baroreceptor afferents play a key role, because hyperthermia-induced changes in the SND pattern were observed after bilateral cervical vagotomy and sinoaortic denervation.

In addition to increasing the level of activity in efferent sympathetic nerves, the respiratory modulation of efferent sympathetic nerve outflow may be physiologically important for several other reasons (10). For example, grouping of impulses may lead to temporal and spatial summation of potentials in postganglionic nerves (10). In addition, the results of several studies demonstrate that bursts of impulses can be more effective than continuous trains at eliciting vasoconstriction in various organs (25, 27, 28). Relative to this point, a recent study by Stauss and Kregel (29) reported that electrical stimulation of the splanchnic nerve at frequencies between 0.1 and 1.0 Hz significantly increased the power in mesenteric resistance. Importantly, these oscillations in mesenteric resistance were translated to arterial blood pressure (29), suggesting that the frequency range of vasomotor activity in peripheral sympathetic nerves can include that of central respiration. It may be that hyperthermia-induced low-frequency SND bursts enhance the effectiveness of sympathetic nerve activity directed toward regionally selective target organs.

The fact that pharmacological blockade of ganglionic transmission during increased Tc produced an immediate and significant reduction in MAP demonstrates that the sympathetic nervous system is a prominent effector in arterial blood pressure regulation during heating. Interestingly, not only did ganglionic blockade during heating eliminate the hyperthermia-induced increases in arterial pressure, it also reduced the basal
level of arterial pressure to values less than those produced by ganglionic blockade at control. The elimination of the heating-induced increase in arterial pressure by ganglionic blockade was expected, because surgical removal of the celiac ganglion has previously been shown to attenuate the pressor response to hyperthermia (20). The fact that the basal level of arterial pressure was less after ganglionic blockade during hyperthermia (20) demonstrates that the basal level of arterial pressure to values less than those produced by ganglionic blockade at control. The elimination of the heating-induced increase in arterial pressure by ganglionic blockade was expected, because surgical removal of the celiac ganglion has previously been shown to attenuate the pressor response to hyperthermia (20). The fact that the basal level of arterial pressure was less after ganglionic blockade during hyperthermia (20) demonstrates that the basal level of arterial pressure to values less than those produced by ganglionic blockade at control. The elimination of the heating-induced increase in arterial pressure by ganglionic blockade was expected, because surgical removal of the celiac ganglion has previously been shown to attenuate the pressor response to hyperthermia (20).

**Perspectives**

Because the sympathetic nervous system plays a key role in the maintenance of physiological homeostasis during periods of acute physical stress, it is important to understand mechanisms involved in sympathetic nerve regulation. Relative to this point, the sympathetic nerve responses to heating provide insight into the dynamic nature of sympathetic neural circuits. First, hyperthermia changes the pattern of efferent SND bursts, demonstrating that sympathetic neural circuits are capable of generating different burst frequencies (i.e., neuronal plasticity) depending on the physiological state of the animal. Second, hyperthermia influences the frequency-domain relationships between respiratory and sympathetic neural circuits, as demonstrated by the enhanced coupling between phrenic nerve and SND bursts during acute heat stress. This is noteworthy, because respiratory modulation of sympathetic nerve activity represents an electrophysiological correlate of the cooperation between the cardiovascular and respiratory systems (32). Third, hyperthermia-induced changes in the SND bursting pattern directly contribute to increasing sympathetic nerve activity, suggesting that pattern transformation is an important central neural strategy for regulating the level of activity in efferent sympathetic nerves. These results demonstrate functional plasticity in sympathetic regulatory mechanisms during acute heat stress.

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