Sympathetic α-adrenergic regulation of blood flow and volume in hamsters arousing from hibernation

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Osborne, P. G., J. Sato, N. Shuke, and M. Hashimoto. Sympathetic α-adrenergic regulation of blood flow and volume in hamsters arousing from hibernation. Am J Physiol Regul Integr Comp Physiol 289: R554–R562, 2005. First published April 21, 2005; doi:10.1152/ajpregu.00004.2005.—Mammals arousing from hibernation display pronounced regional heterothermy, where the thoracic and head regions warm faster than the abdominal and hindlimb regions. We used laser-Doppler flowmetry to measure peripheral hind foot blood flow during hibernation and arousal and gamma imaging of technetium-labeled albumin to measure whole blood volume distribution in hamsters arousing from hibernation. It was discovered that the hibernating hamster responds to physical but not to sound or hypercapnic stimulation with rapid, 73% reduction of hind foot blood flow. Hind foot blood flow vasoconstriction was maintained from the onset of arousal until late in arousal when rectal temperature was rapidly increased. α-Adrenergic blockade early in arousal increased hind foot blood flow by 700%, suggesting that vasoconstriction was mediated by activation of sympathetic tone. Gamma imaging revealed that, by the early phase of arousal from hibernation, the blood volume of the body below the liver is greatly reduced, whereas blood volumes of the thorax and head are much greater than corresponding volumes in anesthetized hamsters. As arousal progresses and cardiac activity increases and regional heterothermy develops, this regional blood volume distribution is largely maintained; however, blood volume slowly decreases in the thoracic region and slowly increases in the shoulder and head regions. The rapid increase in rectal temperature, characteristic of mid- to late-arousal phases, is probably mediated, in part, by reduction of adrenergic tone on abdominal and hindlimb vasculature. Warm blood then moves into the hind body, produces an increase in temperature, blood flow, and blood volume in the hind body and compensatory reductions of blood volume in the neck, head, and thoracic regions.

laser-Doppler flowmetry; gamma imaging; torpor; vasoconstriction

The transition from the low body temperatures of classical hibernation to cenothermia is associated with probably the most remarkable changes in mammalian circulatory physiology, and yet this has been the subject of few in vivo investigations and remains poorly understood. For small mammals in natural hibernation, body temperature, metabolism (20, 24), heart rate (HR), blood pressure (1, 16), and cerebral blood flow (6, 20) are greatly reduced relative to cenothermia. However, despite these enormous reductions in physiological parameters, in vivo studies demonstrate that blood pressure and HR during hibernation remain sensitive to pharmacological activation of α- and β-adrenergic receptors, respectively (16). Evidence for parasympathetic influences on HR and respiration during hibernation is more equivocal and may only be evident in some species that exhibit episodic breathing (10). Respiratory chemosensitivity to hypercapnic gas is retained at low body temperatures during hibernation (14, 18). Collectively, these results are consistent with the functional conservation of some aspects of cenothermic autonomic regulation during hibernation in hamsters, sciurids, and possibly all other small mammalian hibernators.

In hamsters and sciurids, the most intensively studied hibernators, arousal from hibernation to cenothermia appears to be a phenomenon comprising three distinct physiological phases that manifest as heterogeneous warming of the body. The initial warming phase is associated with nonshivering thermogenesis. In the second phase, shivering thermogenesis is recruited after muscles above the diaphragm have attained a temperature of ~20°C, at which point shivering becomes an efficient means of heat production (11). In the final phase, the temperatures of the posterior organs and hindlimbs rapidly increase to cenothermia, metabolic rate decreases (1, 20), the animal presents an EEG profile that can be interpreted as slow-wave sleep (12, 26), and the proteins and mRNA consumed during the preceding arousal phases and hibernation are replenished by reinitiation of gene transcription and translation (21).

In hamsters, the large thermal gradients between anterior and posterior body regions in the arousing animal (1, 20) and their attenuation by nonspecific pharmacological inhibition of sympathetic nervous activity (15) provided in vivo evidence for active vascular control during arousal. Numerous studies on isolated vessels from a variety of small mammalian hibernators have been performed, and the consensus appears to be that aortic, carotid, renal, and femoral vessels retain functional or enhanced sensitivity to adrenergic or purinergic vasoconstriction at low temperatures (4, 9, 19, 22). In contrast, vasodilatory responses at low temperatures have been the subject of few studies. In the hamster, cholinergic endothelium-dependent vasodilation of the carotid artery appears to be depressed in a temperature- or hibernation-dependent manner; at low temperature, sensitivity to nitric oxide donors is retained (23). In marmot heart tissue, sensitivity to endogenous nitric oxide appears to be induced at low temperature (13). These investigative studies demonstrate vessel- and transmitter-specific alterations in function at cold temperatures. However, these pharmacological characterizations can be more accurately assigned a role of physiological relevance once a more holistic understanding of the dynamic nature of circulatory physiology of hibernation and arousal from hibernation has been performed. In this study, we utilized laser-Doppler flowmetry and gamma

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imaging of technetium-labeled albumin for real-time, in vivo measurements of hind foot blood flow (HFBF), whole body regional blood volume, and the effects of pharmacological manipulation on these parameters during arousal from hibernation in hamsters.

MATERIALS AND METHODS

Animals and Housing

The following experiments conformed to the ethical guidelines of the Japanese Physiological Society and Asahikawa Medical University (ethics approval no. 02166). Male hamsters were housed at 22°C with 12:12-h light-dark cycle and ad libitum food and water until 3 mo of age or until body weight was >120 g. Hamsters were then transferred to a 4°C room in constant darkness and housed individually in cages with wood chip nest building material and ad libitum access to rodent chow and water. After 2 mo, hamsters began to hibernate. Thermocouples were used to monitor nest temperature, and infrared motion sensors were used to monitor activity. A computer program calculated the timing and duration of each hibernation bout. Regularly hibernating animals were chosen for experiments.

Animal Surgery

Before experiments, hamsters were anesthetized with pentobarbital sodium (45 mg/kg ip; Dainichi Pharmaceutical), and the skin overlaying the lattissimus dorsi muscle was pierced bilaterally for the permanent placement of silver rings, which were used for electrocardiogram (ECG) recordings. Infusions were via either a polyethylene cannula (PE-10, Becton Dickinson) with a 29-gauge needle tip acutely inserted subcutaneously above the scapular muscles or a chronic femoral vein catheter. Hamsters were cannulated when cenothermic with sterile surgical procedures, under pentobarbital anesthesia supplemented with topical application of xylocaine to the area of the incision. The tip of the silicon tube on the PE-10 femoral vein catheter was positioned in the inferior vena cava. The PE-10 catheter was exteriorized between the shoulder blades and filled with saline containing 5 IU heparin (Novo Nordisk). After surgery, the areas of incisions were irrigated with xylocaine (AstraZeneca) and dilute penicillin G-karium solution (Meiji Pharmaceutical); hamsters were then given a 10 ml/kg ip injection of sterile saline. Hamsters usually reentered hibernation within 7 days after surgery. Thirty to forty hours after the hamster had reentered hibernation, the catheter was flushed with sterile saline-heparin solution (5 IU) and the hamster was aroused from hibernation. These hamsters usually reentered hibernation within 2 days. Hamsters that did not reenter hibernation within 1 wk of surgery or after flushing were allocated to the cold-adapted, hibernation season cenothermic control group for use in urethane-anesthetized gamma imaging blood volume experiments.

HFBF Experiments

HFBF experiments were performed 2 days after the start of hibernation because HR and ECG QRS complex amplitude are relatively constant by the second day of hibernation. A laser probe (0.5 mm diameter) was positioned to make contact with the back of the hamster. Changes in laser light reflected from animal’s back corresponded to respiration and muscle movements. ECG was measured from the implanted silver rings by an oscilloscope. A HR of <10 beats/min confirmed that the connection procedures did not initiate arousal from hibernation. The hibernating hamster’s nest was carefully excavated, and a laser-Doppler flow probe (1 × 2-mm sensing aperture) was glued to the bottom of the hibernating hamster’s rear foot, contralateral to the cannulated leg.

The responses of HFBF, respiratory rate (RR), and ECG to physical stimulation (touch with forceps), 100-decibel loud noise for 30 s (saxophone), and 2 min of breathing of hypercapnic, normoxic gas (20% O₂-10% CO₂-70% N₂; Hokkaido Air and Water) were measured in hibernating hamsters on the third day of hibernation. The effects of a 100-decibel noise for 30 s (saxophone) and 2 min of breathing of hypercapnic, normoxic gas on HFBF, RR, and ECG were also tested in hamsters previously induced to arouse by tactile stimulation when the HR of the hamster was >35–40 beats/min.

In hamsters induced to arouse by tactile stimulation, the effects of 5-min intravenous infusion (0.8 mg/kg) or interscapular subcutaneous infusion (4–5 mg/kg) of α-antagonists (Sigma) or 10-min intravenous infusion of sodium nitroprusside (5–12 µg/kg·l/min; Sigma) or saline control infusions on HFBF, RR, and ECG were measured when HR was >40 beats/min. All drugs were dissolved in sterile physiological saline that had been cooled to the approximate body temperature of the hamster before infusion at 10 µl/min. These doses were chosen on the basis of vasodilatory effects in conscious cenothermic hamsters, rats, and dogs (2, 3, 8). We confirmed, in preliminary experiments, that in urethane-anesthetized hamsters the above doses of α-antagonists blocked hypertension induced by intravenous α-agonists (6 µg/kg phenylephrine, Sigma) for at least 60 min.

HR was recorded on a biophysical amplifier (AYD-10, Nihon Kohden). HFBF was measured with a laser-Doppler flow probe (ALF probe, Advance) connected to a laser-Doppler flowmeter (ALF 21N, Advance). Output from the laser-Doppler flowmeters (volts) and the biophysical amplifier were connected to computer interfaces (MacLab 2e, ADInstruments). Thermocouples were connected to a zero-temperature reference refrigeration unit (Zero-con, Konatsu Electronics) and a thermocouple interface (ICM-7, I-Techno). All data were stored electronically. Saxophone intensity was measured using an integrated sound-level meter (Rion).

Gamma Imaging Experiments

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Gamma Imaging Experiments

Technetium-labeled human serum albumin (99mTc-HSA) was freshly prepared immediately before intravenous injection, per manufacturer’s instructions (Technetium kit, Technet). The specific activity of 99mTc-HSA was >95%. The administered dose was ~5.9 MBq (160 μCi) based on a volume of 150 μl/100 g body wt. Arousing hamsters were fitted with precalibrated rectal, brown adipose tissue (BAT), and cheek pouch thermocouples before they were infused with 99mTc-HSA. In anesthetized hamsters, only rectal temperature was measured. After thermocouples were attached, hamsters were taped, ventral surface downward, with legs spread apart, onto an insulated board. The board had one metal insect pin at each corner. A drop of 99mTc-HSA, sufficient to produce a signal at the gamma-imaging detector, was placed on the top of the pinhead. The board was placed inside an insulated cold box that was positioned over the gamma-imaging detector (Millennium VG, General Electric). The cold box had a transparent roof that allowed RR to be counted and had holes for the circulation of air. The time from removal of the animal from cold home cage, insertion of thermocouples, and connection of cannula to the positioning of the insulated box above the detector was 3–5 min.

Blood volume distribution during anesthesia. Five cold-adapted cenothermic hamsters, each with a chronic femoral vein catheter, were anesthetized with urethane (1.2 ml of 10% urethane ip; Dainichi Pharmaceutical) and positioned in the insulated box maintained at 15°C, so as to provide peripheral cold stimulation. The box was positioned on the gamma imaging stage, and the hamsters were intravenously infused with 150 μl of 99mTc-HSA at 40 μl/min. Rectal temperature and RR were measured simultaneously.

Blood volume distribution, HFBF, and body temperatures during arousal from hibernation. Seven hibernating hamsters with a chronic femoral vein catheter were positioned on an insulated board as outlined above and transferred to an insulated box maintained at 4–8°C positioned above the gamma imaging stage. Positioning the hamster initiated arousal from hibernation. Arousing hamsters were intravenously infused with 150 μl of 99mTc-HSA at 40 μl/min. Under these experimental conditions, the large increase in rectal temperature
associated with the final stages of arousal did not occur until after BAT temperature had reached 30–35°C. Five of the seven arousing hamsters were also used to determine the effect of adrenergic antagonism on blood-volume distribution during arousal. Infusions of the α-adrenergic antagonist phenolamine (0.8 mg/kg in 50 µl; n = 4 hamster) or the α1-adrenergic antagonist prazosin (0.3 mg/kg in 50 µl; n = 1 hamster) began when BAT temperature of the arousing hamsters reached 18–20°C. The effect of the α1-adrenergic antagonist was not different from the effect of the α-antagonist phenolamine. Therefore, results of the α1-adrenergic antagonist experiment were included in the analysis of the α-adrenergic antagonist to increase the sample size. HFBF was also measured in gamma experiments that infused phenolamine.

Dynamic gamma images were acquired every 1 min for up to 90 min, using a low-energy general-purpose collimator, a large field of view gamma camera, and a dedicated data processing unit. Arousing hamsters were monitored until just before the time the hamsters were capable of moving, when mouth temperature was 22°C and rectal temperature was 18°C. At this time, hamsters were given an intravenous infusion of a light anesthetic dose of Nembutal (30 mg/kg), and changes in rectal, BAT, and cheek pouch temperatures and blood volume were monitored for an additional 10 min in the anesthetized hamster. The hamster was killed with an overdose of Nembutal. After the heart had stopped beating, while still taped on the insulated board, the dead hamster was intravenously infused with 0.5 ml of radiopaque solution (Optiray 320, Tyco Healthcare) and then X-rayed.

Analysis of Gamma Measurements

The digitized X-ray and digitized gamma images were size matched by overlaying the metal pins in the X-ray image with the gamma signal from the 99mTc-HSA-coated metal pins. The anatomical features of the digitized X-ray were used to accurately determine the size and position of the regions of interest (ROIs) on the gamma image. ROI were whole body; hind body region below kidneys; kidney region; chest cavity region including lungs, heart, and liver; bladder region; and feet regions starting at the elbow or knee joint. ROIs of regions above liver and regions containing head and shoulders were calculated from the above measurements (see Fig. 4B).

ROI time-activity curves were normalized to control for radioactive decay of 99mTc. Whole body time-activity curves were corrected for accumulation of 99mTc in the bladder by subtracting the bladder time-activity curve. Gamma activities in each ROI in anesthetized and arousing hamsters were presented as the average of five 1-min frames, converted to a percentage of total body activity, commencing 5 min after the end of the 99mTc-HSA infusion. Later during arousal, five of the seven arousing hamsters received an infusion of α-adrenergic blocker. In these hamsters, blood volumes in each ROI were calculated from the average of five frames at times corresponding to 20 min before, at the start, 20 min after phenolamine infusion, and after 10-min anesthesia with Nembutal (30 mg/kg iv). BAT, rectal temperature, and RR for each of these periods are presented in Table 1.

Statistics

Comparisons of HFBF between hibernating and arousing hamsters were by paired Student’s t-test. Analyses of effects of infusions of saline and phenolamine during arousal on HR, RR, and HFBF were by two-way ANOVA, with repeated measures, with Tukey-Kramer correction for multiple comparisons. Comparisons of regional blood volume between anesthetized and arousing hamsters was by unpaired Student’s t-test. Analyses of changes in QRS amplitude during arousal, the effect of adrenergic blockade on blood volume within a ROI between 5-min time periods in arousing hamsters, and changes in rates of temperature were by repeated-measures ANOVA with Tukey-Kramer correction for multiple comparisons. P < 0.05 was considered significant. Values are means ± SE.

Table 1. Regional blood volume in anesthetized and arousing hamster and the effect of adrenergic antagonism and anesthesia on regional blood volume during arousal from hibernation

<table>
<thead>
<tr>
<th>Regional Blood Volume, % of total, 10 min after infusion of 99mTc-HSA</th>
<th>Effect of Intravenous α-Adrenergic Blockade During Arousal From Hibernation on Regional Blood Volume, V</th>
<th>Pre: t = +20 min</th>
<th>Post (anesthesia): t = +30 min</th>
<th>Arousal</th>
<th>Anesthetized</th>
<th>Cenothermic</th>
</tr>
</thead>
<tbody>
<tr>
<td>BAT, °C</td>
<td>11.4 ± 1.2</td>
<td>12.5 ± 0.3</td>
<td>25.1 ± 0.9</td>
<td>26.4 ± 0.6</td>
<td>12.5 ± 0.3</td>
<td>18.7 ± 0.5</td>
</tr>
<tr>
<td>Rectal, °C</td>
<td>34 ± 1</td>
<td>34.0 ± 0.3</td>
<td>34.0 ± 0.3</td>
<td>34.0 ± 0.3</td>
<td>34.0 ± 0.3</td>
<td>34.0 ± 0.3</td>
</tr>
<tr>
<td>RR (breaths/min)</td>
<td>60 ± 3</td>
<td>37 ± 6</td>
<td>51 ± 4</td>
<td>78 ± 5</td>
<td>94 ± 8</td>
<td>37 ± 11</td>
</tr>
<tr>
<td>Number</td>
<td>5</td>
<td>7</td>
<td>5</td>
<td>5</td>
<td>5</td>
<td>5</td>
</tr>
<tr>
<td>Above liver</td>
<td>60.5 ± 1.4</td>
<td>81.4 ± 1.1</td>
<td>80.2 ± 0.9</td>
<td>79.3 ± 0.7</td>
<td>75.3 ± 0.9</td>
<td>74.5 ± 0.9</td>
</tr>
<tr>
<td>Below liver</td>
<td>40.8 ± 1</td>
<td>19.1 ± 0.9</td>
<td>19.8 ± 0.9</td>
<td>20.7 ± 0.9</td>
<td>24.1 ± 0.9</td>
<td>25.2 ± 1.0</td>
</tr>
<tr>
<td>Heart, liver, and lung</td>
<td>42.6 ± 2.3</td>
<td>53.9 ± 1.5</td>
<td>52.6 ± 2.3</td>
<td>49.8 ± 2.0</td>
<td>49.0 ± 2.3</td>
<td>47.8 ± 2.2</td>
</tr>
<tr>
<td>Head &amp; neck</td>
<td>***</td>
<td>26.3 ± 1.0</td>
<td>26.4 ± 1.7</td>
<td>24.9 ± 1.6</td>
<td>25.2 ± 2.2</td>
<td>25.4 ± 2.5</td>
</tr>
<tr>
<td>Front feet</td>
<td>1.7 ± 0.3</td>
<td>1.39 ± 0.1</td>
<td>1.65 ± 0.15</td>
<td>1.85 ± 0.18</td>
<td>1.99 ± 0.18</td>
<td>1.99 ± 0.2</td>
</tr>
<tr>
<td>Hindlimb</td>
<td>17.8 ± 0.6</td>
<td>8.0 ± 0.7</td>
<td>9.1 ± 0.6</td>
<td>9.4 ± 0.6</td>
<td>10.9 ± 0.5</td>
<td>11.5 ± 0.5</td>
</tr>
<tr>
<td>Kidney</td>
<td>23.1 ± 0.9</td>
<td>10.4 ± 0.5</td>
<td>10.1 ± 0.1</td>
<td>11.5 ± 0.1</td>
<td>13.3 ± 0.5</td>
<td>13.9 ± 0.7</td>
</tr>
<tr>
<td>Hind feet</td>
<td>2.2 ± 0.1</td>
<td>1.31 ± 0.22</td>
<td>1.57 ± 0.28</td>
<td>1.68 ± 0.22</td>
<td>2.01 ± 0.24</td>
<td>2.17 ± 0.23</td>
</tr>
<tr>
<td>Bladder</td>
<td>10.1 ± 1.3</td>
<td>3.0 ± 0.5</td>
<td>3.8 ± 0.6</td>
<td>3.9 ± 0.5</td>
<td>4.5 ± 0.6</td>
<td>4.8 ± 0.5</td>
</tr>
</tbody>
</table>

Values are means ± SE. α-Adrenergic blockade was with 0.8 mg/kg iv phenolamine, and anesthesia was with 30 mg/kg iv Nembutal. BAT, brown adipose tissue; HSA, human serum albumin; RR, respiratory rate. Significantly different from arousal initial: *P < 0.05; **P < 0.1; ***P < 0.001. Increase significantly different from t = 0 min: ↑P < 0.05; ↑↑P < 0.01; ↑↑↑P < 0.001. Decrease significantly different from t = 0 min: ↓P < 0.05; ↓↓P < 0.01; ↓↓↓P < 0.001. NS, not significant.
RESULTS

**HFBB Experiments**

In all recordings (30 measurements from 11 hibernating hamsters; mean of 2.8 measurements per animal), arousal induced by tactile stimulation to the hamster’s back on the third day after hibernation was associated with a rapid decrease in HFBF and a slow increase in HR and RR (Fig. 1A). Figure 1B shows that, during hibernation before tactile stimulation, HFBF was 0.327 ± 0.028 V (n = 30) and decreased to a minimum of 0.090 ± 0.007 V during arousal (t = 9.24, df = 29, P < 0.001). HR during hibernation before tactile stimulation was 8.5 ± 0.2 beats/min (n = 30). Cardiac parameters during the initial reduction of HFBF were typically an increase in HR (2–3 beats/min) and no change in ECG QRS complex amplitude. ECG QRS complex amplitude was compared between hibernating hamsters and tactile stimulation-induced aroused hamsters when HR was 10, 15, and 20 beats/min. ECG QRS complex amplitude was 2.9 ± 0.08 V for hibernating animals and had not increased when HR was 10 beats/min but had increased to 4.1 ± 0.13 V when HR was 15 beats/min (P < 0.001) and to 4.8 ± 0.2 V when HR was 20 beats/min [F(3,29) = 75.5, P < 0.001]. In 28 of 30 recordings, touching the animal initiated a prompt decrease in HFBF followed by arousal. Very rarely, animals were less responsive to tactile stimulation and could be touched repeatedly before arousal (Fig. 1A).

Figure 2 shows a typical response to 2-min inhalation of hypercapnic, normoxic gas to a hibernating hamster. In four hibernating hamsters, a 2-min inhalation of hypercapnic, normoxic gas produced an immediate, robust increase in RR so that episodic breathing became continuous. HFBF gradually and transiently increased from 0.345 ± 0.047 V by a maximum of 16% within 10–15 min after exposure to hypercapnic, normoxic gas (t = 3.15, df = 7, P < 0.05). HR nonsignificantly increased from 8.6 ± 0.8 to a maximum of 11.1 ± 1.3 beats/min during the period when HFBF was increased. Breathing returned to an episodic pattern within 5–10 min of replacement of hypercapnic gas with air. HFBF also returned to basal levels but more slowly than RR or HR. All hamsters continued to hibernate. Four hibernating hamsters were exposed to 30 s of 100-dB sound (saxophone), but no changes in RR, HR, or HFBF were recorded and no animal aroused (data not presented).

Once arousal had been initiated and HFBF was reduced, HFBF was not further reduced with additional tactile stimulation (data not presented). The reduced HFBF slowly increased as cardiac activity increased during arousal. Late in arousal, HFBF increased markedly when metabolic rate, as estimated from RR (18), approached maximum (Fig. 3A). Complete recordings from hibernation until the hamster had attained cenothermia could not be recorded because of signal artifacts induced by animal movement in the last phase of arousal, when rectal temperature rapidly rises to cenothermia and the hamster becomes capable of poorly coordinated walking. In five hamsters that had been stimulated to arouse by tactile stimulation, presentation of hypercapnic, normoxic gas for 2–3 min, when RR was ~20 breaths/min and HFBF was reduced, transiently increased RR but did not increase HFBF or affect the increasing HR.
Figure 3 shows that in seven hamsters stimulated to arouse from torpor, a 5-min infusion of phentolamine (4–5 mg/kg sc) commencing when RR was 26–110062 breaths/min and HR was 40–110062 beats/min, increased HFBF from 0.09–110060.01 to a maximum of 0.84–110060.2 V. Comparisons of HFBF at 0 min with later 5-min interval time points demonstrated a significant difference between treatments \( F(1,12) = 9.7, P < 0.01 \) and a significant interaction between time and treatment \( F(7,84) = 12.3, P < 0.001 \). By 20 min after the start of the infusion, the increase became significantly different from the saline infusion (\( P < 0.05 \)). HR and RR increased significantly in both saline- and phentolamine-treated hamsters during the experimental period with no significant treatment effect [both \( F(1,12) < 2.7, P > 0.05 \)]. However, both HR \( F(7,84) = 17.4, P < 0.01 \) and RR \( F(7,84) = 10.2, P < 0.01 \) demonstrated an interaction between treatment and time. By 25 min after the start of the phentolamine infusion, HR (51 ± 3 beats/min) was less than during saline infusion (62 ± 5 beats/min, \( P < 0.05 \)). By 45 min after the start of the phentolamine infusion, RR (46 ± 2 breaths/min) was less than during saline infusion (63 ± 6 breaths/min, \( P < 0.05 \)).

In three hamsters stimulated to arouse from torpor, intravenous infusion of sodium nitroprusside (5–12 \( \mu g/kg^{-1} \cdot min^{-1} \)) commencing when RR was 30–40 breaths/min had no measurable effect on HFBF, HR, or RR. In gamma imaging experiments (see below), intravenous infusion of phentolamine (0.8 mg/kg) to four hamsters stimulated to arouse from hibernation, when RR was 30–40 breaths/min, induced an increase in HFBF from 0.09 ± 0.01 to 0.64 ± 0.11 V.

**Gamma Experiments**

\(^{99m}\text{Tc}-\text{HSA} \) was evenly distributed in the body within 1 min of the end of infusion in anesthetized hamsters and within 2–3 min in hamsters arousing from hibernation. Figure 4 shows normalized eight-color images of whole body gamma activities...
of a urethane-anesthetized cenothermic hamster (Fig. 4A) and a hamster arousing from hibernation (Fig. 4B). The contrast medium-enhanced X-ray of each animal has been overlaid on its gamma image. The positions of ROI and the percentage of total blood volume measured in each ROI in urethane-anesthetized cenothermic hamsters and hamsters arousing from hibernation are also presented in Fig. 4, bottom. Table 1 shows significant differences in regional blood volume between anesthetized, cenothermic, cold-adapted hamsters and hamsters stimulated to arouse from hibernation. Blood volumes in the anterior body regions (t = 11.6, df = 11, P < 0.001), including head-neck (t = 5.1, df = 11, P < 0.001) and heart-liver-lungs (t = 4.6, df = 11, P < 0.01), were significantly greater in hamsters arousing from hibernation than in anesthetized hamsters. On the contrary, blood volumes of hindlimb (t = 10.1, df = 11, P < 0.001), kidney (t = 13.0, df = 11, P < 0.001), and hind feet (t = 3.2, df = 11, P < 0.05) were significantly less in arousing hamsters than in anesthetized hamsters. Urine formation during anesthesia was significantly greater than during arousal (t = 17.0, df = 11, P < 0.001). Table 1 shows that, as the process of arousal proceeded from -20 min to 0 min and HR, RR, and regional heterothermia increased, the blood volumes in the upper body regions (above the liver, P > 0.05) and lower body regions (below the liver, P > 0.05) remained unchanged. However, within these regions blood volume was redistributed. Blood volume was decreased in the regions encompassing heart, lung, and liver (P < 0.005), whereas blood volume in neck-head (P < 0.05), front limbs (P < 0.01), and kidney (P < 0.001) increased. Blood volume of hindlimb (P > 0.05), hind feet (P > 0.05), and bladder (P > 0.05) were not changed during this time period.

Figure 5A shows that a 2-min infusion of α-adrenergic antagonist to five hamsters arousing from hibernation, commencing when BAT, rectal temperature, and RR were 18.6 ± 0.4°C, 10.7 ± 0.4°C, and 78 ± 5 breaths/min, respectively, induced redistribution of blood volume from the upper body [F(3,19) = 17.7, P < 0.001] to the kidneys [F(3,19) = 12.3, P < 0.001] and hind body regions [F(3,19) = 17.3, P < 0.001]. Table 1 shows that antagonism of α-adrenergic receptors significantly increased blood volume in the lower body, hindlimb, hind feet, kidney, and bladder regions by 16–25% [F(3,19) > 12.3 for all regions, P < 0.001] (blood volume of bladder includes underlying tissue). Blood volume of heart-liver region [F(3,19) = 34.9, P < 0.0001] and neck-head region [F(3,19) = 4.5, P < 0.025] decreased by 2% and 12%, respectively. Figure 5B shows the profile of HFBF measured simultaneously with gamma imaging in four of the five hamsters arousing from hibernation. Intravenous infusion of the α-adrenergic antagonist phenolamine (0.8 mg/kg) induced an increase in HFBF in all hamsters from a basal value of 0.09 ± 0.01 to 0.64 ± 0.1 V (n = 4) by 10 min after the termination of the 2-min infusion. Figure 5C shows that infusion of the α-antagonist induced a transient decrease in the mean rate of change of BAT temperature. Maximum rate of change of BAT at 24 min was significantly decreased compared with that at 32 min (P < 0.05) during phenolamine infusion [F(7,28) = 4.1, P < 0.01]. Over the same time interval, the mean rate of change of rectal temperature was increased [F(7,28) = 4.1, P < 0.01] but did not attain statistical significance at any time point. Nembutal anesthesia (30 mg/kg iv) induced a less specific reduction in vascular tone and accentuated the changes in blood volume (Table 1). HFBF transiently increased to a maximum of 1.84 ± 0.3 V (n = 4), and rectal temperature increased despite a reduction in BAT temperature and metabolic rate.
DISCUSSION

These experiments demonstrate for the first time that hibernating hamsters, with a body temperature of 6°C, respond promptly to tactile stimulation of the skin but not to 2-min hypercapnic or 30-s sound stimulation with neurogenically derived vasoconstriction of hindlimb blood vessels. After arousal has been initiated, this vasoconstriction is maintained, despite increasing cardiac and metabolic rates, until the late phases of arousal. Vasoconstriction appears to be mediated by α-adrenergic receptor activation that contributes substantially to the maintenance of the thermal heterogeneity required for arousal from hibernation. Organ bath experiments have implicated a role for corelease of purines in vasoconstriction of the hamster hindlimb vessels at temperatures that approximate those of hibernation (22). As such, an in vivo role for nonadrenergic transmitters colocalized in sympathetic terminals in the peripheral vasoconstriction associated with arousal from hibernation is also probable.

In hibernators, large conducting arteries appear to retain sensitivity to the vasodilatory effects of nitrous oxide at low temperatures (13, 23), yet hamsters appear to have temperature-dependent loss of endothelium-dependent vasodilation (23). Our preliminary experiments in only three animals demonstrated that sodium nitroprusside infusion failed to alter arousal-induced vasoconstriction in the hindlimb during early arousal. This probably reflects the fact that it is generally considered that nitric oxide has a significantly reduced role in mediating vasodilation in smaller arteries and peripheral vessels. This is circumstantially supported by the finding that endogenous nitric oxide appears not to influence blood flow to organs other than the heart during hibernation (13).

The decrease in HFBF at the onset of arousal from hibernation was also associated with a simultaneous gradual increase in HR and delayed increase in QRS complex amplitude. This suggests that cardiac activation at arousal is mediated by central mechanisms that stimulate the activity of the atrioventricular node and increase the number or synchronicity of ventricular cells involved in contraction. Consistent with previous reports (14, 27), breathing of hypercapnic, normoxic gas during hibernation evoked a large, transient increase in RR. We also recorded a small, transient increase in HR and HFBF that were probably secondary to the large increase in rate and amplitude of ventilation. It is interesting to note that, in response to their appropriate stimuli, both the muscles of respiration and vascular beds and their innervating nerves demonstrate rapid functional responses at the low body temperatures of hibernation or early arousal. In contrast, HR and QRS during the initial stages of arousal appear capable of only slow increases. This raises the possibility that local regulation of vascular tone and venous return rather than changes in HR may be the mechanisms by which cardiac output and regional blood flow are matched to regional metabolism during hibernation.

From a teleological perspective, peripheral and hindlimb vasoconstriction appears necessary to partition warm blood between the anterior of the animal, which contains essential organs such as heart, brain, and BAT, and the cooler posterior of the animal, which contains considerable muscle mass and little nonshivering thermogenic tissue. Gamma imaging of 99mTc-HSA was used to examine the redistribution of regional blood volume that resulted from the induction of adrenergically mediated peripheral vasoconstriction induced at arousal from hibernation and the time course of changes in distribution of blood volume as arousal progressed.

Nuclear imaging techniques impose positional and movement constraints during measurements that are incompatible with the measurement of regional blood volume in hibernating and nonanesthetized, cenothermia hamsters. Such measurements were restricted to hamsters in the early stages of arousal from hibernation, which remained incapable of movement, and in anesthetized hamsters. In the absence of data from conscious unrestrained hamsters, urethane-anesthetized, cold-adapted hamsters from the same cohort and season were employed as controls. Urethane anesthesia was chosen because, of the available general anesthetics, it has favorable properties of preservation of autonomic control of visceral functions, excitatory and inhibitory reflexes and blood pressure, and minimal depression of central nervous system activity and RR (17). It does produce skeletal muscle relaxation and does reduce renal blood flow (17).

In theory, skeletal muscle relaxation induced by anesthesia will increase blood volumes in limbs and muscular head-neck regions with compensatory reductions in other regions when compared with conscious, unrestrained animals. However, experimental studies focused on this issue have not been performed, and the magnitudes of the discrepancies in regional blood volume between anesthetized and conscious animals are not known. As such, the anesthetized hamster is not an ideal control. However, because blood volume of the muscular neck head region of the arousing hamster is significantly greater than that in the anesthetized hamster, in which blood pooling theoretically should occur, and because the blood volume of the nonurine forming kidney is significantly less than in the arousing hamster, we are confident that the differences in regional blood volume distribution between hamsters in the two states do not result solely from anesthesia. In addition, the effective redistribution of blood volume in the arousing hamster after infusion of α-antagonist also demonstrates that regional blood distribution during arousal is a consequence of the unique circulatory adaptations associated with arousal from hibernation.

Figure 4 shows that, early after the onset of arousal, the blood volumes of the hind body, hindlimb, kidney, and hind feet are greatly reduced, suggesting that the reduced HFBF associated with the induction of arousal from hibernation results from vasoconstriction that is applied to deep and peripheral hind body vasculature. The initial blood volume measurements correspond to the early phase of arousal, and it appears that at this stage the principal blood reserves are the thermogenically active lung-heart-liver and neck-head regions.

It is well established that arousal from hibernation is metabolically demanding (20, 24). In the imaging period before infusion of the α-antagonist, RR increased from 51 to 78 breaths/min and BAT temperature rose from 12 to 19°C; however, the regional blood volume distribution between upper and lower body changed little, as did the rectal temperature, which increased from 9.5 to 10.6°C. In this interval, the blood volume in heart-lung-liver gradually decreased, whereas blood volume in the head-neck and kidney regions increased, possibly consistent with an increased metabolic role for these tissues.
Although animal studies are not available, human graded exercise by cycling experiments have shown that blood-volume distribution (measured by gamma imaging of 99mTc-labeled red blood cells) is correlated with regional oxygen consumption. The largest increase in blood volume during exercise was recorded in the thorax (lung > heart), with a smaller increase in the legs; blood volume, however, decreased in the abdomen, kidney, and spleen (5, 7). This profile of change in regional blood volume in response to exercise and increasing metabolic rate contrasts markedly with the changes in regional blood volume in the hamster arousing from hibernation. Together, these data suggest that the profile of regional blood volume distribution during arousal from hibernation is a complex adaptation balancing metabolic and vasoconstrictor influences.

The last phase of arousal is associated with a rapid increase in rectal temperature. Measurement of blood volume during the last phase was not possible because by this time the brain of the arousing hamster is cenothermic and functional, and the animals struggle when they become aware that their immediate environment is unfamiliar. To some extent, we were able to mimic the increase in HFBF and rectal temperature characteristic of the late arousal phase during the middle arousal phase while the animals remained incapable of movement.

Laser-Doppler experiments demonstrated that α-adrenergic receptor antagonism during the early arousal phase induced an increase of HFBF. Gamma imaging experiments revealed that α-adrenergic receptor antagonism during the middle arousal phase induced a whole body redistribution of blood volume, blood flow, and regional temperature gradients. Receptor-antagonized vasodilation of lower body vasculature was associated with increased HFBF, an increased blood volume of the hind body, and an increase in rectal temperature. The pharmacologically induced increase in rectal temperature was small compared with that measured in the late arousal phase because the thermal reservoir in the upper body and the animal’s metabolic rate are less in the middle arousal phase. Simultaneously, blood volume of the neck and head region was reduced, and the rate of increase of BAT temperature was reduced. Additional nonspecific reduction of vascular tone by anesthesia further increased HFBF, increased rectal temperature, and induced measurable production of urine. The blood volume in the hindlimbs and kidney increased with a corresponding reduction of blood volume in heart-lung-liver so that the overall blood volume distribution tended to approach the pattern observed in cenothermic anesthetized hamsters.

Hibernation is a polyphyletic phenomenon, and species differences are apparent in a number of aspects of hibernation physiology among small mammals. However, at low temperatures in vitro, sensitivity to adrenergic vasoconstriction is retained by vessels of hamsters (22), hedgehogs (4), ground squirrels (9), and marmots (19) and may be characteristic of all small mammal hibernators in vivo. In hamsters (20) and sciurids (25), the induction and maintenance of thermal gradients in the body during arousal from hibernation appear to be a common strategy. Comparative studies are not available; speculatively, however, hamsters may be representative of other small mammalian hibernators in that the regulation of blood flow and the redistribution of regional blood volume by sympathetic vasoconstriction on initiation of arousal from hibernation is mediated by α-adrenergic receptors. This sympathetic tone is relaxed late in arousal. This scenario is probably the principal mechanism by which the body temperature gradients produced during arousal from hibernation are regulated.

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