HIGHLIGHTED TOPIC | Neural Changes Associated with Training

Exercise training attenuates increases in lumbar sympathetic nerve activity produced by stimulation of the rostral ventrolateral medulla

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Mueller PJ. Exercise training attenuates increases in lumbar sympathetic nerve activity produced by stimulation of the rostral ventrolateral medulla. J Appl Physiol 102: 803–813, 2007. First published October 19, 2006; doi:10.1152/japplphysiol.00498.2006.—Exercise training (ExTr) has been associated with blunted activation of the sympathetic nervous system in several animal models and in some human studies. Although these data are consistent with the hypothesis that ExTr reduces the incidence of cardiovascular diseases via reduced sympathoexcitation, the mechanisms are unknown. The rostral ventrolateral medulla (RVLM) is important in control of sympathetic nervous system activity in both physiological and pathophysiological states. The purpose of the present study was to test the hypothesis that ExTr results in reduced sympathoexcitation mediated at the level of the RVLM. Male Sprague-Dawley rats were treadmill trained or remained sedentary for 8–10 wk. RVLM microinjections were performed under Inactin anesthesia while mean arterial pressure, heart rate, and lumbar sympathetic nerve activity (LSNA) were recorded. Bilateral microinjections of the GABA$_A$ antagonist bicuculline (5 mM, 90 nl) into the RVLM increased LSNA in sedentary animals (169 ± 33%), which was blunted in ExTr animals (100 ± 22%, $P < 0.05$). Activation of the RVLM with unilateral microinjections of glutamate (10 mM, 90 nl) increased LSNA in sedentary animals (76 ± 13%), which was also attenuated by training (26 ± 2%, $P < 0.05$). Bilateral microinjections of the ionotropic glutamate receptor antagonist kynurenate (40 mM, 90 nl) produced small increases in mean arterial pressure and LSNA that were similar between groups. Results suggest that ExTr may reduce increases in LSNA due to reduced activation of the RVLM. Conversely, we speculate that the relatively enhanced activation of LSNA in sedentary animals may be related to the increased incidence of cardiovascular disease associated with a sedentary lifestyle.

sympathetic nerve activity; treadmill training; microinjection

EXERCISE TRAINING (ExTr) is associated with alterations in neural control of the circulation (9, 58, 66). Studies in humans and animals have demonstrated that ExTr alters autonomic control of the heart and the vasculature (4, 13, 21, 22, 40, 43, 53, 58). Interestingly, in animal studies, there appears to be a consistent effect of increased physical activity (i.e., spontaneous wheel running or treadmill training) to reduce sympathoexcitation in response to various stimuli, including arterial baroreceptor unloading (4, 13, 21, 22, 40, 53). Although not as consistent in human studies (24, 26, 60), similar evidence for reduced baroreflex-mediated sympathoexcitation (1) and sympathoexcitation produced by other stimuli (54) exists. Taken together, these data suggest that the beneficial effects of physical activity may be due, at least in part, to reductions in sympathetic nervous system activity. Conversely, with the recent association with physical inactivity and chronic disease (10, 42), these data may also suggest that increases in sympathetic nervous system activity contribute to the increased incidence of cardiovascular diseases associated with a sedentary lifestyle (e.g., obesity, hypertension, etc.) (7, 65).

The rostral ventrolateral medulla (RVLM) is one of the primary brain regions involved in the generation of resting and reflex-mediated changes in sympathetic outflow (18, 27). The RVLM contains bulbospinal neurons that innervate sympathetic preganglionic neurons, and the activity of these RVLM neurons is critically important in the regulation of resting sympathetic outflow and arterial blood pressure (18, 27). The activity of RVLM neurons is regulated by both excitatory and inhibitory neurotransmitters (18, 27), and alterations in both excitation and inhibition of RVLM neurons have been proposed to contribute to various physiological and pathophysiological states (5, 11, 12, 31, 32, 61). However, little is known regarding the effects of ExTr on control of sympathetic outflow at the level of the RVLM. A recent report has demonstrated pressor responses to activation of the RVLM are blunted in swim-trained rats (44). Therefore, it is reasonable to suggest that alterations in the excitation or inhibition of RVLM neurons contribute to alterations in sympathoexcitatory mechanisms produced by changes in physical activity.

We hypothesized that ExTr results in reduced sympathoexcitation mediated at the level of the RVLM. To test this hypothesis, RVLM microinjections were performed in sedentary (Sed) and ExTr rats, while mean arterial pressure (MAP), heart rate (HR), and lumbar sympathetic nerve activity (LSNA) were recorded. Our results suggest that increases in LSNA in response to disinhibition of the RVLM or direct excitation of the RVLM are blunted by ExTr. These data are consistent with the hypothesis that ExTr limits sympathoexcitation at the level of the RVLM by reducing activation of neurons involved in control of sympathetic outflow. Furthermore, we hypothesize that blunted sympathoexcitation contributes in part to the reduced incidence of cardiovascular disease associated with a physically active lifestyle. Conversely, we also speculate that a sedentary lifestyle may lead to increased risk of cardiovascular diseases associated with a physically active lifestyle.
disease, in part by increased activation of the sympathetic nervous system.

**METHODS**

All surgical and experimental procedures were conducted in accordance with the American Physiological Society’s “Guiding Principles in the Care and Use of Animals” and approved by the Institutional Animal Care and Use Committee of the University of Missouri-Columbia.

**ExTr.** Male Sprague-Dawley rats (100–125 g, n = 34) were trained to run on a motor-driven treadmill for 8–10 wk using a progressive exercise protocol described previously by our laboratory (51). This endurance-type training protocol was adapted from McAllister and coworkers (45) and has been shown to produce a significant training effect, as evidenced by an increase in citrate synthase activity in soleus muscle (45, 51). Briefly, animals began the protocol with a familiarization period that consisted of short bouts of walking at 15 m/min at a 15% grade for 1 wk. The duration of these bouts was initially 5 min and then was increased by 2–3 min/day for the first week. After the first week, animals were selected based on their willingness to exercise on the treadmill. Animals that did not tolerate the protocol were excluded from further study. Animals that were willing to exercise were randomly assigned to either a Sed group (groups of six to eight) or an ExTr group (groups of six). Several groups of Sed and ExTr animals were required to attain the total number of animals used in this study (Sed, n = 17; ExTr, n = 17). Animals assigned to the exercise protocol continued running at an increasing speed (1 m·min⁻¹·day⁻¹) and duration (5 min/day) over a 3-wk period until they were running at 30 m/min (15% grade) for 60 min/day. This workload is estimated to be 85 ml·kg⁻¹·min⁻¹, according to Armstrong and colleagues (2), for rats acclimated to a treadmill. One-hour sessions continued for 5 days/wk for the remainder of the 8- to 10-wk protocol. In rare instances (~5–10%), animals in the exercise group that exhibited signs of stress (i.e., porphyrin rings, lack of grooming, etc.) or refusal to run were removed from the study. During the training period, Sed rats and trained animals were housed together to achieve similar housing conditions. In addition, Sed animals were placed in Plexiglas lanes on a nonmoving area of the treadmill during training sessions to expose animals to similar noise, vibration, and handling. Before acute experimentation, animals were allowed a minimum of 24–48 h to recover from their most recent bout of exercise to minimize alterations in cardiovascular control due to an acute bout of exercise (35, 41).

**Surgical procedures.** On the day of experimentation, animals were instrumented for microinjection studies and MAP and LSNA recordings similar to previous studies from our laboratories (50, 51). Briefly, animals were first anesthetized with isoflurane (2% in 100% O2), and arterial and venous catheters were implanted. Femoral arterial and venous catheters were used for measurement of arterial pressure and cardiac output. Arterial and venous catheters were implanted. Femoral arterial and venous catheters were used for measurement of arterial pressure and cardiac output. Femoral arterial and venous catheters were implanted. Femoral arterial and venous catheters were used for measurement of arterial pressure and cardiac output. Femoral arterial and venous catheters were implanted. Femoral arterial and venous catheters were used for measurement of arterial pressure and cardiac output.

When all surgical manipulations were completed, Inactin (0.025 ml/min, 100 mg/kg iv) was infused slowly over 20–30 min, and the isoflurane was reduced. Once the infusion was complete, supplemental doses of Inactin (5 mg iv) were given as necessary to prevent withdrawal to toe pinch, and the gas anesthetic was withdrawn completely. Artificial ventilation was continued (60–80 breaths/min) with a mixture of room air supplemented with 100% O2. Arterial blood gases were preserved by adjusting the rate or volume of the ventilator (PO2 > 100 Torr, PCO2 between 35 and 40 Torr). Body temperature was maintained within normal limits with a heating pad. Experiments were performed within a Faraday cage to decrease electrical noise.

**Microinjections.** Microinjections were performed similar to previous studies in our laboratories (50, 51). Before microinjections, the brain stem was oriented in a horizontal plane by deflecting the animal’s head ventrally so that calamus scriptorius was located 2.4 mm posterior to the interaural line (36, 48). Multibarrel glass pipettes (3 or 5 barrels, outside tip diameter 40–70 μm) containing appropriate drugs were inserted into the RVLM under visual observation with the aid of a dissecting microscope. Target stereotaxic coordinates for the RVLM were 0.9–1.1 mm rostral and 1.7–2.2 mm lateral to calamus scriptorius, and 3.5–3.7 mm ventral to the dorsal surface of the medulla. In some animals, microinjections of glutamate (30 nl, 10 mM) were performed in the nucleus tractus solitarius (NTS) to test the adequacy of GABA_A receptor blockade in the RVLM, as described previously (67). Target stereotaxic coordinates for the NTS were 0.5 mm rostral and 0.5 mm lateral to calamus scriptorius, and 0.5 mm ventral to the dorsal surface of the medulla, similar to previous studies (50, 51). Microinjections were performed using a custom-built pressure microinjection system with a 1-nl resolution. Microinjection volumes were measured directly by monitoring the fluid meniscus in the micropipette barrel with the aid of a ×150 compound microscope equipped with a fine reticule. The RVLM was functionally identified bilaterally by pressor and sympathoexcitatory responses to glutamate (10 mM, 30 nl). The NTS was functionally identified bilaterally by depressor and sympathoinhibitory responses to glutamate (10 mM, 30 nl). Glutamate dose-response curves in the RVLM were performed utilizing unilateral 30-nl injections of increasing concentrations of glutamate (1–10 mM) ejected from different barrels of the same pipette. Experiments examining overall tonic inhibitory or excitatory transmitter emissions utilized bilateral 90-nl microinjections of antagonists. Bilateral microinjections were made serially such that, following the initial injection, the pipette was withdrawn and reinserted into the brain on the contralateral side. Bilateral injections were made within ~1 min of each other.

**Protocol 1: Effect of glutamatergic excitation in RVLM.** To test whether responses to activation of the RVLM are altered by ExTr, the excitatory amino acid (EAA), glutamate, was microinjected unilaterally in the left RVLM at increasing concentrations (1–10 mM, 30 nl or 30–300 pmol) to elicit dose-dependent increases in MAP, HR, and LSNA. Different concentrations of glutamate were microinjected in a random order from separate barrels of the same pipette. A minimum of 5 min was allowed between injections.

**Protocol 2: Effect of GABAergic receptor blockade in the RVLM.** To test whether ExTr altered the influence of tonic GABA_A-mediated transmission, the GABA_A receptor antagonist bicuculline (5 mM, 90 nl or 450 pmol per side) was microinjected bilaterally into the RVLM. The dose and volume of bicuculline were based on previous studies in which bicuculline was used to inhibit tonic GABA_A receptor activation in the RVLM (29, 47, 48). GABA_A receptors in the RVLM are believed to be required for arterial baroreflex-mediated changes in sympathetic nerve activity (8, 19, 27, 63). Therefore, in a subset of animals, we tested the ability of bilateral microinjections of bicuculline in the RVLM to block sympathoexcitatory responses to decreases in MAP with sodium nitroprusside (5 μg/kg iv). In the remaining animals, we tested the ability of bilateral microinjections of bicuculline in the RVLM to block depressor and sympathoinhibitory responses to microinjections of glutamate (10 mM, 30 nl or 300 pmol) into the NTS, as demonstrated previously (67).
Protocol 3: Effect of ionotropic glutamate receptor blockade in RVLM. To test whether ExTr altered tonic ionotropic glutamate-mediated transmission, the ionotropic glutamate receptor antagonist kynurenate (40 mM, 90 nl or 3.6 nmol total per side) was microinjected bilaterally in the RVLM. This dose and volume of kynurenate were based on previous studies in which similar doses of kynurenate were used to inhibit ionotropic EAA-mediated excitation in RVLM (37, 47).

Histology. In addition to functional identification of the RVLM with glutamate injections in every animal, injection sites within the RVLM were marked with 2% pontamine sky blue dye (30 nl) in a subset of animals (Sed, n = 6; ExTr n = 7) used in these studies. Following an overdose with euthanasia solution (Beuthanasia, 0.2 ml), brains were removed and placed in 10% phosphate-buffered formalin solution containing sucrose. After at least 1 wk of fixation, the hindbrain was frozen and sectioned into 40-μm coronal slices on a cryostat. Sections were mounted on gel-coated slides, and the center of the dye spot was determined using a ×40 microscope. A rat brain atlas (56) was used to aid in the identification of anatomical landmarks. The location of the center of the dye spot was transferred to a modified graphical representation of the RVLM and surrounding structures based on the rat atlas (56).

Data collection and analysis. Experimental data were acquired using a commercially available computer data acquisition system (Power Lab, ADInstruments, Colorado Springs, CO). A Tektronix oscilloscope was used to monitor raw LSNA. LSNA was amplified with a Grass preamplifier (P511), which was filtered using a 3-kHz low-pass filter and a 30-Hz high-pass filter. LSNA was rectified and integrated using a root mean square converter with a time constant of 28 ms. The rectified, integrated LSNA was averaged electronically and was used to analyze changes in LSNA as a percentage of the control level of LSNA before each microinjection. Ganglionic blocking agents (hexamethonium, 30 mg/kg + atropine methyl nitrate, 1 mg/kg iv) were administered to determine background noise. LSNA was defined after subtracting out the background noise recorded in each animal.

Statistical analysis. Body weights and baseline hemodynamic variables were analyzed by Student’s t-test. Data comparing the change in MAP, HR, or LSNA before and after agonist or antagonists were analyzed by two-way ANOVA with repeated measures. When ANOVA indicated a significant interaction, differences between individual means were assessed by post hoc Holm-Sidak test, according to a commercially available software package (SigmaStat 3.0, SPSS, Chicago, IL). A probability of P < 0.05 was considered statistically significant, and P values <0.1 are reported and noted as trends (16). Data are expressed as means ± SE.

Drugs. Inactin, 1-glutamate, bicuculline methiodide, sodium nitroprusside, and kynurenate were obtained from Sigma Chemical (St. Louis, MO). With the exception of sodium nitroprusside and kynurenate, all drugs were dissolved directly in artificial cerebrospinal fluid (aCSF). Sodium nitroprusside was made fresh each day for intravenous injection by dissolving 0.05 mg per 1 ml of 0.9% saline. Kynurenate was first dissolved in a few drops of 1 N NaOH before being diluted in aCSF. All drugs were pH adjusted to 7.3–7.5 using sodium hydroxide or hydrochloric acid and filtered before microinjection. Drugs used for microinjection were relatively short acting, with recovery typically within 30 min or less. In cases where more than one antagonist was used in an individual animal, a full hour of recovery was given between each protocol. Drugs were given in a randomized fashion to prevent an “order” effect from occurring in any particular group.

RESULTS

Effects of ExTr. Table 1 contains data regarding body weights, absolute and relative soleus muscle weights, and baseline hemodynamic parameters in Sed and ExTr animals.

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<tr>
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<th>Sedentary Control</th>
<th>Exercise Trained</th>
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<tr>
<td>n</td>
<td>17</td>
<td>17</td>
</tr>
<tr>
<td>Body weight, g</td>
<td>416 ± 29</td>
<td>409 ± 7</td>
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<tr>
<td>Soleus muscle, mg</td>
<td>0.156 ± 0.0048</td>
<td>0.162 ± 0.0045</td>
</tr>
<tr>
<td>Soleus muscle/body weight, mg/g</td>
<td>0.375 ± 0.0109</td>
<td>0.396 ± 0.0103*</td>
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<tr>
<td>Resting MAP, mmHg</td>
<td>114 ± 1</td>
<td>109 ± 2*</td>
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<tr>
<td>Resting HR, beats/min</td>
<td>279 ± 4</td>
<td>288 ± 7</td>
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Values are means ± SE; n, no. of rats. There were trends for significant changes in relative soleus weight (*P = 0.078) and resting mean arterial pressure (MAP; †P = 0.057). HR, heart rate.

Body weights and absolute soleus weights were not different between groups. Relative soleus muscle weights had a tendency to be higher in ExTr rats (P = 0.078). Baseline MAP also demonstrated a strong trend to be significantly lower in ExTr rats (P = 0.057). However, baseline HR was not different between groups in our Inactin-anesthetized preparation.

Identification of the RVLM. To functionally identify the RVLM, we used unilateral microinjections of glutamate (30 nl, 10 mM or 300 pmol) into the right and left RVLM. Figure 1 represents individual examples of glutamate microinjections into the RVLM of one Sed and one ExTr rat. Pressor and sympathoexcitatory responses were observed in individual animals with variable effects on HR. Table 2 represents averaged control responses to glutamate in identified portions of the RVLM of all Sed (n = 17) and ExTr (n = 17) rats used in this study. As shown, glutamate produced pressor responses that were not different between groups, independent of the side of RVLM injected. HR responses were small and variable and overall were not significant in either group. In contrast, glutamate produced sympathoexcitatory responses in both groups. ExTr rats, however, exhibited blunted sympathoexcitatory responses to this single dose of glutamate.

Protocol 1: Effect of glutamatergic excitation in RVLM. To more fully characterize the reduced sensitivity to glutamate observed during functional identification of the RVLM, we performed unilateral microinjections of glutamate at increasing doses (1–10 mM in 30 nl or 30–300 pmol) into the RVLM of Sed (n = 8) and ExTr (n = 6) rat (Fig. 2). Glutamate produced dose-related pressor responses in both groups. Although the pressor response to the highest dose of glutamate (10 mM) tended to be attenuated in the trained group, this trend did not reach statistical significance (P = 0.089). Glutamate microinjections produced small and inconsistent HR responses that were not dose related and did not differ between groups.

Sympathoexcitatory responses to glutamate were dose dependent in Sed animals. However, sympathoexcitatory responses to glutamate in ExTr animals were minimal at all doses and were not dose dependent. In addition, there was a significant reduction in the sympathoexcitatory response to the highest dose of glutamate (10 mM) and a trend for the intermediate dose of glutamate (3 mM) to be attenuated (P = 0.068).

Protocol 2: Effect of GABAergic receptor blockade in the RVLM. To test whether ExTr influenced sympathoexcitatory responses to removal of GABAergic tone in the RVLM, bilateral microinjections of the GABA A antagonist bicuculline methiodide (5 mM, 90 nl or 450 pmol per side) were performed.
into the RVLM of Sed and ExTr rats (Fig. 3). Figure 3 represents data comparing responses to bicuculline over time after bilateral microinjections into the RVLM of groups of Sed (n = 15) and ExTr rats (n = 12). Bicuculline produced an increase in MAP and LSNA and a decrease in HR in both groups. Although the pressor response to bicuculline in ExTr rats appeared to be somewhat reduced, neither the effect of training (P = 0.259) nor the interaction between training and time reached significance (P = 0.08). The latter result precluded testing for differences at individual time points. In contrast, bradycardic responses to bicuculline were significantly enhanced by ExTr at 5 and 15 min by post hoc analysis. Sympathoexcitatory responses to bicuculline were significantly reduced at 5 and 15 min by post hoc analysis. Responses at 3, 4, 10, and 20 min demonstrated trends for attenuation (P = 0.071, 0.052, 0.062, and 0.058, respectively) but did not achieve statistical significance.

To functionally evaluate the effectiveness of GABA_A receptor blockade in the RVLM and verify that blockade was similar between groups, we assessed sympathoexcitatory responses to nitroprusside (5 μg/kg iv) were reduced equivalently by bicuculline in a subset of Sed and ExTr rats. Figure 4 represents responses to intravenous sodium nitroprusside before and after bilateral microinjections of bicuculline in the RVLM of Sed (n = 6) and ExTr (n = 5) rats. Under baseline conditions, acute depressor responses to nitroprusside (5 μg/kg iv) produced sympathoexcitation in both groups. Again, the pressor and sympathoexcitatory responses to NTS microinjections of glutamate were reversed to slight pressor and sympathoexcitatory responses in both groups. For Effect of vehicle microinjections in RVLM. To control for vehicle and volume-related effects of microinjections, we performed bilateral microinjections of aCSF (90 nl) in a subset of Sed (n = 6) and ExTr (n = 7) animals. Bilateral microinjections of bicuculline produced sympathoexcitation in both groups. Remaining effects of nitroprusside after bicuculline were not different between groups.

We also functionally compared the effectiveness of GABA_A receptor blockade to abolish depressor and sympathoexcitatory responses to microinjections of glutamate into the NTS. Figure 5 demonstrates the responses to glutamate microinjections into the left (Fig. 5A) or right (Fig. 5B) NTS of Sed (n = 9) and ExTr (n = 7) rats, before and after bilateral RVLM microinjections of bicuculline. Overall, there was an effect of ExTr to influence the depressor and pressor responses to glutamate before and after bicuculline, respectively. Similar to a previous study from our laboratory (51), microinjections of glutamate into the NTS produced sympathoexcitatory responses that were similar between Sed and ExTr animals. Interestingly, depressor and sympathoexcitatory responses were also similar between injected sides of the NTS within and across groups, despite the fact that we consistently recorded from the left lumbar nerve (compare Fig. 5A vs. B). Following bilateral microinjections of bicuculline into the RVLM, depressor and sympathoinhibitory responses to NTS microinjections of glutamate were reversed to slight pressor and sympathoexcitatory responses in both groups. Again, the pressor and sympathoexcitatory responses to NTS glutamate microinjections following RVLM microinjections of bicuculline were similar, whether injections were performed into the left (Fig. 5A) or right (Fig. 5B) NTS.

**Protocol 3: Effect of ionotropic glutamate receptor blockade in RVLM.** We tested whether ExTr influenced the response to removal of tonic EAA tone in the RVLM by performing bilateral microinjections of the ionotropic glutamate receptor antagonist kynurenate (40 mM, 90 nl or 3.6 nmol per side) into the RVLM of Sed and ExTr rats (n = 6 and 7, respectively) (Fig. 6). Bilateral microinjections of kynurenate produced small but significant increases in MAP and LSNA in both groups (Fig. 6). Although visually there appeared to be differences in the depressor and sympathoexcitatory responses to kynurenate, these were not significantly different between groups. Kynurenate had small and inconsistent effects on HR that were not significant and did not vary between groups.

| Table 2. Hemodynamic responses to microinjections of glutamate (30 nl, 10 mM) into the left or right RVLM in sedentary and exercise-trained rats |
|---------------------------------|-----------------|
| Sedentary Control | Exercise Trained |
| n | 17 | 17 |
| Left RVLM | | |
| ΔMAP, mmHg | 17±2 | 15±1 |
| ΔHR, beats/min | -1±4 | -2±2* |
| ΔLSNA, % | 66±2 | 48±7* |
| Right RVLM | | |
| ΔMAP, mmHg | 16±1 | 15±1 |
| ΔHR, beats/min | 3±3 | -1±3 |
| ΔLSNA, % | 68±8 | 45±4* |

Values are means ± SE; n, no. of rats. RVLM, rostral ventrolateral medulla; Δ, change; LSNA, lumbar sympathetic nerve activity. *P < 0.05, sedentary vs. exercise trained.
tions of aCSF (90 nl) produced small and variable changes in MAP, HR, or LSNA that were not significant between groups (data not shown). aCSF did produce a small depressor effect (≤5 mmHg in both groups), which was associated with a small but significant increase in LSNA (<12% in both groups). Importantly, the pressor and sympathoexcitatory responses noted with kynurenate microinjections at 15 and 20 min were significantly greater than the depressor and sympathoexcitatory responses noted with aCSF (P < 0.05).

**Verification of injection sites.** We functionally identified the RVLM with microinjections of glutamate and by testing the effectiveness of bicuculline to block sympathoinhibitory and sympathoexcitatory responses mediated by GABA_A receptors in the RVLM. In addition to these functional tests, we confirmed our microinjection sites by comparing the stereotaxic coordinates used in each group of animals. Average stereotaxic coordinates for the left and right RVLM in Sed and ExTr rats are shown in Table 3. When compared statistically, the coordinates between the two groups did not significantly differ.

We also confirmed our microinjection sites histologically by analyzing dye injections in a subset of rats used in these studies.
(Sed, n = 6; ExTr, n = 7). Figure 7 represents a composite of our injection sites. Histological analysis of these microinjection sites verified that the pipettes were located within cardiovascular regions of the RVLM that have been described in previous microinjection studies (29, 47).

DISCUSSION

The purpose of the present study was to test the hypothesis that an increase in physical activity produced by treadmill ExTr was associated with alterations in the regulation of RVLM neurons important in control of sympathetic nervous system activity. Our results suggest that sympathoexcitation produced by either direct excitation or disinhibition of the RVLM is blunted significantly by ExTr. These data suggest that ExTr limits sympathoexcitation at the level of the RVLM by reducing the excitation of neurons involved in control of sympathetic outflow. We speculate that these mechanisms contribute to the reduced incidence of cardiovascular diseases in physically active humans. Conversely, since Sed animals exhibited exaggerated sympathoexcitatory responses relative to the trained rats, we also speculate that the increased risk of cardiovascular disease associated with a sedentary lifestyle may be due, in part, to increased activation of the sympathetic nervous system.

We sought to determine whether GABAergic inhibition of the RVLM was altered by ExTr. RVLM neurons are under tonic GABAergic inhibition that is mediated by both arterial baroreceptor-dependent and arterial baroreceptor-independent mechanisms (18, 27). It is possible that greater remaining GABAergic inhibition of RVLM neurons during baroreceptor unloading contributes to blunted baroreflex-mediated sympathoexcitation observed in previous studies (13, 21, 22, 53). A similar mechanism has been proposed to explain reductions in sympathetic outflow and RVLM activity following acute bouts of exercise (35). If RVLM neurons are under enhanced GABAergic inhibition following ExTr, we would predict that pressor and sympathoexcitatory responses to blockade of①

Fig. 4. Effect of bilateral microinjections of bicuculline into the RVLM on sympathoexcitatory responses to decreases in MAP with sodium nitroprusside (5 μg/kg iv). Control responses to nitroprusside (nonhatched bars) produced similar decreases in AP and sympathoexcitation in Sed (nonshaded bars, n = 6) and ExTr (shaded bars, n = 5) rats. Following bilateral RVLM microinjections of bicuculline sympathoexcitatory responses to nitroprusside (hatched bars) were virtually abolished. #P < 0.05, effect of bicuculline.

Fig. 5. Effect of bilateral microinjections of bicuculline into the RVLM on depressor and sympathoinhibitory responses to glutamate microinjections into the left (A) or right (B) nucleus tractus solitarius (NTS) in Sed (nonshaded bars) or ExTr rats (shaded bars). Control injections of glutamate (nonhatched bars) produced depressor and sympathoinhibitory responses that were completely blocked by bilateral bicuculline microinjections into the RVLM of both groups (hatched bars). Effects were consistent across parameters and groups, whether the left or right NTS was injected. P < 0.05; #effect of bicuculline; *effect of exercise training.
GABA<sub>A</sub> receptors in the RVLM of ExTr rats would have been enhanced. However, bilateral blockade of GABA<sub>A</sub> receptors with bicuculline resulted in attenuated pressor and sympathoexcitatory responses in ExTr rats. These data suggest that GABA<sub>A</sub>ergic inhibition is not augmented by ExTr. Furthermore, a reduced level of excitation of RVLM neurons following removal of GABA<sub>A</sub>ergic tone in the RVLM may be responsible for diminished sympathoexcitatory responses. These data suggest that the reductions in sympathoexcitatory that occur with chronic ExTr are mediated by different mechanisms than acute reductions in sympathetic outflow observed during post-exercise hypotension (35).

We performed a series of functional tests that require GABA<sub>A</sub> receptors in the RVLM to verify that microinjections of bicuculline were producing a similar level of blockade of GABA<sub>A</sub> receptors in the RVLM of Sed and ExTr rats. Sympathoexcitatory responses to intravenous nitroprusside and sympathoinhibitory to glutamate microinjections in the NTS were abolished by bicuculline, suggesting strong evidence that our dose and volume of bicuculline were effective in producing functional blockade of GABA<sub>A</sub> receptors in the RVLM. Importantly, these responses were blocked to a similar degree in both Sed and ExTr rats, consistent with a similar degree of blockade of GABA<sub>A</sub> receptors by bicuculline in both groups. Along with the fact that our microinjection coordinates and dye spots were similar between groups, we believe that differences in the response to bicuculline (and other drugs used in this study) are due to physiological differences produced by ExTr rather than differences in experimental procedures.

Diminished sympathoexcitation in ExTr animals that occurs following reductions in GABA<sub>A</sub>ergic tone in the RVLM, either by baroreceptor unloading (13, 21, 22, 53) or blockade of GABA<sub>A</sub> receptors (present study), could be due to enhanced non-GABA<sub>A</sub>-mediated inhibition or diminished excitatory transmission within the RVLM. To test the latter hypothesis, we evaluated whether the RVLM was less sensitive to generalized excitation. Sympathoexcitatory responses to glutamate were significantly blunted in ExTr animals, and at the highest dose there was a trend for pressor response to glutamate to be attenuated ($P = 0.089$). These results are effectively similar to previous studies in which pressor responses to glutamate in the RVLM of swim-trained rats were attenuated (44) and suggest that RVLM neurons may be less sensitive to generalized excitation following increases in physical activity. Diminished sensitivity to glutamate could be explained by a variety of possibilities, including differences in pre- or postsynaptic signaling involving glutamate and other neurotransmitters. Included within these possibilities is the theory that glutamate drives both excitatory and inhibitory processes within the RVLM to influence arterial blood pressure regulation, as suggested by Sved and colleagues (33, 64). It is possible that, following ExTr, glutamate may drive a greater proportion of inhibitory processes in the RVLM that act to offset the direct sympathoexcitatory effects of glutamate.

To assess whether inhibitory processes that are driven by EAAs in the RVLM are tonically active in ExTr animals, we blocked ionotropic glutamate receptors in the RVLM. Similar to previous studies (33, 37), blockade of ionotropic glutamate receptors with kynurenate had only modest effects initially on sympathoexcitatory responses and variable effects on HR in both groups. Fig. 6. Group data for ΔMAP, ΔHR, and ΔLSNA responses to blockade of ionotropic excitatory amino acid transmission with bilateral microinjections of kynurenate (40 mM, 90 nl or 3.6 nmol per side) in the RVLM of Sed (•; $n = 6$) and ExTr (○; $n = 7$) rats. Kynurenate produced similar pressor and sympathoexcitatory responses and variable effects on HR in both groups.

Table 3. Average stereotaxic coordinates relative to calamus scriptorius used for left and right RVLM microinjections in sedentary and exercise-trained rats

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<th>Sedentary Control</th>
<th>Exercise Trained</th>
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<tr>
<td>$n$</td>
<td>17</td>
<td>17</td>
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<tr>
<td>Left RVLM</td>
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</tr>
<tr>
<td>Rostral, mm</td>
<td>1.09±0.03</td>
<td>1.09±0.03</td>
</tr>
<tr>
<td>Lateral, mm</td>
<td>1.77±0.04</td>
<td>1.79±0.03</td>
</tr>
<tr>
<td>Ventral, mm</td>
<td>3.48±0.05</td>
<td>3.49±0.06</td>
</tr>
<tr>
<td>Right RVLM</td>
<td></td>
<td></td>
</tr>
<tr>
<td>Rostral, mm</td>
<td>1.07±0.03</td>
<td>1.09±0.03</td>
</tr>
<tr>
<td>Lateral, mm</td>
<td>1.72±0.03</td>
<td>1.76±0.03</td>
</tr>
<tr>
<td>Ventral, mm</td>
<td>3.55±0.03</td>
<td>3.48±0.06</td>
</tr>
</tbody>
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Values are means ± SE; $n$, no. of rats.
arterial pressure and sympathetic outflow that were not different between Sed and ExTr animals. However, over time, we observed significant increases in MAP and LSNA after blockade of ionotropic glutamate receptors. These data suggest that EAAs in the RVLM tonically suppress sympathetic outflow under our experimental conditions. Previous evidence supporting a tonic net sympathoinhibitory action of EAA has been reported by Keily and Gordon (36), who observed that blockade of the N-methyl-D-aspartate subtype of ionotropic receptors in the RVLM increased arterial pressure in anesthetized rats. Therefore, although the idea that EAAs support arterial pressure has been questioned recently (29), the previous study by Kiely and Gordon and the present study provide evidence that EAAs can provide an overall sympathoinhibitory influence under certain experimental conditions.

The mechanisms by which ExTr alters neural control of the circulation are likely to be numerous and complex. Nevertheless, alterations within the central nervous system have been proposed previously and are reinforced by the present study. Based on several elegant studies, DiCarlo and colleagues (14, 21, 59) concluded that central nervous system alterations contributed to blunted baroreflex-mediated sympathoexcitation following ExTr. This conclusion was based on findings that chronic exercise had no influence on arterial baroreceptor (14) or cardiopulmonary afferent sensitivity (59), despite producing blunted baroreflex-mediated sympathoexcitation (13, 21, 22).

The present studies support and extend these conclusions and suggest that alterations at the level of the RVLM may contribute importantly to ExTr-induced reductions in sympathoexcitation during baroreceptor unloading. In the RVLM, EAAs mediate a number of sympathoexcitatory reflexes (18). Therefore, reduced responsiveness of the RVLM to glutamate suggests that ExTr may produce a generalized reduction in sympathoexcitation produced by a number of stimuli. Although controversial, this possibility is supported by some studies in humans (54) and animals (49, 51, 55), where pressor responses to other types of sympathoexcitation are blunted in trained subjects.

It is likely that alterations within other cardiovascular brain regions contribute to blunted sympathoexcitatory responses following ExTr, including those observed in the present study. For example, ExTr appears to reduce the elevated firing rate of caudal hypothalamic neurons in spontaneously hypertensive rats (3). This effect may occur via a restoration of GABA transmission within the caudal hypothalamus (38). In addition, ExTr restores diminished nitric oxide synthase in the paraventricular nucleus of spontaneously hypertensive rats (23) and rats and rabbits with heart failure (68, 69). ExTr is also associated with reductions in elevated sympathetic outflow in heart failure animals (69). These types of effects in forebrain nuclei could certainly contribute to blunted sympathoexcitation following ExTr via hypothalamic projections to the RVLM (20). However, it is important to note that the above mechanisms have been established for ExTr in diseased animal models, whereas the present study was carried out in “normal” animals. It is clear then that further studies are necessary to examine whether alterations in other brain regions impact control of sympathetic outflow at the level of the RVLM in response to ExTr in normal individuals. In addition, it is worth repeating that a unique aspect of these experiments was the fact that ExTr in otherwise healthy animals produced alterations in control of sympathetic outflow. As mentioned, if interpreted in a slightly different manner, our results provide evidence that a sedentary lifestyle may lead to increased risk of cardiovascular disease, in part by increased activation of the sympathetic nervous system.
We found it intriguing that glutamate produced similar levels of sympathoinhibition from either the left NTS or the right NTS. In addition, functional identification of the RVLM with glutamate microinjections resulted in similar sympathoexcitatory responses, whether it was the right or left RVLM being identified. These results were interesting and noteworthy, given that LSNA was only recorded from the left lumbar chain. These data may provide functional evidence of contralateral projections from the NTS and the RVLM to neurons that influence sympathetic nerve activity. Whether responses observed from the NTS were due to contralateral projections to the RVLM or other nuclei, including the NTS or caudal ventrolateral medulla, cannot be determined by this study. It is also beyond the scope of this study whether responses elicited from the RVLM are due to contralateral projections to the other RVLM or due to contralateral projections to the spinal cord or other cardiovascular brain regions.

As with any study, our conclusions need to be taken in the context of our experimental design. To record directly from efferent sympathetic nerves while making discrete and consistent microinjections into the RVLM and the NTS of an intact animal, the use of anesthesia was required. Although it is well known that anesthesia can influence cardiovascular responses, our results demonstrating blunted sympathoexcitation in anesthetized ExTr animals are consistent with findings in conscious ExTr animals, including rats (13, 44, 53). Furthermore, blunted activation of the RVLM in response to an acute bout of exercise in conscious rats has been observed previously (30). Finally, preliminary data from our laboratory suggest that ExTr reduces the number of activated neurons in the RVLM of conscious rats during baroreceptor unloading (52). Thus, although anesthesia may have modified the magnitude of our responses, we believe it does not explain the differences observed between Sed and ExTr animals. Reduced activation of RVLM neurons following ExTr observed in previous studies (30, 52) suggests that reduced sympathoexcitation is due, at least in part, to changes at the level of the RVLM. However, it is also possible that alterations at the intermediolateral cell column or sympathetic ganglia could be responsible for the responses observed.

Although differences in arterial pressure and lumbar sympathetic activity were qualitatively similar in magnitude and direction between groups, blunted lumbar sympathetic nerve responses were not always associated with blunted pressor responses in ExTr animals. Arterial pressure is a complex variable that is a product of a number of factors, several of which are likely to be altered by ExTr. In particular, cardiac adaptations to ExTr include augmented contractile responses to sympathomimetic agents, such as β-adrenergic agonists (28, 62). Therefore, a greater cardiac output response during activation of the sympathetic nervous system may have offset reduced peripheral vasoconstrictor responses. It is also possible that ExTr has a specific effect on lumbar sympathetic outflow vs. other sympathetic outflows. We believe this to be unlikely, however, since ExTr has also been shown to reduce measures of resting (39, 46, 53) and baroreflex-mediated (21, 22, 55) increases in renal sympathetic outflow in animal (39, 53) and human studies (46). Nonetheless, it is clear that further studies are warranted to determine how blood pressure regulation is altered by ExTr and whether cardiac adaptations or responsive-ness of other sympathetic nerves contribute importantly to blood pressure responses observed in the present study.

We speculate that the reductions in efferent sympathetic outflow observed in this study and others are of functional importance due to the direct influence of the sympathetic nervous system on vasoconstriction, peripheral vascular resistance, and arterial pressure. In addition, the sympathetic nervous system also affects vascular smooth muscle via trophic effects (17) and arrhythmogenesis at the level of the heart (6, 15). Previous studies have demonstrated increased sympathetic nervous system activity in the absence of an associated blood pressure increase and suggest that it may be a mechanism for cardiovascular disease, despite adequate blood pressure control (25). Therefore, reductions in efferent sympathetic outflow produced by ExTr may have much broader implications in prevention of cardiovascular disease beyond its well-known blood pressure lowering effects (6, 34, 57).

In summary, the present study has demonstrated that sympathoexcitatory responses due to disinhibition of the RVLM or direct excitation of the RVLM are blunted by ExTr. These data suggest that ExTr may limit sympathoexcitation at the level of the RVLM by reducing activation of neurons involved in control of sympathetic outflow. It is possible that blunted sympathoexcitation contributes to the reduced incidence of cardiovascular disease associated with a physically active lifestyle. Finally, we speculate that a sedentary lifestyle leads to increased risk of cardiovascular disease, in part, by activation of the sympathetic nervous system.

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