GABA_A receptor activation at medullary sympathetic neurons contributes to postexercise hypotension

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Kajekar, Radhika, Chao-Yin Chen, Tatsushi Mutoh, and Ann C. Bonham. GABA_A receptor activation at medullary sympathetic neurons contributes to postexercise hypotension. Am J Physiol Heart Circ Physiol 282: H1615–H1624, 2002. First published January 17, 2002; 10.1152/ajpheart.00725.2001.—A single bout of exercise results in a postexercise hypotension (PEH) that is accompanied by a reduced baroreflex function. Based on the role of rostral ventrolateral medulla (RVLM) neurons in controlling sympathetic nerve activity (SNA) and blood pressure, the role of γ-aminobutyric acid (GABA) in controlling RVLM neuronal activity, and the reduced baroreflex-SNA relationship during PEH, we determined whether: 1) RVLM neuronal activity is decreased during PEH, 2) GABA_A receptor mechanisms mediate the decrease, and 3) baroreflex control of RVLM activity is reduced. Spontaneously hypertensive rats (SHR) were subjected to 40 min of treadmill or sham exercise (Sham PEH). PEH lasted 10 h in conscious and anesthetized SHR, indicating that the anesthetics did not affect the expression of PEH. Extracelluar RVLM neuronal activity having a cardiac and sympathetic rhythm, lumbar SNA, and blood pressure were recorded at rest and during baroreflex function curves. Resting RVLM neuronal activity was lower and was increased to a greater extent by GABA_A-receptor antagonism in PEH versus Sham PEH (P < 0.05). Baroreflex control of RVLM neuronal activity operated with a reduced gain (P < 0.05). Thus increased GABA signaling at RVLM neurons may contribute to PEH.

spontaneously hypertensive rats; central sympathetic network; extracellular recording

HYPERTENSION is a major antecedent of stroke, heart failure, and end-stage renal disease. After decades of improvement in its control with pharmacological treatments, national reports now indicate that hypertension is adequately controlled in only about 25% of individuals in the United States (55). This downward trend in pharmacological management has led to renewed efforts to reduce the prevalence of hypertension with nonpharmacological approaches (5). In individuals with hypertension, a single bout of mild to moderate dynamic exercise can lead to a postexercise decrease in blood pressure (7, 13, 20, 28–30, 35, 48). This phenomenon, termed postexercise hypotension (PEH), can restore blood pressure back toward normal (30, 35) and can persist for up to 12 h (19, 30). Understanding the mechanisms of PEH may be a first step in increasing an awareness of this strategy for controlling hypertension and might lead to a greater emphasis on lifestyle modification compared with a sole reliance on pharmacological therapy. The spontaneously hypertensive rat (SHR) appears to be an ideal model for investigating the mechanisms; following single bouts of mild to moderate exercise (treadmill or spontaneous wheel running), SHR exhibit PEH of the same magnitude and duration observed in hypertensive humans (10, 14, 38, 43).

Both neural and humoral changes have been explored in hypertensive humans and SHR to help explain PEH; these include activation of the endogenous opioid system (8, 9), enhanced cardiopulmonary reflex-mediated sympathoinhibition (14), reduced vascular resistance (13, 29, 32, 33, 38) and reactivity (33), or thermoregulatory-induced increases in skin blood flow (21).

There are two salient characteristics of PEH: 1) a reduction in sympathetic nerve activity (20, 29, 32, 38) and 2) a requirement for an intact baroreflex system (10). To begin to unravel the mechanisms underlying PEH, a good starting point is to determine the mechanism for the reduced sympathetic nerve discharge. There is now considerable evidence that sympathetic nerve activity is generated by a group of neurons in the rostral ventrolateral medulla (RVLM) and that cardiovascular reflex and central descending changes in sympathetic nerve activity and arterial blood pressure (ABP) are relayed through these neurons (27). These RVLM neurons, characterized by their sympathetic-related rhythm and cardiac rhythm from entrainment by baroreceptor input (27), are under a tonic inhibition, largely mediated by γ-aminobutyric acid (GABA) acting at GABA_A receptors (2, 15, 24, 26, 40, 45). In that regard, blockade of GABA_A receptors in the RVLM can increase the baseline activity of the individual RVLM neurons (23) and can also increase sympathetic nerve activity and arterial blood pressure (25, 47, 54, 61). The
GABA-ergic input arises mostly from baroreceptor reflex pathways through the nucleus tractus solitarius and caudal ventrolateral medulla (2, 15, 24, 26, 45); but there is also evidence for a GABA-ergic inhibitory input independent of the baroreceptors (40). Thus GABA-ergic mechanisms in the RVLM could contribute to the development of PEH.

Regarding the second salient feature of PEH (i.e., the requirement for an intact baroreflex system), while the baroreflex system is essential, regulatory events in the reflex pathway must be altered, given that sympathetic nerve activity is reduced rather than elevated as might be expected during the lower ABP. Studies in humans and SHR report significant decreases in muscle sympathetic nerve activity, associated with a downward and leftward shift of the baroreflex-sympathetic nerve activity relationship (11, 29). These findings raise the possibility of a centrally mediated reduced gain and resetting of the baroreflex system, which might be expected to manifest at RVLM neurons.

Thus, based on the fundamental role of the RVLM neurons in controlling sympathetic vasomotor tone and blood pressure, the regulatory role of GABA in controlling the baseline activity of those neurons and the reduced baroreflex function during PEH, the purpose of this study was to address three questions: 1) Does a decrease in RVLM cardiovascular sympathetic neuronal activity accompany PEH? 2) Is the reduced RVLM sympathetic output (and hence PEH) mediated by an increase in RVLM GABA_A-receptor mechanisms? 3) Does a reduced gain of the baroreflex-control of RVLM neuronal activity contribute to the decreased gain in the baroreflex-sympathetic nerve activity relationship?

**METHODS**

All experimental protocols in this work were reviewed and approved by the Institutional Animal Care and Use Committee in compliance with the Animal Welfare Act and in accordance with Public Health Service Policy on Humane Care and Use of Laboratory Animals. All experiments were performed in male SHR (250–350 g; Charles River Laboratories) randomly assigned to an exercise group (PEH) that were subjected to a single bout of exercise, each rat was anesthetized (intraperitoneally) with an initial dose of a mixture of α-chloralose (80 mg/kg) and urethane (500 mg/kg). The adequacy of anesthesia was assessed every 20–30 min by pinching the hindlimb paw and monitoring for hindlimb flinch or for fluctuations in ABP (>5 mmHg). When needed, supplemental doses of α-chloralose (16 mg/kg iv) and urethane (100 mg/kg iv) were given.

**General animal preparation for neuronal recordings in anesthetized SHR.** Immediately after the exercise or sham exercise, each rat was anesthetized (intraperitoneally) with an initial dose of a mixture of α-chloralose (80 mg/kg) and urethane (500 mg/kg). When needed, supplemental doses of α-chloralose (16 mg/kg iv) and urethane (100 mg/kg iv) were given. The adequacy of anesthesia was assessed every 20–30 min by pinching the hindlimb paw and monitoring for hindlimb flinch or for fluctuations in MABP (>5 mmHg) or heart rate (HR) (>10%). After the initial anesthetic dose, a catheter was inserted into the tail vein for intravenous administration of supplementary doses of anesthetic and a constant infusion of intravenous fluids (8.4% sodium bicarbonate, 0.05 ml/min, and a mixture of 5% dextrose and 0.2% sodium chloride, 0.06 ml/min). A catheter was inserted into the femoral artery and advanced to the abdominal aorta to record ABP and withdraw samples for monitoring arterial blood gases. Three hundred microliters of arterial blood were taken for blood gas measurement every 30 min at the beginning of the experiment and every 2–3 h once the blood gases were stabilized.

**All experiments were performed in male SHR (250–350 g; Charles River Laboratories) randomly assigned to an exercise group (PEH) that were subjected to a single bout of exercise on a motor-driven treadmill at 15 m/min, 10° for 40 min and to a sham-exercise group (Sham PEH) placed on the treadmill for 40 min with no exercise.**

**Studies on PEH in conscious and anesthetized SHR.** In preliminary experiments, the time course of PEH and the effect of anesthetic on the expression of PEH were examined. SHR were anesthetized with ketamine (50 mg/kg) and xylazine (8 mg/kg), and a catheter was inserted via the left carotid artery and advanced to the descending aorta. The other end of the catheter was tunneled beneath the skin and exteriorized at the back of the neck for recording ABP and mean APB (MABP). The rats were allowed 3–5 days to recover from surgery and were then randomly assigned in a crossover design to PEH or Sham PEH groups. The rats were further divided into anesthetic and nonanesthetic (conscious) groups. One SHR studied in the conscious state was also studied in the anesthetized state.

On the day of the experiment, each rat was placed on the treadmill, and the arterial catheter was connected to a blood pressure transducer. The rats were on the treadmill for a minimum of 40 min and up to 60 min before the exercise or sham exercise was commenced. During the preexercise period, a MABP reading was taken over a 10–20-s period every 10 min. After three consecutive MABP readings that were within 10%, the rat was subjected to exercise or sham exercise. MABP was measured every 10 min during the 40 min of exercise or sham exercise and over the next 10 h after exercise or sham exercise in both conscious and anesthetized SHR. After exercise or sham exercise, rats in the conscious PEH and Sham PEH groups were returned to their individual home cages, and rats in the anesthetized group were anesthetized (intraperitoneally) with an initial dose of a mixture of α-chloralose (80 mg/kg) and urethane (500 mg/kg). The adequacy of anesthesia was assessed every 20–30 min by pinching the hindlimb paw and monitoring for hindlimb flinch or for fluctuations in ABP (>5 mmHg). When needed, supplemental doses of α-chloralose (16 mg/kg iv) and urethane (100 mg/kg iv) were given.

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branes. After completion of the surgical preparation, at the commencement of recording, the animals were paralyzed with gallamine (10 mg/kg iv). During neuromuscular blockade, the adequacy of anesthesia was assessed every 20–30 min by pinching the hindlimb paw and monitoring for fluctuations in ABP or HR.

**Extracellular RVLM unit recording and iontophoresis.** Extracellular recordings of single unit activity were made through a single glass electrode filled with 0.5 M sodium acetate. For the iontophoresis studies, the recording electrode was affixed to a three-barrel electrode at a 20° angle with the tip extending 25 μm below the remaining barrels. Of the nonrecording barrels, one contained normal saline (0.9%) for balancing the ejection currents and the other two contained the GABA<sub>A</sub> receptor agonist, muscimol (10 mM; pH 6) and the GABA<sub>B</sub> receptor antagonist, bicuculline methiodide (20 mM, pH 3.0), respectively. Ejection and balancing currents were produced by a constant current source (NeuroPhore BH 2, Medical Systems; Greenvale, NY). The drugs were retained in the electrode by applying a holding current of ~5 nA and were ejected with cathodic waves.

RVLM neurons were located using an approach previously described by Guyen et al. (27). Briefly, stimulation (5 V, 1 Hz for 100 ms) of the mandibular branch of the facial nerve with a bipolar hook electrode generated a field potential recorded in the facial nucleus with a glass electrode. The caudal site of the facial motor nucleus was identified, and the recording electrode was moved ~400 mm caudally to the RVLM. The searched RVLM region extended from 1.3 to 1.8 mm lateral and 0.8 to 1.5 mm rostral to calamus scriptorius, and from 2.0 to 4.5 mm dorsal to the ventral surface (caudorostral inclination of electrode was 2.5°).

**RVLM neuronal recording protocols.** To determine whether PEH was due to a decrease in tonic central sympathetic output, we recorded the spontaneous baseline activity of sympathetic cardiovascular RVLM neurons during PEH and Sham PEH. All RVLM neuronal recordings were commenced at ~4 h after the exercise or sham-exercise period, owing to the transient anesthetic-induced drop in MABP (that lasted ~2 h) (see Fig. 1).

Once a stable recording was obtained, a temporal correlation to the rhythmic discharge of LSNA by triggering averaged LSNA by unitary spikes of the RVLM neuron (22). A cardiac rhythm was established for the same neuron by constructing a post-R-wave (ECG)-triggered histogram using EGAA/Computerscope software (RC Electronics; Goleta, CA). A period of at least 2 min of spontaneous discharge of neurons displaying a sympathetic and cardiac rhythm was acquired to determine the baseline spontaneous firing rate.

To determine whether baroreflex control of RVLM neuronal activity was altered during PEH, complete baroreflex stimulus-response curves were generated by alternatively increasing or decreasing (in random order) MABP by progressively infusing phenylephrine (100 μg/ml) or nitroprusside (100 μg/ml) while simultaneously monitoring RVLM single unit activity and LSNA. The rate of MABP change was controlled to ~1.2 mmHg/s. Only neurons on which complete baroreflex stimulus curves were performed (range of MABP at least 50–180 mmHg) were included in the study.

**Bicuculline iontophoreses protocols.** In separate experiments from those used to establish the baroreflex function in SHR during PEH, we determined whether a GABA<sub>A</sub> receptor mechanism contributed to the tonic decrease in central sympathetic output of RVLM neurons and hence PEH. We compared the maximal effect of bicuculline on the baseline RVLM neuronal activity in PEH and Sham PEH animals. Each neuron was first tested with two doses (a low and high current) of muscimol before and during iontophoresis of bicuculline to confirm that 1) the neurons had GABA<sub>A</sub> receptors and that 2) the amount of bicuculline that maximally increased the neuronal spiking activity was sufficient to block GABA<sub>A</sub>-receptor-mediated decreases in neuronal activity (to verify that increases in spiking activity attributed to bicuculline were GABA<sub>A</sub> receptor mediated and not due to nonspecific effects of current injection or instability in the recording during iontophoresis). After a 2-min recovery period after muscimol ejections, bicuculline was iontophotically applied with an ejection current starting at 10 nA. The current was progressively increased in 10- to 30-nA increments every 60- to 90-s interval until the minimum current was reached that maximally increased the spiking activity. Once that current was established, in every case, the current was then further increased by 10 nA to verify that further increase in the amount of antagonist had no further effect on spiking activity. Under constant bicuculline iontophoresis, RVLM unit activity was monitored over 2 min, and the response to muscimol injected was retested.

**Data analysis.** For the initial studies to determine whether PEH lasted 10 h in the conscious state, we used a two-way ANOVA with PEH and Sham PEH as one within factor and time as the other within factor. To determine whether PEH lasted 10 h in the anesthetized state, we also used a two-way ANOVA with PEH and Sham PEH as one between factor and time as the other within factor. To determine whether anesthesia affected the development of PEH, we averaged the 3 MABP values taken immediately before exercise and the 36 MABP values taken every 10 min between 4 and 10 h after exercise (PEH). The data were compared by using a two-way ANOVA with MABP as a within factor and with conscious versus anesthetized as a between factor. ANOVAs were followed by post-hoc tests when appropriate. Animal weights and blood gases were compared using an unpaired t-test.

For comparing spontaneous baseline RVLM neuronal firing frequency, unit activity was collected in 1-s bins, expressed as impulses per second (Hz), averaged over 2 min, and compared for neurons recorded during PEH and Sham PEH by using an unpaired t-test. Recording times for RVLM unit activity from the onset of anesthesia were also compared using an unpaired t-test. Raw LSNA voltage was rectified, integrated, and expressed as microvolts times seconds. Background instrumentation noise was determined after the rat was killed, and spontaneous LSNA was eliminated; this represented the zero reference point and was subtracted from LSNA before further analysis. LSNA, MABP, and HR averaged over 2 min were compared during PEH and Sham PEH using an unpaired t-test. RVLM-LSNA ratios and time from the R-wave to the peak unit activity and LSNA for PEH and Sham PEH were compared using unpaired t-tests.

Complete stimulus-response curves were generated for RVLM unit activity and LSNA as a function of MABP. Reflex changes in LSNA and RVLM single unit activity to changes in MABP were fit to a sigmoid logistic function as described by Kent et al. (36) using SigmaPlot (Jandel) to allow for the comparison of various parameters used to assess baroreflex sensitivity between animals (18): the steady-state maximum activity (Max), the steady-state minimum activity (Min), the range of nerve activity, pressure at which neuronal activity was first decreased (P<sub>ah</sub>), and pressure at which neuronal activity was first maximally inhibited (P<sub>final</sub>). The maximum slope was used as an index of the sensitivity or maximum gain (G<sub>max</sub>) of the baroreflex function. The above parameters determined from curve-fit analysis of the function curves of PEH and Sham PEH groups were compared using an un-
paired t-test. We further determined whether \( C_{\text{max}} \) depended on a baseline firing rate in PEH or Sham PEH by linear regression analysis. To determine whether RVLM and LSNA activity remained related during PEH, LSNA was plotted as a function of RVLM neuronal activity for each neuron recorded during PEH and Sham PEH during phenylephrine- and nitroprusside-induced changes in MBP. Data points were fit by linear regression, and the slopes of the regression lines from PEH and Sham PEH were compared using an unpaired t-test.

To determine whether GABA acting at GABA\(_A\) receptors contributed to the reduction in baseline RVLM unit activity in PEH, we compared the maximum bicuculline-induced increase in unit activity over baseline activity in PEH and Sham PEH using an unpaired t-test. The ejection currents used to achieve maximal unit responses to bicuculline during PEH and Sham PEH were compared with an unpaired t-test. The high-dose muscimol-induced decreases in unit activity were compared in each group using a paired t-test. The extent to which bicuculline prevented the muscimol-evoked unit response was determined in each group by using an unpaired t-test. The currents used to achieve the high-dose muscimol inhibition of unit activity in PEH and Sham PEH were compared with an unpaired t-test. Linear regression analysis was used to determine whether the bicuculline-induced increases in firing activity depended on the baseline activity.

Unless stated, all data are expressed as means ± SE. For all statistical tests, significance was claimed in analyses when the probability of a type 1 error was <0.05.

**RESULTS**

**PEH in conscious and anesthetized SHR.** We confirmed previous findings (38, 43) demonstrating that PEH follows mild dynamic exercise in conscious SHR and extended those findings to show that the PEH lasted for at least 10 h \((n = 4)\) in exercised rats and was not observed during sham exercise \((n = 4)\) (Fig. 1A, two-way ANOVA with two-way repeated measures, \( P < 0.05 \), PEH vs. Sham PEH; \( P < 0.05 \), time; \( P < 0.05 \), interaction). At 10 h after exercise MABP was still lower than the control (preexercise values) in the PEH group (Fishers least significant difference, \( P < 0.05 \)) and was never lower than control in the Sham PEH group (Fishers least significant difference, \( P > 0.05 \)).

We further showed that PEH was still expressed when the SHR were anesthetized with mixture of \( \alpha \)-choloralose (80 mg/kg) and urethane (500 mg/kg) following exercise (Fig. 1B, two-way ANOVA with one-way repeated measures, \( P < 0.05 \), PEH vs. Sham PEH; \( P < 0.05 \), time; \( P < 0.05 \), interaction). As shown in Fig. 1B, the anesthetic induced a transient fall in MABP in SHR in both the PEH \((n = 4)\) and Sham PEH \((n = 5)\) groups that lasted ~2 h. However, as was the case with the conscious animals, at 10 h after exercise, MABP was still lower than the control (preexercise values) in the PEH group (Fishers least-significant difference, \( P < 0.05 \)). In the Sham PEH group after the initial anesthetic-induced decrease in MABP, the MABP was never lower than control (Fishers least-significant difference, \( P > 0.05 \)).

Finally, after the transient anesthetic-induced fall in MABP, the magnitude and duration of PEH were not different in the conscious and anesthetized rats \((P > 0.05)\), conscious PEH vs. anesthetized PEH; \( P < 0.05 \), time; \( P > 0.05 \), interaction). In the conscious PEH group, MABP was 170 ± 7 mmHg before exercise and 138 ± 9 mmHg after exercise (averaged between 4 and 10 h). In the anesthetized PEH group, MABP was 168 ± 5 mmHg before exercise and was 143 ± 5 mmHg after exercise (and the administration of the anesthetic) (averaged between 4 and 10 h). In the conscious Sham PEH group, MABP was 169 ± 10 mmHg before sham exercise and 173 ± 12 mmHg after exercise (averaged between 4 and 10 h); and in the anesthetized Sham PEH group, MABP was 165 ± 4 mmHg before sham exercise and 166 ± 10 mmHg during the corresponding 4- to 10-h period after sham exercise.

**Sympathetic cardiovascular RVLM neuronal activity during PEH and Sham PEH.** A total of 59 RVLM neurons considered to be cardiovascular sympathetic neurons based on a temporal relationship to LSNA and to the R-wave of the ECG were recorded in 37 SHR. Twenty-nine neurons were recorded from 18 SHR in the exercise group (PEH) and 30 neurons were recorded from 19 SHR in the sham-exercise group (Sham PEH). All neurons were studied between 4 and 10 h after anesthesia to avoid nonspecific changes in MABP owing to anesthetic. The mean time in which the RVLM neuronal recordings were made after anesthesia was 6.23 ± 0.27 h (range = 4.15–8.20 h) in the PEH.
group and 6.28 ± 0.27 h (range = 4.15–9.30 h) in the Sham PEH group. (P > 0.05 PEH vs. Sham PEH). Both PEH and Sham PEH animals had a similar weight distribution (PEH = 312 ± 9 g, Sham PEH = 309 ± 5 g; P > 0.05) and arterial blood gases (pH = 7.40 ± 0.01 vs. 7.39 ± 0.01; PCO2 = 39 ± 1.0 mmHg vs. 42 ± 2 mmHg; PO2 = 388 ± 29 mmHg vs. 363 ± 34 mmHg, respectively; P > 0.05).

Figure 2 shows examples of neurons recorded from Sham PEH and PEH. The spike-triggered averages of LSNA in Fig. 2A (top trace) illustrate the temporal relationship of the neuron and LSNA in a SHR during PEH and Sham PEH. In contrast, LSNA exhibited no consistent temporal relationship to random triggers delivered by a pulse generator at the same average frequency as that of the RVLM unit (Fig. 2A, bottom trace). As shown by the R-wave-triggered histograms (Fig. 2B), the neurons also displayed a cardiac rhythm, as did ABP and LSNA. The baseline firing activity, recorded at resting ABP, was 4.1 Hz for the neuron recorded during PEH and 12.7 Hz for the neuron recorded during Sham PEH (Fig. 2C, insets). The curve-fit data (Fig. 2C) show that the Gmax was 0.21 Hz/mmHg in the SHR with PEH and 0.34 Hz/mmHg in the SHR during the corresponding Sham PEH period. The maximum activity was 11.0 Hz during PEH and 28.1 Hz during Sham PEH.

The group data confirmed that sympathetic cardiovascular RVLM neurons showed a reduced baseline firing activity (Fig. 3) and reduced responsiveness to baroreceptor input during PEH compared with Sham PEH (Fig. 4). Sympathetic cardiovascular RVLM neurons discharged with significantly lower frequencies during PEH (6 ± 1 Hz, n = 29) compared with Sham PEH (11.7 ± 3 Hz, n = 30) (P < 0.05) (Fig. 3A). The cardiac rhythm displayed by the neurons was not different in neurons recorded during PEH or Sham PEH; the neuronal activity peaked at 51.0 ± 5 ms after the R wave of the ECG during PEH and at 48.5 ± 4 ms during Sham PEH (P > 0.05). The corresponding LSNA was also lower; 0.39 ± 0.05 μV·s during PEH compared with 1.00 ± 0.09 μV·s during Sham PEH.

![Fig. 2. Examples of rostral ventrolateral medulla (RVLM) neuronal recordings made during PEH and Sham PEH. A: RVLM unit activity and average lumbar sympathetic nerve activity (LSNA) showed a temporal relationship in both neurons. LSNA was synchronized to 1,500 spontaneous unitary spikes (Unit trigger→LSNA) but not to 1,500 random triggers (Random trigger→LSNA) delivered by a signal generator at the same average frequency as the unit. Calibration bar, 15 μV. B: each RVLM neuron had a cardiac rhythm as evidenced by the R-wave-triggered histograms, as did LSNA and MABP. C: insets show action potential (AP) discharge rates for each neuron. Fitted curves show RVLM unit activity plotted as a function of MABP.](http://ajpheart.physiology.org/)

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maximum activity was $10.9 \pm 1.1$ vs. $17.2 \pm 1.2$ Hz, range was $10.0 \pm 0.7$ vs. $15.1 \pm 1.2$ Hz, and $G_{\text{max}}$ was $0.126 \pm 0.01$ vs. $0.214 \pm 0.02$ Hz/mmHg, respectively. There was no relationship between baseline firing activity and $G_{\text{max}}$ in either group; the $r^2$ value for the Sham PEH group was 0.05 ($P = 0.41$); the $r^2$ value for the PEH group was 0.07 ($P = 0.44$). In addition, for neurons with similar baseline activities ($6.6 \pm 1.5$ (SD) Hz in the PEH group and $7.6 \pm 1.1$ (SD) Hz in the Sham PEH group), the $G_{\text{max}}$ was still lower in the PEH group ($0.13 \pm 0.01$ Hz/mmHg for PEH vs. $0.21 \pm 0.02$ Hz/mmHg for Sham PEH, $P = 0.02$).

LSNA recorded in the same animals (9 Sham PEH, 9 PEH SHR) showed a similarly reduced maximum and range, and the baroreflex curve showed a reduced $G_{\text{max}}$ (Fig. 4B; $P < 0.05$). Maximum LSNA was $0.88 \pm 0.2$ vs. $1.59 \pm 0.3$ μV·s, the range was $0.77 \pm 0.2$ vs. $1.33 \pm 0.3$ μV·s, and $G_{\text{max}}$ was $0.013 \pm 0.01$ vs. $0.025 \pm 0.01$ μV·s/mmHg$^{-1}$, respectively. Neither the $P_{\text{th}}$ nor $P_{\text{cutoff}}$ were different in the PEH or Sham PEH groups ($P > 0.05$).

The relationship between RVLM neuronal activity and LSNA was not changed during PEH. When LSNA was plotted as a function of RVLM neuronal activity for each neuron, the mean data yielded slopes that were not different (0.09 ± 0.03 and 0.07 ± 0.02 for PEH and Sham PEH neurons, respectively; $P = 0.45$).

**Contribution of GABAA receptors on RVLM neuronal activity during PEH.** The effects of GABAA-receptor antagonism on the baseline spiking activity of sympathetic cardiovascular RVLM neurons were studied in 32 RVLM neurons from 19 SHR (18 neurons were recorded from 9 SHR in the PEH group and 14 from 10 SHR in the Sham PEH group). All neurons were tested

Fig. 3. Group data from RVLM neurons showing average resting spontaneous firing activity, LSNA, and MABP during PEH (filled bars) and Sham PEH (open bars). A: resting RVLM neuronal activity was significantly lower in PEH compared with the Sham PEH group neurons recorded at resting blood pressure (*$P < 0.05$) as was LSNA (B) and MABP (C).

(Fig. 3B) ($P < 0.05$), as was MABP ($120 \pm 2$ mmHg in the PEH group and $137 \pm 3$ mmHg in the Sham PEH group) ($P < 0.05$) (Fig. 3C) and HR (362 ± 7 beats/min in the PEH group and 409 ± 12 beats/min in the Sham PEH group) ($P < 0.05$). The cardiac rhythm of LSNA, like RVLM neuronal activity, was also not different in the two groups: 128 ± 6 and 135 ± 4 ms after the R wave in the PEH and Sham PEH group, respectively, ($P > 0.05$).

The curve fit data show that baroreflex control of RVLM neuronal activity operated with a reduced gain and range in SHR during PEH compared with Sham PEH (Fig. 4). Figure 4 shows the group curve-fit data relating changes in RVLM unit activity and LSNA measured simultaneously to changes in MABP in the PEH and Sham PEH groups. RVLM neuronal activity exhibited a significantly reduced maximum activity and range in the PEH ($n = 11$ neurons in 9 SHR) compared with the Sham PEH group ($n = 16$ neurons in 9 SHR) (Fig. 4A; $P < 0.05$). The baroreflex control of RVLM unit activity also operated with a reduced $G_{\text{max}}$ in PEH compared with Sham PEH ($P < 0.05$). Maxi-

Fig. 4. Curve fit data for RVLM unit activity and LSNA. A: RVLM maximum unit activity, range of activity (maximum-minimum activity) (top) and maximal gain ($G_{\text{max}}$, bottom) were significantly lower during PEH (solid line, $n = 11$ neurons) compared with Sham PEH (dashed line). B: LSNA maximum activity, range of activity (top), and $G_{\text{max}}$ (bottom) were also significantly lower during PEH compared with Sham PEH.
with muscimol before and during bicuculline to verify the presence of GABA_A receptors and to confirm that the amount of bicuculline that maximally increased the neuronal spiking activity was sufficient to block GABA_A-receptor-mediated decreases in neuronal activity. An example of the neuronal responses to muscimol and bicuculline in an SHR from the PEH and Sham PEH group is shown in Fig. 5.

Muscimol was tested at two currents. The higher of the two currents was selected as one that decreased unit activity to about the same extent in both groups. At the high current, muscimol significantly decreased RVLM neuronal activity in the PEH group by 68 ± 9% (P < 0.05, n = 8 neurons, baseline vs. muscimol) and by 65 ± 9% in the Sham PEH group (P < 0.05, n = 6 neurons, baseline vs. muscimol) (data not shown). The high-dose muscimol ejection currents were not different in the two groups (P > 0.05), 37 ± 3 nA in the PEH group and 42 ± 6 nA in the Sham PEH group. The muscimol-induced decrease in neuronal activity, tested during the constant iontophoresis of bicuculline that maximally increased neuronal activity, was abolished in both groups (P < 0.05, before and during bicuculline).

The group data showing the maximal bicuculline-induced increases (Δ) in firing activity over the baseline firing activity in RVLM neurons in the PEH and Sham PEH groups are shown in Fig. 6. In the PEH group (n = 18), bicuculline increased the baseline activity (4.9 ± 1.1 Hz) to a maximum of 33.1 ± 4.4 Hz (Δ = 28 ± 4 Hz); the increase over baseline was significantly greater compared with that in the Sham PEH group (n = 14), where bicuculline increased the baseline activity of 14.8 ± 4.5 Hz to a maximum of 28.1 ± 5.1 Hz (Δ = 13 ± 2 Hz) (P < 0.05, ΔPEH vs. ΔSham PEH). The maximal level of firing activity was not different in the PEH and Sham PEH groups; however, the baseline activity, as expected, was lower in the PEH compared with the Sham PEH group (P < 0.05). The bicuculline-induced increase in firing rate in neurons in the Sham PEH or PEH group was not dependent on the baseline firing rate in either group (r² = 0.02; P = 0.61 for Sham PEH; r² = 0.001, P = 0.90 for PEH). In addition, in neurons with similar baseline activities recorded during PEH [5.6 ± 1.2 (SD) Hz] and Sham PEH [7.5 ± 1.2 (SD) Hz] the bicuculline-induced increase was still greater in PEH compared with Sham PEH (29.2 ± 5.7 Hz for PEH vs. 14.4 ± 2.6 Hz for Sham PEH, P = 0.04). The maximum current for ejecting bicuculline was not different in the two groups, averaging 71 ± 5 nA in the PEH group and 72 ± 7 nA in the Sham PEH group (P > 0.05).

**DISCUSSION**

This study provides new data on central neural mechanisms contributing to PEH. First, cardiovascular sympathetic neurons in the RVLM demonstrated a significant decrease in their spontaneous firing activity along with a reduced LSNA during PEH. Second, this reduced RVLM neuronal activity was mediated, at least in part, by a GABA_A-receptor mechanism. Third, baroreceptor reflex control of the RVLM sympathetic innervation of the baroreceptor afferents.

**Fig. 5.** Representative traces illustrating the effect of muscimol and bicuculline on RVLM AP discharge frequency during PEH (A) and Sham PEH (B). Resting spontaneous activity was lower in PEH compared with Sham PEH. Muscimol produced current-related decreases and bicuculline produced current-related increases in unit activity during PEH and Sham PEH. Constant bicuculline ejected with the current, which maximally increased unit activity, abolished the unit responses to the high dose of muscimol in PEH and Sham PEH SHR. Traces represent the maximum response observed to drug application during a 5-s period.

**Fig. 6.** Group data showing bicuculline-induced maximal increases in RVLM neuronal activity during PEH and Sham PEH. Maximal bicuculline-induced increase in RVLM neuronal firing activity (filled bars) over the baseline firing activity (open bars) was significantly greater in neurons recorded during PEH compared with the maximal increase in neurons recorded during Sham PEH. Resting unit activity was significantly lower in PEH compared with Sham PEH. Maximal unit activity was not significantly different in the two groups. *P < 0.05, baseline PEH vs. baseline Sham-PEH, ΔPEH vs. Sham-PEH.
cardiovascular neuronal activity and sympathetic output operated with a significantly reduced gain and range during PEH.

A reduction in sympathetic nerve activity is a salient feature of PEH (20, 29, 32, 38) and has been proposed to mediate PEH (10). One important site where the level of sympathetic nerve activity can be decreased is the RVLM central sympathetic network. During PEH, RVLM sympathetic cardiovascular neurons exhibited a significantly reduced resting activity compared with the corresponding Sham PEH period; this reduced activity was accompanied by reduced LSNA, suggesting that a decreased central sympathetic outflow from the RVLM contributes to PEH. To allow for recording from the RVLM neurons, the experiments were performed in α-chloralose- and urethane-anesthetized SHR. After an initial anesthetic-induced fall in ABP, both the magnitude and duration of PEH were the same in the anesthetized and conscious SHR.

To our knowledge, this is the first study to implicate an exaggerated contribution of GABA A signaling at functionally identified cardiovascular sympathetic RVLM neurons as a mechanism contributing to PEH. It is well established that RVLM neurons are under a tonic GABA-ergic inhibitory influence, largely (2, 15, 24, 26, 45), though not exclusively, via a baroreceptor input (40). In the present study, the maximal increase in firing activity of RVLM neurons in response to bicuculline was significantly greater during PEH compared with Sham PEH, suggesting that a tonically active GABA A-receptor mechanism contributed to the reduction in RVLM neuronal output. Had other non-GABA-ergic mechanisms contributed importantly, the maximal dose of bicuculline should have increased the neuronal activity to the same extent during PEH or Sham PEH. In addition, even in neurons with similar baseline activities recorded during PEH and Sham PEH, the bicuculline-induced increase was still greater in the PEH neurons, suggesting that a reduced baseline activity alone did not explain the exaggerated bicuculline responses in PEH.

A limitation of the iontophoretic ejection technique is that the actual amount of drug ejected is not determined; however, the ranges for iontophoretic ejection currents for bicuculline were the same in the recordings from neurons in the PEH and Sham PEH group. In addition, after neuronal activity was judged to be maximally increased, increasing the bicuculline ejection current further had no effect on the spiking activity. For these reasons, it seems unlikely that different amounts of drug were ejected in the two groups, but rather more likely that equivalent amounts of bicuculline had a greater effect at RVLM synapses during PEH compared with Sham PEH.

The critical nature of an intact baroreflex system for the development of PEH was explicitly demonstrated by DiCarlo’s group (10). Whether the absence of baroreceptor afferent input directly altered the behavior of neurons in the central baroreflex network or somehow triggered other central mechanisms is not known. Whatever the nature of the effects, the gain of baroreflex control of sympathetic nerve activity appears to be blunted both in humans and SHR (10, 29). In the current study, the baroreceptor stimulus-response curves demonstrated a marked reduction in the gain of baroreflex control of RVLM neuronal activity, which most likely accounted for the reduced gain of LSNA. The question arises as to whether the increased GABA inhibitory influence on baseline RVLM neuronal activity during PEH contributed to the attendant reduced baroreflex gain, or were the two unrelated? Whereas the baroreflex function curve represents dynamic changes, it seems reasonable to consider that a tonic enhancement of nonbaroreceptor GABA-mediated inhibition of RVLM neuronal activity during PEH could reduce the maximal activity associated with baroreceptor unloading, thereby decreasing the range of baroreflex function and hence the gain. On the other hand, for RVLM neurons with similar baseline activities in the PEH and Sham PEH groups, the G max was still significantly lower in the PEH group, suggesting that the reduced G max cannot be explained by a reduced range. Thus, on balance, the data are consistent with the idea that an enhanced GABA-ergic influence contributed to the reduced baroreflex gain.

Given that hypertension and exercise are the two key elements for the development of PEH, the relationship of the baroreflexes and sympathetic nerve activity in hypertension and during exercise may be relevant to resolving the mechanisms of PEH. There is extensive evidence that the baroreflex system, viewed as a whole, resets to higher blood pressures during hypertension (39) and exercise (16, 46, 50, 53).

The resetting in hypertension has been suggested to occur at the level of the peripheral baroreceptors (4, 12, 37, 42, 52, 57), although there are also data for central resetting (1, 12, 39). The resetting during exercise has been uniformly proposed to occur in the central nervous system, whether attributed to central command (51) or to skeletal muscle afferents (49, 50) or to both (41). It is tempting to speculate that exercise against a background of hypertension sets in motion some immediate, yet relatively long-lasting (up to 10 h) changes, including in GABA signaling, in the central nervous system that triggers the development of PEH. In that regard, Overton and colleagues (44) reported that microinjection of the GABA A receptor agonist, muscimol, in the posterior hypothalamus during exercise produced postexercise behavioral effects, including a mild sedation, that was attributed to GABA. Viewed collectively, the findings suggest that GABA signaling may be altered at various neural networks during exercise.

A number of other central nervous system neurotransmitter systems could contribute to PEH, including one other inhibitory neurotransmitter glycine (58). On the other hand, the opioid system in PEH has received considerable attention, particularly since elevated endorphins have been reported during exercise (3). The opioid antagonist, naloxone, given intravenously has been shown to attenuate PEH to varying degrees following exercise in humans (31) and SHR (56). These findings have led to the suggestion that...
central endorphins might initiate the decrease in central sympathetic outflow during PEH. Both GABA_A (15, 24, 45, 58, 59) and μ-opioid receptors are located on RVLM sympathetic cardiovascular neurons and have been colocalized in rat brain stem (34). In addition, results from physiological and neurochemical studies provide evidence for an interaction between GABA_A and μ-opioid receptor systems (6, 17, 60). These findings raise the possibility that redundant and interactive opioid and GABA_A receptor mechanisms in the RVLM may contribute to PEH.

In conclusion, the results of this study suggest that upregulation of aspects of GABA signaling at sympathetic cardiovascular RVLM neurons lead to a decreased neuronal output that may contribute to the decrease in sympathetic outflow and hence PEH. Moreover, the effect appears to last at least 10 h.

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