RVLM and raphe differentially regulate sympathetic outflows to splanchnic and brown adipose tissue

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Morrison, Shaun F. RVLM and raphe differentially regulate sympathetic outflows to splanchnic and brown adipose tissue. Am. J. Physiol. 276 (Regulatory Integrative Comp. Physiol. 45): R962–R973, 1999.—To determine whether neurons in the rostral raphe pallidus (RPa) specifically control the sympathetic nerve activity to brown adipose tissue (BAT SNA), thereby regulating adipocyte metabolism and BAT thermogenesis, the responses in BAT SNA to disinhibition of RPa neurons and to disinhibition of neurons in the vasomotor region of the rostral ventrolateral medulla (RVLM) were compared with those in splanchnic (Spl) SNA, which primarily regulates visceral vasoconstriction. In urethane-chloralose-anesthetized ventilated rats, both acute hypothermia and microinjection of bicuculline into RPa produced significantly larger increases in BAT SNA (542 and 1.949% of control) than in Spl SNA (19 and 24% of control). The enhanced burst discharge in BAT SNA was not coherent with that in Spl SNA or with the arterial pressure (AP) at any frequency except the central respiratory frequency. Microinjections of bicuculline into RPa evoked increases in Spl SNA (86% of control) and AP (32 mmHg), but reduced BAT SNA to low, normothermic levels. Microinjections of muscimol into RVLM reduced Spl SNA (−82% of control) and AP (−59 mmHg), but did not prevent the increase in BAT SNA after disinhibition of RPa neurons. These results indicate that the neural networks generating BAT SNA in response to disinhibition of RPa neurons are independent of those generating basal Spl SNA and support a model in which sympathetic outflow to tissues involved in thermoregulation and metabolism is regulated by central pathways, including neurons in RPa, that are distinct from those involved in the sympathetic control of the cardiovascular system.

thermogenesis; fever; bicuculline; sympathetic rhythms; hypothermia

EARLY CONCEPTS of the regulation of autonomic outflow were focused on the sympathetic nervous system as a monolithic effector providing a global enhancement of organ function in response to stress (6). More recently, with improved opportunities to record from the sympathetic nerves to a variety of tissues, to selectively stimulate small populations of central neurons, to assess through c-fos induction which neurons are activated by particular stimuli, and to determine using viral tracing the elements of central circuits contributing to the sympathetic regulation of individual organs, an organizational concept has emerged that emphasizes the differential central control of the sympathetic outflows to functionally specific targets (15). In particular, in regard to medullary regulation of sympathetic outflows, stimulation of subpopulations of sympathetic premotoneurons in the rostral ventrolateral medulla (RVLM) can selectively increase the sympathetic nerve activity (SNA) to particular vascular beds (7, 23, 25), neurons have been identified in this region that are more strongly correlated to specific sympathetic outflows (2), and evidence has been provided for distinct, but coupled, central neural oscillators generating the activity on the sympathetic nerves to a variety of cardiovascular targets (9).

Although considerable information is available on the brain stem regulation and the differential sympathetic control of cardiovascular tissues, relatively little is known about the brain stem networks determining the sympathetic outflow to noncardiovascular targets and their interactions with those governing SNA to the heart and blood vessels. On the basis of the large increases in the SNA to brown adipose tissue (BAT) produced by disinhibition of local neurons with bicuculline, the rostral raphe pallidus (RPa) has recently been identified as a brain stem region containing neurons that are important in the sympathetic regulation of tissues involved in thermoregulation and metabolism (29). The present study examined the specificity of the RPa regulation of BAT SNA by comparing basal and evoked BAT SNA with that of the splanchnic (Spl) nerve, involved predominantly in cardiovascular regulation through innervation of visceral vascular tissues. The results suggest a similar organization in the neural mechanisms generating BAT and Spl SNAs, as well as a common modulation by central respiratory networks; however, the differences in frequency characteristics, baroreceptor modulation, and responses to hypothermia and disinhibition of RPa neurons, as well as the absence of a significant coherence between BAT and Spl SNAs, indicate that they are generated by separate brain stem networks. These data support a model in which neurons in RPa are predominantly involved in the regulation of sympathetic outflows to targets with a thermoregulatory and metabolic function compared with neurons in the RVLM that exert selective premotor control of the sympathetic nerve activities to cardiovascular target tissues. A preliminary report of these results has been made (26).

METHODS

Sprague-Dawley rats (300–450 g, 11 male, 2 female) were anesthetized intravenously with urethan (0.8 g/kg) and chlоро
cose (80 mg/kg) after induction with 3% isoflurane in 100% O2 and cannulation of a femoral artery, a femoral vein, and the trachea. Arterial pressure (AP) was measured with a transducer (Cobe) connected to the femoral arterial cannula, and heart rate was derived from the AP signal (Gould Biotech). Animals were positioned prone in a stereotaxic frame (incisor bar: −11.0 mm) with a spinal clamp on the T10.
vertebra. Animals were paralyzed with ω-tubocurarine (0.3 mg initial dose, 0.1 mg/h supplements) and artificially ventilated with 100% O2 (50 cycles/min, tidal volume: 3 ml). Small adjustments in minute ventilation were made as necessary to maintain end-tidal CO2 between 4 and 5%. Throughout most of the experiment, colonic temperature was maintained at 37.5°C with a heat lamp and a heating plate beneath the animal. To provide a natural stimulus for the activation of the sympathetic nerve discharge to BAT, body temperature was lowered once in each animal by turning off the heat sources and, in some cases, placing dry ice in contact with the metal heating plate beneath the animal. This caused temperature to fall from 37.5°C to between 34 and 35°C within 10 min, at which time the heat sources were turned on and body temperature returned to 37.5°C.

Postganglionic SNA to BAT was recorded from the central cut end of a small nerve bundle dissected from the ventral surface of the right interscapular BAT after dividing the fat pad along the midline and reflecting it laterally. With the use of a dorsal approach, postganglionic SNA was recorded from the central cut end of the left splanchic nerve as it exited from the suprarenal ganglion. The left phrenic nerve was dissected medial to the scapula in the vicinity of the brachial plexus. Anesthesia may dampen sympathetic outflow and thermoregulation, but sympathetic nerve recordings in conscious animals and in humans indicate similar burst characteristics, frequency components, and reflex responses to those seen in anesthetized animals. In some animals, the vagi were sectioned bilaterally to eliminate synchronization of the artificial ventilation and the phrenic nerve activity through the input from pulmonary stretch afferents. The sympathetic nerve activities were filtered from 1 to 300 Hz, and the phrenic nerve was filtered from 300 to 3,000 Hz, amplified with a Cyberamp 380 (Axon Instruments), and digitized (22 kHz) and recorded (Neurodata) on VCR tape along with the AP and stimulus trigger pulses. The experimental protocol consisted of 1) an initial control recording period at a normal body temperature of 37.5°C, 2) a reduction in body temperature, 3) a return to normal body temperature and a second control recording period (30 min after the hypothermia), 4) microinjection of bicuculline into RPa, and 5) microinjection of bicuculline into RVLM or 6) microinjection of muscimol into RVLM followed by a second microinjection of bicuculline into the RPa. No differences were observed between the parameters recorded during the first and second control periods.

A stimulating electrode or a microinjection pipette (tip outside diameter: 20 µm) was positioned stereotaxically in the RPa or the RVLM after a partial occipital craniotomy and reflection of the atlantooccipital membrane. Relative to the calamus scriptorius, the coordinates for the RPa were anterioposterior: 3.0 mm, mediolateral (ML): 0.0 mm, dorsosventral (DV): −2.6 mm, and those for the RVLM were anterioposterior: 2.6 mm, ML: 1.9 mm, DV: −2.3 mm. At the end of each experiment, a pipette containing a 1% solution of fast green dye was stereotaxically positioned at the sites of the microinjections and dye was electrophoretically deposited (15 µA anodal direct current for 15 min). After perfusion and histological processing, the locations of the microinjection sites in the RPa and RVLM were plotted on camera lucida drawings of sections through the rostral medulla (32).

After digitization at 1 kHz, signals were analyzed with software written in the ASYST programing environment. The amplitudes of the SNA to the splanchic nerve and to BAT were derived from autospectral analysis. For each experimental condition, average autospectra of the sympathetic nerve activities were obtained by dividing 20.5-s data records into nine 4.1-s segments with a 50% overlap. The value of the autospectra at each frequency was computed as the mean value of the powers at that frequency in the individual autospectra of these nine segments. The amplitudes of the sympathetic nerve activities were taken as the root mean square value of the total power in the 1- to 10-Hz band of the averaged autospectra. Ordinary and partial coherences among nerve activities were computed according to published algorithms (3, 11). Statistical significance was assessed with the Student’s paired t-test, with P < 0.05.

RESULTS

In the 13 rats studied, the resting mean AP was 110 ± 6 mmHg and the heart rate was 374 ± 17 beats/min in the intact animals (n = 8) and 407 ± 17 beats/min in the vagotomized animals (n = 5). As illustrated in the example in Fig. 1A, at normal body temperature (37.5°C), there was little spontaneous discharge on the sympathetic nerve to BAT, whereas the splanchic nerve exhibited a robust discharge synchronized to the cardiac cycle and modulated over the course of the respiratory cycle. The autospectra of the AP and the splanchic nerve activity had prominent peaks at the heart rate frequency, and there was a near-maximum coherence between the AP and the splanchic nerve activity at the heart rate frequency (AP—Spl coherence value: 0.98 at 6.35 Hz; Fig. 1B). Similarly, the autospectra of the phrenic nerve activity and the splanchic nerve activity had prominent peaks at the central respiratory frequency, and there was a significant coherence between the phrenic and the splanchic nerve activities at the central respiratory frequency (Phr—Spl coherence: 0.94 at 0.73 Hz; Fig. 1B). Albeit of low amplitude, the BAT SNA at normothermic temperature was frequently correlated with the AP at the heart rate frequency (AP—BAT coherence: 0.64 at 6.35 Hz; Fig. 1B), suggesting the presence of a baroreceptor reflex modulation of the BAT SNA. Under these conditions, Spl SNA and BAT SNA were coherent at the frequency of the heart rate (Spl—BAT coherence: 0.64 at 6.35 Hz; Fig. 1B). In all such cases, this coherence was eliminated by “subtracting” the common influence of the baroreceptor reflex through partialization of the coherence between Spl SNA and BAT SNA with respect to the AP (Spl—BAT/AP coherence: 0.01 at 6.35 Hz; Fig. 1B). This result suggests that the neural network generating or controlling the component of BAT SNA present under normothermic conditions may share a common modulatory influence of the baroreceptor reflex with the network regulating Spl SNA but that, beyond this common reflex modulation, there is no detectable interaction between them. The group mean coherence values under normothermic control conditions are presented in Table 1.

As body temperature was lowered, large bursts of spontaneous activity emerged on the sympathetic nerve to BAT (Fig. 1C). These bursts were similar in character to those occurring continuously on the splanchic nerve, suggesting that a similar neural mechanism underlies the synchronization of the discharge of axons in the splanchic nerve and in the sympathetic nerve bundle to BAT. As illustrated in the example in Fig. 1C,
Fig. 1. Effect of acute hypothermia on sympathetic nerve activities to brown adipose tissue (BAT SNA), splanchnic (Spl) SNA, and phrenic nerve activity (Phr, top trace is integrated phrenic nerve activity), arterial pressure (AP), autospectra (as) of these 4 signals, and ordinary coherences between pairs of signals (e.g., Phr → Spl). Both panels are from same experiment. A: 4-s records of Phr, AP, Spl SNA, and BAT SNA during normothermic control conditions (colonic temperature: 37.5°C). B: autospectra and coherences of Phr, AP, Spl, and BAT and partial coherence between Spl SNA and BAT SNA with respect to AP (Spl → BAT/AP) during normothermic conditions. C: same traces as in A, during acute hypothermia (34.4°C). D: same traces as in B, during hypothermia. Horizontal scale bar represents 1 s in A and C. Vertical scale bar represents 100 µV for Phr and 150 µV for Spl SNA in A and C and 60 µV and 100 µV for BAT SNA traces in A and C, respectively.
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Table 1. Effects of acute hypothermia, disinhibition of RPA neurons, and inhibition of RVLM neurons on relationship between Spl and BAT and their central respiratory and baroreceptor modulation

<table>
<thead>
<tr>
<th>Condition</th>
<th>Phr → Spl</th>
<th>AP → Spl</th>
<th>Phr → BAT</th>
<th>AP → BAT</th>
<th>Spl → BAT</th>
</tr>
</thead>
<tbody>
<tr>
<td>Control (37.5°C)</td>
<td>0.79 ± 0.04</td>
<td>0.89 ± 0.03</td>
<td>0.26 ± 0.06</td>
<td>0.76 ± 0.05</td>
<td>0.70 ± 0.06</td>
</tr>
<tr>
<td>Hypothermia (34.5°C)</td>
<td>@0.92 ± 0.11</td>
<td>@6.63 ± 0.20</td>
<td>@0.86 ± 0.08</td>
<td>@6.58 ± 0.23</td>
<td>@6.49 ± 0.22</td>
</tr>
<tr>
<td>Bicuculline in RPA</td>
<td>@0.77 ± 0.04</td>
<td>@0.90 ± 0.03</td>
<td>@0.77 ± 0.04</td>
<td>@6.23 ± 0.22</td>
<td>@2.75 ± 0.82</td>
</tr>
<tr>
<td>Muscimol in RVLM</td>
<td>@0.84 ± 0.03</td>
<td>@0.70 ± 0.15</td>
<td>@0.85 ± 0.04</td>
<td>@7.14 ± 0.17</td>
<td>@0.84 ± 0.04</td>
</tr>
<tr>
<td>Bicuculline in RPA after muscimol in RVLM</td>
<td>NA</td>
<td>@0.61 ± 0.11</td>
<td>NA</td>
<td>0.08 ± 0.03</td>
<td>0.19 ± 0.07</td>
</tr>
</tbody>
</table>

Values are means ± SE of maximum ordinary coherence values and corresponding frequencies (Hz) at which they occurred. NA, not applicable because treatment eliminated phrenic nerve activity (Phr). Spl, splanchnic nerve activity; AP, arterial pressure; BAT, brown adipose tissue sympathetic nerve activity; RPA, raphe pallidus; RVLM, rostral ventrolateral medulla.

during the maximum hypothermic response, the bursts in the SNA to BAT occurred between 1 and 4 Hz, with the autospectrum indicating a mean burst frequency of 3.1 Hz (Fig. 1D) and an increase in amplitude of 1.08% of the normothermic control level (Fig. 1B) of BAT SNA. The bursts in BAT SNA during hypothermia were only weakly correlated with the cardiac cycle, as indicated by the low coherence value between the AP and BAT SNA at the heart rate frequency (AP → BAT, coherence value: 0.03 at 6.35 Hz; Fig. 1D). The bursts in BAT SNA during hypothermia were only weakly correlated with the central respiratory cycle, as indicated by the low coherence value between the phrenic nerve activity and BAT SNA at the phrenic burst frequency (Phr → BAT, coherence value: 0.24 at 0.73 Hz; Fig. 1D). These results suggest that the mechanisms generating the bursts in BAT SNA during acute hypothermia are not modulated to a significant degree by either the baroreceptor reflex or by the central respiratory generating networks.

In contrast to the changes in BAT SNA, acute hypothermia elicited only a slight increase in the amplitude of SPl SNA (25% of control; Fig. 1C), which remained strongly synchronized to the AP (Fig. 1D; AP → Spl, coherence value: 0.99 at 6.35 Hz) and the central respiratory cycle (Fig. 1D, Phr → Spl, coherence value: 0.92 at 0.73 Hz). In this animal, hypothermia caused a maximum increase in mean blood pressure of 8 mmHg and in heart rate of 10 beats/min. The bursts in SPl SNA and those in BAT SNA exhibited only a weak coherence during the acute hypothermic stimulus (Fig. 1D; Spl → BAT, maximum coherence value: 0.24 at 1.46 Hz, a harmonic of the respiratory frequency). Thus the neural network generating the discharge in BAT SNA under the physiological conditions of hypothermia does not appear to be coupled to that generating SPl SNA.

In nine animals, a reduction in body temperature from 37.5°C to a mean of 34.5 ± 0.1°C resulted in an increase in BAT SNA of 542 ± 157% of control, which was significantly (P < 0.01) greater than the 19 ± 1.8% control increase that occurred in the Spl SNA. On average, acute hypothermia also produced a rise in mean AP of 7 ± 1.6 mmHg (P < 0.01) and in heart rate of 17 ± 4 beats/min (P < 0.01). The mean coherence values are presented in Table 1. During hypothermia, the mean coherence values between the AP and BAT SNA and between the phrenic nerve and BAT SNA were significantly less than those between the AP or the phrenic nerve activity and Spl SNA. The mean maximum coherence value between Spl and BAT sympathetic nerve activities indicated the absence of a correlation during acute hypothermia.

Another stimulus that evokes a sympathetic discharge to BAT is disinhibition of neurons in the RPa, a medullary site mediating BAT thermogenesis (29). Microinjection of bicuculline (60 nl, 500 μM) into the RPa induced a large and continuous bursting discharge on the sympathetic nerves to BAT (Fig. 2A), as well as a small increase in the amplitude of the bursts in Spl SNA and a rise in AP and heart rate. As shown in Fig. 2A, the bursts in BAT SNA generated by disinhibiting neurons in RPa occurred more frequently and more regularly than those induced as a reflex response to hypothermia (compare Fig. 1C). The autospectrum of BAT SNA (Fig. 2B) indicates a mean burst frequency of 3.9 Hz and an increase in amplitude of 2,250% of control (control was similar to Fig. 1, A and B, which are from the same experiment as Fig. 2, A and B). After bicuculline microinjection into RPa, there was a low coherence value between the AP and BAT SNA (Fig. 2B, AP → BAT, coherence value: 0.12 at 6.84 Hz), suggesting the absence of a baroreceptor reflex influence on the central neural network generating or transmitting the bursts in BAT SNA. Similarly, the BAT SNA arising from disinhibition RPa neurons exhibited only a weak coherence with the phrenic nerve activity (Fig. 2B; Phr → BAT, coherence value: 0.24 at 0.73 Hz). In this experiment, augmented discharge of RPa neurons increased Spl SNA by 13% of the control level, without a change in the frequency characteristics of the splanchnic nerve activity. The coherence values between the AP and Spl SNA (Fig. 2B; AP → Spl, 0.99 at 6.84 Hz) and between the phrenic nerve activity and Spl SNA (Fig. 2B; Phr → Spl, 0.96 at 0.73 Hz) remained at the high levels that characterized the splanchnic nerve discharge during control conditions (Fig. 1B). The maximum coherence value between Spl SNA and BAT SNA (Fig. 2B; Spl → BAT, coherence value: 0.31 at 2.20 Hz)
indicates a weak correlation at a harmonic of the phrenic burst frequency.

In several of the rats with intact vagi and in all of the animals with sectioned vagi (n = 5), there was a strong correlation between the bursts in BAT SNA induced by disinhibition of RPa neurons and those on the phrenic nerve (Fig. 2C). This resulted in a prominent component in the BAT SNA autospectrum (Fig. 2D) at the
central respiratory frequency (0.73 Hz) and a high coherence value between phrenic and BAT nerve activities (Fig. 2D; Phr→BAT, coherence value: 0.95 at 0.73 Hz). As expected from the high coherences between the phrenic nerve activity and both BAT and Spl SNAs, splanchnic and BAT nerve activities were strongly coherent at the phrenic frequency (Fig. 2D; Spl→BAT, coherence value: 0.89 at 0.73 Hz). Partialization of the coherence between Spl SNA and BAT SNA with the phrenic nerve activity eliminated the coherence at the phrenic frequency (Fig. 2D; Spl→BAT/Phr, coherence value: 0.01 at 0.73 Hz) and did not reveal a significant relationship between BAT SNA and Spl SNA at any other frequency.

In 13 animals, microinjection of bicuculline into the rostral RPa resulted in a mean maximum increase in BAT SNA of 1,949 ± 604% of control, which was significantly greater (P < 0.001) than the 24 ± 7.2% of control increase that occurred in the Spl SNA. The mean coherence values after disinhibition of RPa neurons are presented in Table 1. During the maximum response, the mean coherence between the AP and BAT SNA was significantly (P < 0.001) less than that between the AP and Spl SNA (Table 1). In contrast, the mean coherence value between the phrenic nerve and BAT SNA was comparable to the value observed between the phrenic nerve activity and Spl SNA (Table 1), particularly if only the vagotomized animals are considered (Phr→BAT, mean coherence value: 0.87 ± 0.03, n = 5). The maximum ordinary coherence values between Spl and BAT sympathetic nerve activities occurred at the phrenic frequency (Table 1). After partialization of the coherence between Spl SNA and BAT SNA with the phrenic nerve activity, there were no frequencies at which the two nerve activities were coherent. These results suggest that the networks generating Spl and BAT sympathetic nerve activities share a common input from the central respiratory generating network (whose effect is enhanced in the absence of a vagal input) but are not otherwise coupled.

To determine whether BAT SNA would be influenced by an increase in the discharge of neurons in the RVLM, including the cardiovascular sympathetic premotoneurons located there (1, 4, 21, 24, 28), changes in BAT SNA were observed after bilateral sympathetic microinjection (60 nl, 500 µM) into the RVLM. Disinhibition of vasoconstrictor sympathetic premotoneurons in the RVLM resulted in a large increase in Spl SNA (Fig. 3B; 53% of control) and a brisk pressor response (Fig. 3B; 38 mmHg). Bicuculline microinjection into the RVLM did not result in any change in the low levels of BAT SNA present under normothermic control conditions (data not shown). To establish a level of control BAT SNA to test for an inhibitory effect of disinhibiting RVLM neurons, bicuculline was microinjected into the RPa before application of bicuculline to the RVLM. As described above, this resulted in a sustained bursting discharge on the sympathetic nerve to BAT (Figs. 2, A and C, and 3A). Subsequent microinjection of bicuculline into the RVLM produced a prompt inhibition of the BAT SNA amplitude (Fig. 3B; BAT amplitude: 5% of elevated control in Fig. 3A) to the level seen under normothermic conditions (compare with Fig. 1A).

In 10 rats, bilateral bicuculline microinjection into the RVLM increased Spl SNA by 86 ± 14.4% of prebicuculline control levels and increased mean AP by 32 ± 4.5 mmHg. In eight of the rats, heart rate fell by 35 ± 6.8 beats/min but was increased by 29 and 14 beats/min in the other two rats that were vagotomized. This suggests that the reduced heart rate in the majority of the rats (whose vagi were intact) may have arisen from a bicuculline-induced increase in the vagal preganglionic neurons in the region of the nucleus ambiguus immediately dorsal to the RVLM. Bicuculline microinjection into the RVLM reduced BAT SNA to 13 ± 2.9% of the enhanced control level immediately before the microinjections.

To determine whether the BAT SNA generated by disinhibition of RPa neurons is dependent on neurons in RVLM, bicuculline was microinjected into RPa after local lesions in the RVLM were inhibited with bilateral microinjections of the GABA<sub>A</sub> agonist muscimol (60 nl, 1 mM). As expected from the inhibition of sympathetic premotoneurons in the RVLM, Fig. 4C illustrates that within 5 min after microinjecting muscimol into the RVLM, the bursting discharge on the Spl SNA was nearly abolished and the amplitude had fallen to a minimum of 17% of the premicroinjection control level (Fig. 4A). This was accompanied by a fall in arterial pressure of 46 mmHg and in heart rate of 118 beats/min. The amplitude of BAT SNA was unchanged. Phrenic nerve activity was eliminated, presumably through the effect of muscimol on elements of the respiratory generating network in the vicinity of the
RVLM. In five rats, muscimol reduced Spl SNA to $18 \pm 3.5\%$ of control and decreased mean AP by $59 \pm 5.7$ mmHg to a basal level of $68 \pm 4.3$ mmHg. BAT SNA was not different from the premicroinjection control levels. The mean coherence values are presented in Table 1. After muscimol microinjection into RVLM, a reduced coherence remained between the AP and the Spl SNA (Fig. 4D), although there were no coherent frequencies
between the AP and BAT SNA or between the Spl SNA and the BAT SNA (Fig. 4D).

Subsequent microinjection of bicuculline into the RPa during the maximum effects of muscimol microinjections in the RVLM elicited a large increase in BAT SNA (Fig. 4E) with similar magnitude and frequency characteristics to the BAT SNA responses to disinhibition of RPa neurons under initial control conditions (e.g., Figs. 1A and 2A). In the experimental data illustrated in Fig. 4E, bicuculline microinjection into RPa induced bursts in BAT SNA with a mean burst frequency of 2.8 Hz (Fig. 4F), which increased BAT SNA amplitude by 2,309% of the control amplitude (Fig. 4C). Application of bicuculline into the RPa also increased Spl SNA (156% of the reduced “control” level after muscimol microinjection into RVLM) but to a much smaller extent than BAT SNA. Of particular interest, the frequency components in the Spl SNA induced by disinhibition of RPa neurons after inhibition of RVLM neuronal activity were very similar to those in the BAT SNA response. This was indicated by the common pattern of burst occurrence in the BAT SNA and Spl SNA seen in the oscillographic records in Fig. 4E and by the similarity in the relative power profile of the component frequencies in their autospectra (Fig. 4F). These observations were confirmed by the significant coherence between Spl SNA and BAT SNA (Fig. 4F, Spl—BAT, coherence values: 0.39–0.68 at 1.46–3.17 Hz) in the frequency range of the burst occurrence in BAT SNA. These results indicate not only that the generation of the augmented sympathetic discharge to BAT after disinhibition of RPa neurons is not dependent on the activity of neurons in the RVLM but also that the splanchnic sympathetic nerve contains a small population of fibers whose activity is controlled by the activity of RPa neurons and is synchronized to the discharge of fibers in the sympathetic nerves to BAT.

Electrical stimulation (twin pulses, 6-ms interval, 1-ms duration, 0.25 Hz, 50 µA) in RPa evoked excitatory potentials in both the BAT SNA and the Spl SNA (Fig. 5). The mean onset and peak latencies of the excitatory potential evoked in BAT SNA (Fig. 5, peak 3) were 136 ± 3 and 169 ± 3 ms (n = 13), respectively. In nearly all cases, two potentials were evoked in Spl SNA by RPa stimulation: an early potential (Fig. 5, peak 1) with mean onset and peak latencies of 53 ± 3 and 80 ± 3 ms (n = 10), respectively, and a late potential (Fig. 5, peak 3), usually rising from the postexcitatory depression after the early potential, with a mean peak latency of 167 ± 4 ms (n = 13). In three animals, RPa stimulation did not evoke an early potential in the Spl SNA.

In Fig. 6, top, the positions of the approximate centers of the bicuculline microinjections into the RPa in the 13 animals described in this study are plotted on a single medullary cross-section (32) corresponding to the median rostral-caudal location of the injection sites. These sites were clustered in the rostral RPa and the overlying raphe magnus at the level of the caudal half of the facial motor nucleus. The positions of the bicuculline microinjections (n = 10) and the muscimol microinjections (n = 5) in the RVLM are plotted in Fig. 6,
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which elicited an increase in Spl SNA and in AP, their identification in RPa remains to be determined whether the SNA on the two nerves arises from a common oscillator impinging on target tissues involved in metabolism and thermoregulation while the activity of neurons in the RVLM regulates the sympathetic outflow specifically to cardiovascular targets such as the blood vessels and heart. First, within the network oscillators generating BAT and Spl SNAs, evidence for distinct populations of sympathetic premotoneurons is provided by two observations. At normal body temperature, the BAT SNA is almost completely inhibited, whereas that on the splanchnic nerve exhibits a robust tonic discharge. Because basal sympathetic tone in both nerves is controlled primarily from supraspinal sites, this finding is consistent with the existence of distinct populations of bulbospinal, premotoneurons providing the excitatory drive to the sympathetic preganglionic neurons for BAT, and to those for the splanchnic nerve. Additionally, because baroreceptor modulation of SNA arises primarily through inhibition of sympathetic premotoneurons, the marked difference in the baroreceptor modulation of BAT and Spl SNAs noted by comparing their coherences with the AP (Figs. 1D and 2B) supports separate premotor neuronal populations for these sympathetic outflows.

Second, the ability of bicuculline microinjections in the RPa to increase the SNA to BAT after neuronal activity in the RVLM was inhibited with muscimol microinjections indicates that RVLM neurons, including the well-characterized sympathetic premotoneurons located there, are not required for the BAT sympathetic excitation mediated by increases in neuronal activity in the RPa. Although the existence of RPa neurons that project to the spinal intermediolateral nucleus (22, 36) and that have axonal conduction velocities appropriate to mediate the RPa stimulus-evoked responses in BAT SNA (27, 29) suggests that the BAT sympathetic excitation induced by local application of bicuculline arises from disinhibition of sympathetic premotoneurons controlling BAT SNA, their identification in RPa remains to be demonstrated.

Third, microinjection of bicuculline into the RVLM, which elicited an increase in Spl SNA and in AP, reduced the elevated BAT SNA produced by disinhibition of RPa neurons. This finding, suggesting an inhibitory influence of neurons in RVLM on the network generating BAT SNA, argues against a role for increased activity of RVLM neurons in the BAT sympathoexcitation occurring either during hypothermia or in response to disinhibition of RPa neurons. Furthermore, because muscimol application to RVLM did not produce an increase in BAT SNA (Fig. 4C), it does not appear that RVLM neurons are the source of the GABAergic input to RPa that maintains BAT SNA at the low level observed during normothermia. Anatomic evidence for a projection from RVLM to medullary raphe nuclei has been provided (31). The reduction in BAT SNA during excitation of vasomotor sympathetic premotoneurons in the RVLM may reflect a brain stem mechanism for reducing energy expenditure and body temperature during hypotension (e.g., hemorrhage), a situation in which there would be a baroreceptor reflex-mediated increase in the discharge of RVLM sympathoexcitatory neurons. A similar mechanism for conserving metabolic fuel in such situations has been proposed (14) for hypothalamic thermoregulatory neurons: warm- and cold-sensitive cells in preoptic/anterior hypothalamus were excited and inhibited, respectively, during the reduced baroreceptor input present during hypotension or blood loss (20), leading to activation of mechanisms to reduce body temperature and metabolism under these conditions.

Fourth, during periods of increased BAT SNA induced physiologically with hypothermia or pharmacologically by disinhibition of RPa neurons, there was no coherence between BAT and Spl SNAs (except for a shared central respiratory modulation). The absence of a linear correlation between these two signals suggests that they are generated by separate brain stem neural circuits (3, 19), often modeled as oscillators because of the regular bursting discharge that characterizes SNA. The extent to which the functional subsets of neurons comprising the oscillators generating BAT and Spl SNAs are anatomically separate is unknown; the present data indicate only that, at a minimum, the populations of output (i.e., bulbospinal) neurons of the oscillator are unique. These results are compatible with either of the current hypotheses for generation of basal SNA. That is, differences in the properties between the bulbospinal neurons controlling BAT and Spl SNAs could result in different and uncorrelated burst frequencies on the two nerves whether SNA is generated through network interactions among neurons (8, 21) or through pacemaker potentials in sympathetic premotoneurons (12). In regard to the former mechanism, it remains to be determined whether the SNA on the two nerves arises from a common oscillator impinging on distinct populations of bulbospinal premotoneurons or from neural networks that are entirely separate.

Although coherence analysis did indicate correlations between BAT and Spl SNAs under three conditions, these results do not conflict with the conclusion just described. The first instance was the coherence at the heart rate between the basal SNA to BAT at normal
body temperature and the Spl SNA (Spl—BAT; Fig. 1B). In the absence of a stimulus for thermogenesis at normal body temperature, the component of BAT SNA to the adipocytes is expected to be inhibited, as indicated by the very low amplitude of the sympathetic discharge to BAT (Figs. 1A and 4A). However, the sympathetic nerves to BAT also contain the axons of ganglion cells innervating the BAT blood vessels (5), which would be expected to exhibit characteristics similar to other vasomotor nerves: a tonic discharge maintained by the activity of neurons in the RVLM and modulated by the baroreceptor reflex. On this basis, it would not be surprising to find a correlation between the vasomotor component of BAT SNA and the Spl SNA. It should be emphasized that this was a minor component of the sympathetic outflow to BAT and that the large-amplitude, thermogenic response in the SNA to BAT during acute hypothermia or disinhibition of RPa neurons had no cardiac-related component (Figs. 1D and 2B) and was not inhibited during baroreceptor reflex activation (29).

The second instance was the coherence, albeit weak in most instances, between BAT and Spl SNAs at the respiratory frequency, a relationship that was enhanced in vagotomized animals (Fig. 2D). The absence of a respiratory-related peak when the coherence between Spl and BAT SNAs was partialized with respect to the phrenic nerve activity suggests that the peak is due to an input from the central respiratory generator that is shared by the networks generating BAT and Spl SNAs (see Refs. 10 and 17 for a similar analysis related to baroreceptor influences). These data imply that vagotomy removes an afferent input that reduces the influence of the central respiratory drive to the oscillator generating BAT SNA.

The third instance was the coherence between BAT and Spl SNAs after disinhibition of RPa neurons after RVLM neurons were inhibited with muscimol microinjection (Spl—BAT; Fig. 4F). The strong correlation between the large, thermogenic bursts in BAT SNA and the smaller bursts that were evoked in the Spl SNA during bicuculline microinjection into RPa despite prior elimination of neuronal activity in the RVLM (Fig. 4E) suggests that there is a population of axons within the splanchnic nerve whose activity is determined by the level of excitation of RPa neurons. The ability of RPa stimulation to evoke an excitatory potential in the Spl SNA with a long latency similar to that evoked in the BAT SNA is also consistent with this interpretation. Two possibilities may explain these results. First, in addition to the principal, interscapular depot of BAT, there are significant retroperitoneal deposits of BAT as well, particularly surrounding the adrenals and kidneys (30, 35). While the course of the sympathetic axons to these latter deposits of BAT is not known, it is likely that they receive their sympathetic regulation via axons in the splanchnic nerve. Thus the small component of Spl SNA correlated to the SNA to the interscapular BAT and similarly driven by an increase in the activity of RPa neurons may represent the synchronous discharge of axons within the splanchnic nerve that innervate adipocytes within such local brown fat deposits. In light of these results, the finding that leptin caused an increase in the discharge of several sympathetic nerves in addition to that to interscapular BAT (13) may, in part, have arisen from the activation of sympathetic fibers within those nerves that innervate widely distributed BAT deposits. The second possibility is that the present results may be indicative of an RPa stimulus-evoked increase in splanchnic sympathetic nerve fibers to endocrine tissue such as the pancreas, which would also be expected to participate in metabolic or thermoregulatory control.

**Perspectives**

Figure 7 summarizes a model derived from these results, in which RPa neurons, depicted as raphe-prefrontal neurons projecting to the intermediolateral nucleus, excite sympathetic preganglionic neurons that control metabolic heat production in deposits of brown adipose tissue throughout the body. RPa neurons may also regulate the sympathetic outflow to non-BAT tissues involved in thermoregulation and metabolism. The axons of sympathetic ganglion cells that innervate brown adipocytes comprise a much larger fraction of the fibers in the sympathetic nerve to interscapular BAT than in the splanchnic nerve. Conversely, sympathetic ganglion cells with a putative vasoconstrictor function and exhibiting a cardiac-related discharge mediated by neurons in the RVLM, constitute a much larger fraction of the axons in the splanchnic nerve than in the sympathetic nerve to interscapular BAT. The absence of a strong linear correlation between BAT (thermoregulatory/metabolic) and Spl (vasoconstrictor) SNAs during periods of increased excitatory drive to BAT suggests that the networks generating these sympathetic outflows are independent and, at least in terms of hypothermic and baroreceptor stimulation,
capable of independent, well-differentiated responses to reflex inputs.

The results of recordings from multiple sympathetic nerves in the cat suggest a coupling among the neural oscillators generating the activity of sympathetic nerves with similar, i.e., cardiovascular, functions, but with distinct targets (9, 18). In conjunction with the results of the present study, these findings suggest an organizational concept in which oscillators (or at least their spinally projecting output neurons) generating the activity on sympathetic nerves with similar functions (e.g., cardiovascular: cardiac and vasoconstrictor) are coupled, perhaps by virtue of their anatomic proximity (e.g., within the RVLM), but are not coupled to oscillators generating activity on sympathetic nerves controlling noncardiovascular functions (e.g., cellular and homeostatic metabolic events) that are located in anatomically remote sites (e.g., rostral RPa). While control by RPa neurons of non-BAT tissues with thermoregulatory and metabolic functions and the potential for coupling of oscillators within RPa remain to be investigated, it is of interest that the sympathetic outflow to the tail artery, regulating blood flow and thus heat loss in this organ, exhibits several characteristics similar to those in BAT SNA. Tail artery SNA is not under baroreceptive regulation, but is modulated by central respiratory circuits (16), and anatomic (34) and functional (33) evidence has suggested that neurons in the rostral ventromedial medulla, including the RPa, are important in controlling this thermoregulatory sympathetic outflow as well.

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