Putative role of the NTS in alterations in neural control of the circulation following exercise training in rats

Patrick J. Mueller1,2 and Eileen M. Hasser,1,2,3

1Dalton Cardiovascular Research Center and 2Departments of Biomedical Sciences and
3Medical Pharmacology and Physiology, University of Missouri-Columbia, Columbia, Missouri

Submitted 28 June 2005; accepted in final form 17 September 2005

Mueller, Patrick J., and Eileen M. Hasser. Putative role of the NTS in alterations in neural control of the circulation following exercise training in rats. Am J Physiol Regul Integr Comp Physiol 290: R383–R392, 2006.—Exercise training (ExTr) has been associated with alterations in neural control of the circulation, including effects on arterial baroreflex function. The nucleus tractus solitarius (NTS) is the primary termination site of cardiovascular afferents and critical in the regulation of baroreflex-mediated changes in heart rate (HR) and sympathetic nervous system outflow. The purpose of the present study was to determine whether ExTr is associated with alterations in neurotransmitter regulation of neurons involved in control of cardiovascular function at the level of the NTS. We hypothesized that ExTr would increase glutamatergic and reduce GABAergic transmission in the NTS and that, collectively, these changes would result in a greater overall sympathoinhibitory drive from the NTS in ExTr animals. To test these hypotheses, male Sprague-Dawley rats were treadmill trained or maintained under sedentary conditions for 8–10 wk. NTS microinjections were performed in Inactin-anesthetized animals instrumented to record mean arterial pressure (MAP), HR, and lumbar sympathetic nerve activity (LSNA). Generalized activation of the NTS with unilateral microinjections of glutamate (1–10 mM, 30 nl) produced dose-dependent decreases in MAP, HR, and LSNA that were unaffected by ExTr. Bilateral inhibition of NTS with the GABA_A agonist muscimol (1 mM, 90 nl) produced increases in MAP and LSNA that were blunted by ExTr. In contrast, pressor and sympathoexcitatory responses to bilateral microinjections of the ionotropic glutamate receptor antagonist, kynurenic acid (40 mM, 90 nl), were similar between groups. Bradycardic responses to bilateral microinjections of the GABA_A antagonist bicuculline (0.1 mM, 90 nl) were attenuated by ExTr. These data indicate that alterations in neurotransmission at the level of the NTS contribute importantly to regulation of HR and LSNA in ExTr animals. In addition to alterations at NTS, these experiments suggest indirectly that changes in other cardiovascular nuclei contribute to the observed alterations in neural control of the circulation following ExTr.

Microinjection; sympathetic nerve activity; nucleus tractus solitarius; treadmill

It is well established that exercise training (ExTr) produces changes in neural control of the circulation (4, 36, 51). For example, studies in both humans and animals have reported that ExTr alters autonomic control of the heart and the vasculature (3, 7, 10, 11, 22, 29, 33, 36). In particular, arterial baroreflex-mediated sympathoexcitation appears to be blunted by ExTr in animal models (3, 7, 10, 11, 22, 33) and in some (1), but not all (14, 15, 41), human studies.

The nucleus tractus solitarius (NTS) is the primary termination site for cardiovascular afferents (9) and is an important brain region involved in both resting and reflex control of arterial pressure and sympathetic nervous system activity (2, 37, 43). Inhibition of neurons in the NTS blocks several cardiovascular reflexes and produces increases in arterial pressure and sympathetic outflow, indicating a tonic sympathoinhibitory influence of the NTS (13, 38, 44, 49). Similar to other brain regions, glutamate appears to be the primary excitatory neurotransmitter and GABA appears to be the primary inhibitory neurotransmitter in the NTS (2, 26, 33, 37). Reductions in glutamatergic and enhancement of GABAergic neurotransmission in the NTS would tend to increase sympathetic nervous system activity and have been implicated in various forms of experimental hypertension (32, 40, 46).

It is possible that ExTr alters excitatory and inhibitory processes in the NTS to affect regulation of sympathetic outflow. It has been reported that animal models of ExTr exhibit reduced resting (24) and blunted baroreflex-mediated sympathoexcitation (3, 7, 10, 11, 22, 33). We hypothesized that ExTr results in an increase in the sympathoinhibitory influence of the NTS. This increase in the sympathoinhibitory influence of the NTS could occur via increases in excitatory or decreases in inhibitory regulation of NTS neurons involved in sympathoinhibition, or both mechanisms could occur concomitantly. To test these possibilities, we performed NTS microinjections in sedentary (Sed) and ExTr rats while recording mean arterial pressure (MAP), heart rate (HR), and lumbar sympathetic nerve activity (LSNA). Our results suggest that sympathoinhibitory responses to exogenous excitation of the NTS are not altered by ExTr. In contrast, sympathoexcitatory responses to direct inhibition of the NTS were blunted by ExTr. ExTr also appears to reduce tonic GABA_A-mediated inhibition of NTS neurons involved in control of HR, whereas tonic ionotropic glutamate transmission in the NTS appears to be unaltered. Finally, it is possible that changes in other brain regions also contribute to the observed alterations in neural control of the circulation after ExTr.

METHODS

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

Training protocol. Male Sprague-Dawley rats (100–125 g) were exercise trained on a treadmill for 8–10 wk according to a progressive exercise protocol adapted from Laughlin and colleagues (31). Briefly, animals were acclimatized to a custom-built treadmill by walking at 15 m/min at a 15% grade for 1 wk. The duration of walking (initially

http://www.ajpregu.org
0363-6119/06 $8.00 Copyright © 2006 the American Physiological Society
R383

The costs of publication of this article were defrayed in part by the payment of page charges. The article must therefore be hereby marked “advertisement” in accordance with 18 U.S.C. Section 1734 solely to indicate this fact.
increasing speed (1 m/min).

ExTr group continued with the exercise protocol and ran 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 speed and this duration of running were continued for 5 days/wk throughout the remainder of the 8- to 10-wk protocol. Although rare (~5–10%), ExTr animals that exhibited overt signs of stress (i.e., porphyrin rings, lack of grooming, etc.) or refusal to run during the training program were removed from the study. During the training period, Seds were pair-housed with ExTr animals. To expose animals to similar noise, vibration, and handling, Seds were placed in Plexiglas lanes on a nonmoving area of the treadmill during training sessions. Before they were studied acutely, animals were given 24–48 h to recover from the last bout of exercise to minimize the effects of an acute bout of exercise on cardiovascular control (20, 25).

On the day of experimentation, animals were anesthetized initially with halothane or isoflurane (2% in 100% O₂). Femoral arterial and venous catheters were implanted for measurement of arterial pressure and administration of drugs, respectively. To record LSNA, a midline laparotomy was performed, and a portion of the lumbar chain was located caudal to the left renal vein. The lumbar sympathetic chain was isolated and placed on the electrodes, which were composed of two Teflon-insulated silver wires (Medwire, 0.005 in. diameter, 36 gauge) passed through Silastic tubing (0.025 in. ID). The electrode-nerve complex was covered with polyvinylsiloxane gel (Coltene President), which was allowed to solidify before closure. After closure of the midline incision, a tracheostomy was performed. The rats were then placed in a Kopf stereotaxic apparatus and ventilated mechanically with a continuous mixture of halothane or isoflurane and oxygen (2% in 100% O₂). The dorsal surface of the medulla was exposed by a midline incision. After overlying muscle was dissected, the occipital bone was partially removed. Finally, an incision was made through the atlanto-occipital membrane to expose the brain stem.

At the completion of all surgical manipulations, Inactin (0.025 ml/min, 100 mg/kg iv) was infused slowly over 20–30 min, and the gas anesthetic was withdrawn. After the infusion, supplemental doses of Inactin (5 mg iv) were given as necessary to prevent withdrawal to toe pinch. Animals continued to be ventilated (60–80 breaths/min) with a mixture of room air supplemented with 100% O₂. Arterial blood gases were maintained (PO₂ > 100 Torr, PCO₂ between 35 and 40 Torr) by adjusting the rate or volume of the ventilator. Body temperature was monitored with a rectal probe and maintained within normal limits with a heating pad. A wire was attached to the skin to serve as a ground for the LSNA electrode, and experiments were performed within a Faraday cage to decrease electrical noise.

**Microinjections.** Multibarrel glass pipettes (3 or 5 barrel, outside tip diameter of 30–80 μm) were placed into the NTS with the aid of a dissecting microscope. Target stereotoxic 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. Microinjections were performed with a custom-built pressure microinjection system. The volume of drug delivered was determined by monitoring the movement of the meniscus in each barrel of known diameter using a 150× microscope with a calibrated reticle. The NTS was identified functionally by depressor and sympathoinhibitory responses to unilateral microinjection of glutamate (30 nl, 10 mM). Subsequent unilateral injections were made in volumes of 30 nl. Bilateral microinjections were made in volumes of 90 nl and were made serially (i.e., first on one side of the medulla and then the other). Bilateral injections were given within 1 min of each other.

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

**Protocol 2. Effect of bilateral inhibition of NTS.** To assess the overall tonic influence of the NTS on cardiovascular function in ExTr rats, the NTS was inhibited by bilateral microinjections of the GABA_A agonist muscimol (1 mM, 90 nl or 90 pmol per side). This dose and volume were used based on previous studies examining inhibition of the NTS with muscimol (6, 38). In addition, to verify the extent to which this dose and volume of muscimol inhibited the NTS, sympathetic arterial baroreflex responses to phenylephrine (5 μg/kg iv) were tested before and after bilateral muscimol in a subset of animals.

**Protocol 3. Effect of ionotropic glutamate receptor blockade of NTS.** To test whether ExTr altered the influence of 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 into the NTS. This dose and volume of kynurenate were based on previous studies in which kynurenate was used to inhibit ionotropic EAA-mediated excitation in NTS (27, 28, 48). In addition, to verify the extent to which this dose and volume of kynurenate blocked EAA-mediated transmission in the NTS, sympathetic arterial baroreflex responses to phenylephrine (5 μg/kg iv) were tested before and after bilateral kynurenate in a subset of animals.

**Protocol 4. Effect of GABAergic receptor blockade of NTS.** To test whether ExTr altered the influence of tonic GABA_ε-mediated transmission, the GABA_ε receptor antagonist bicuculline (0.1 mM, 90 nl or 9 pmol per side) was microinjected bilaterally into the NTS. This dose and volume of bicuculline were based on previous studies in which bicuculline was used to inhibit tonic GABA_ε receptor activation in the NTS (30, 47).

**Histology.** In addition to functional identification of NTS injection sites with glutamate in every animal, we marked injection sites in the NTS with 2% pontamine sky blue dye (30 nl) in a subset of animals (n = 7 each group) used in these studies. After the rats were overdosed with anesthesia (Beuthanasia, 0.2 ml), the brains were removed and placed in 10% phosphate-buffered formalin containing sucrose. After a minimum of 1 wk, the medulla was blocked, frozen, and then sectioned into 40-μm coronal slices. Slices were then mounted on microscope slides, and the center of the dye spot was determined using a 40× microscope. Using a rat brain atlas (34), we identified anatomical landmarks and denoted the center of the dye spot on a graphical representation of the NTS and surrounding structures.

**Assessment of training effect.** To determine whether a training effect occurred in ExTr animals, citrate synthase activity was assessed in the soleus muscle from the noncatheterized leg of animals from both groups. After euthanasia, muscles were removed and stored at −70°C. Citrate synthase activity was measured from whole muscle soleus homogenate using a spectrophotometric method (42). As published previously for the exercise protocol used in this study (31), a significantly higher citrate synthase activity in the ExTr group was considered to be indicative of a training effect.

**Data collection and analysis.** All experimental data were acquired using a computer data acquisition system (PowerLab, ADInstruments, Colorado Springs, CO). A Grass preamplifier (P511) was used to amplify the LSNA signal, which was then filtered using a high-pass frequency level of 30 Hz and a low-pass frequency level of 3 kHz. Raw LSNA was monitored with a Tektronix oscilloscope and on the data acquisition system. 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. Background noise was determined after administering ganglionic blocking agents.
LSNA was defined as the amount of recorded nerve activity after subtracting out the background noise recorded. LSNA responses were analyzed as a percentage of the control level of LSNA before each microinjection.

Although muscimol and kynurenate have both been shown to inhibit cardiovascular reflexes through the NTS (5, 6, 16, 27, 28, 38, 48), we determined the extent to which these agents blocked sympathoinhibitory responses to intravenous injections of phenylephrine (5/262). Because the change in arterial pressure to phenylephrine was not always identical before and after bilateral microinjections, we used the ratio of the change in LSNA to the change in MAP as an estimate of baroreflex-mediated sympathoinhibition. The percent inhibition of the baroreflex by muscimol or kynurenate was then calculated by dividing the ratio before, or by the ratio after, the bilateral microinjections.

**Statistical analysis.** Body weights, soleus muscle citrate synthase activity, heart weight to body weight ratios, 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 and LSNA/MAP ratios elicited by phenylephrine before and after muscimol or kynurenate were analyzed by two-way ANOVA with repeated measures. When ANOVA indicated a significant interaction, differences between individual means were assessed by post hoc Tukey’s test. All statistical analyses were performed using a commercially available software package (SigmaStat, SPSS, Chicago, IL). A probability of $P < 0.05$ was considered statistically significant. Data are expressed as means ± SE.

**Drugs.** Inactin, l-glutamic acid, kynurenate, bicuculline methiodide, and muscimol were obtained from Sigma (St. Louis, MO). With the exception of kynurenate, all drugs were dissolved directly in artificial cerebrospinal fluid (aCSF). 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.

**RESULTS**

**Baseline effects of ExTr.** Table 1 contains data regarding body and organ weights, soleus muscle citrate synthase activity, and baseline hemodynamic parameters in Sed and ExTr animals. As expected, body weights were significantly lower and soleus muscle citrate synthase activity was higher in ExTr rats. In addition, the total heart-to-body weight ratio was higher in ExTr animals. In our Inactin-anesthetized preparations, neither baseline MAP nor HR values were different between groups.

**Protocol 1. Effect of glutamatergic excitation of NTS.** Figure 1 demonstrates the effects of unilateral glutamate microinjec-
tion (10 mM, 30 nl, or 300 pmol) into the NTS of one Sed and one ExTr rat while recording MAP, HR, and LSNA. Glutamate produced similar depressor, bradycardic, and sympathoinhibitory responses in both animals. Figure 2 represents average data from groups of Sed (n = 10) and ExTr (n = 7) animals in which glutamate dose-response relationships were determined. Glutamate produced dose-dependent reductions in MAP, HR, and LSNA in both groups. There was no main effect of training and no interactions between dose and group, suggesting that ExTr had no significant effect on the response to glutamatergic excitation of NTS neurons.

Protocol 2. Effect of bilateral inhibition of NTS. To assess the overall tonic influence of the NTS on cardiovascular function in ExTr rats, bilateral microinjections of the GABA<sub>A</sub> agonist muscimol were performed to inhibit neuronal activity in the NTS of Sed and ExTr rats. Figure 3 demonstrates the effects of bilateral microinjections of muscimol into the NTS of one Sed and one ExTr rat while recording MAP, HR, and LSNA. Bilateral microinjections of muscimol (1 mM, 90 nl, or 90 pmol per side) produced increases in MAP and LSNA with little or no change in HR in both animals. However, the increase in MAP and LSNA appeared to be reduced in the ExTr vs. the Sed animal.

Consistent with these results, microinjection of muscimol in groups of Sed (n = 8) and ExTr (n = 9) animals produced increases in MAP and LSNA (Fig. 4). However, by 3 and 5 min after the injection of muscimol, MAP and LSNA responses, respectively, were significantly blunted in ExTr rats. These differences persisted for 15 and 10 min, respectively, for MAP and LSNA responses and suggest that ExTr significantly reduced the response to inhibition of NTS neurons with the GABA<sub>A</sub> agonist. Muscimol had small and inconsistent effects on HR that were not significant and did not vary between groups (Fig. 4).

We determined the extent to which muscimol inhibited the NTS in both groups by examining sympathoinhibitory responses to phenylephrine before and after muscimol in a subset of animals (n = 6 each group). Table 2 contains data regarding the ratios of LSNA to MAP produced by phenylephrine and the percent inhibition of this ratio produced by bilateral microinjections of muscimol. There was a significant overall effect of muscimol to reduce the ratio of LSNA to MAP but no overall effect of training. In addition, the percent inhibition of this ratio was not significantly different between groups (Table 2). These data suggest that ExTr neither modified baroreflex-mediated sympathoinhibition nor the ability of muscimol to inhibit baroreflex-mediated sympathoinhibition.

To control for vehicle or volume effects of our microinjections, we also performed bilateral microinjections of aCSF (90 nl) in a subset of Sed and ExTr animals. Bilateral microinjections of aCSF (90 nl) produced small and inconsistent changes in MAP, HR, or LSNA in Sed (n = 4) or ExTr (n = 7) animals (Fig. 5). These data suggest that responses to our microinjections are not simply due to a vehicle or volume effect on the excitatory state of neurons in the NTS.

Protocol 3. Effect of EAA receptor blockade of NTS. We tested whether ExTr influenced tonic effects of EAA receptor activation in the NTS by performing bilateral microinjections of the ionotropic glutamate receptor antagonist kynurenate (40 mM, 90 nl, or 3.6 nmol per side) into the NTS of Sed and ExTr rats (n = 11 and 9, respectively). Bilateral microinjections of kynurenate produced significant increases in MAP and LSNA in both groups consistent with removal of EAA tone in the NTS (Fig. 6). In contrast to the effects of muscimol, the increases in MAP and LSNA with kynurenate were similar between groups. Kynurenate had small and inconsistent effects on HR that were not significant and did not vary between groups (Fig. 6). These data suggest that interruption of EAA-mediated transmission results in similar levels of sympathoexcitation in Sed and ExTr animals.

We determined the extent to which kynurenate functionally blocked ionotropic EAA-mediated transmission to the NTS by examining sympathoinhibitory responses to phenylephrine before and after kynurenate in a subset of animals (n = 8 each group). Table 2 contains data regarding the ratios of LSNA to MAP produced by phenylephrine and the percent inhibition of this ratio produced by bilateral microinjections of kynurenate. There was a significant overall effect of kynurenate to reduce

Fig. 2. Group data for peak changes in mean arterial pressure (MAP), HR, and LSNA to increasing doses of glutamate (1–10 mM, 30 nl, or 3–300 pmol total) in the NTS of Sed (n = 10) and ExTr (n = 8) rats. Glutamate produced dose-dependent decreases in all variables (P < 0.05) that were not affected by ExTr.
the ratio of LSNA to MAP without a significant effect of ExTr.
In addition, the percent inhibition of this ratio was not signif-
icantly different between groups (Table 2). These data suggest
that ExTr neither modified baroreflex-mediated sympathoinhi-
bition nor the ability of kynurenate to inhibit baroreflex-
mediated sympathoinhibition between groups. In addition, the
percent inhibition of the LSNA-to-MAP ratio was similar with
muscimol and kynurenate between and across groups (Ta-
ble 2).

Protocol 4. Effect of GABA\(\alpha\)ergic receptor blockade of NTS.
To test whether ExTr influenced the tonic effects of GABA\(\alpha\)
receptor activation, we performed bilateral microinjections of
the GABA\(\alpha\) antagonist, bicuculline methiodide (0.1 mM, 90 nl,
or 9 pmol per side) into the NTS of Sed and ExTr rats (\(n = 8\)
and 7, respectively). Bicuculline produced significant de-
creases in MAP, HR, and LSNA in both Sed and ExTr animals
(Fig. 7). There was a significant overall effect of training to
blunt the decreases in HR produced by bicuculline. MAP
responses to bicuculline were not significantly different (\(P >\)
0.1), and LSNA responses to bicuculline were nearly identical.
These data suggest that ExTr reduces tonic GABA\(\alpha\)ergic
control of HR but has no significant effect on GABA\(\alpha\)ergic
control of MAP or LSNA.

Histology. In addition to functionally identifying the NTS
with microinjections of glutamate (30 nl, 10 mM) in every
animal, we injected dye in the NTS of a subset of rats to

![Fig. 3. Raw figure of the effects of bilateral muscimol micro-
injections (Musc; 90 nl, 1 mM, or 90 pmol/side) into the NTS
of one Sed (A) and one ExTr (B) rat. MAP, HR, and LSNA
were recorded. Muscimol produced increases in MAP and
LSNA in both animals with little or no change in HR in either
animal. Increases in MAP and LSNA were blunted in the ExTr
animal.](http://ajpregu.physiology.org/Downloadedfrom)
anatomically verify our injection sites. Histological analysis of the microinjection sites marked with pontamine sky blue (n = 7 for each group) verified that the pipettes were within the NTS (Fig. 8). Pipette tips were located within the intermediate NTS, lateral to the area postrema ~500 μm rostral to calamus scriptorius (Fig. 8).

**DISCUSSION**

The purpose of the present study was to determine whether ExTr is associated with alterations in neurotransmitter regulation of NTS neurons involved in control of cardiovascular function. The results of our experiments suggest that ExTr does not alter overall ionotropic EAA neurotransmission at the NTS since sympathoinhibitory responses to glutamate were similar in both groups and the effects of bilateral NTS blockade of ionotropic EAAs also were similar. In contrast, ExTr appears to reduce tonic GABA<sub>A</sub>ergic inhibition of NTS neurons involved in control of HR. Finally, ExTr attenuates sympathoexcitation produced by generalized inhibition of the NTS. Together, these data suggest that ExTr induces alterations in cardiovascular regulation at the level of the NTS. As outlined below, our data also suggest indirectly that other brain regions contribute to alterations in neural control of the circulation following ExTr.

Several reports have demonstrated that baroreceptor unloading produces blunted sympathoexcitation in ExTr rats and rabbits (7, 10, 11, 33). In contrast, baroreceptor-mediated sympathoinhibition appears unaltered (7, 10, 11, 33). Because baroreceptor afferents terminate at the level of the NTS (9), it is reasonable to hypothesize that the NTS may be involved in blunted sympathoexcitation produced by ExTr. Baroreceptor afferents utilize glutamate as their primary neurotransmitter (27, 28, 48) and alterations in glutamatergic transmission could contribute to changes in baroreceptor function following ExTr. In the present study, sympathoinhibitory responses to glutamate were normal, suggesting that the response to general excitation of the NTS was unaltered by ExTr. These data are consistent with baroreflex-mediated sympathoinhibition being normal after ExTr (7, 10, 11, 33).

Because baroreflex-mediated sympathoexcitation is blunted by ExTr, one might initially expect that blockade of EAA receptors (i.e., withdrawal of glutamatergic excitation) in the NTS might also produce blunted sympathoexcitation in ExTr rats. However, in the present study, blockade of EAA receptors in the NTS produced similar pressor and sympathoexcitatory responses in Sed and ExTr animals. The most straightforward explanation of these data is that overall tonic ionotropic glutamatergic excitation of the NTS is unchanged by ExTr. This could also be interpreted to mean that arterial baroreceptor signaling is unchanged at the level of the NTS. However, there

<table>
<thead>
<tr>
<th>Table 2. Effect of bilateral nucleus tractus solitarius microinjections of muscimol or kynurenate on phenylephrine-induced changes in MAP and LSNA in sedentary and exercise-trained animals</th>
</tr>
</thead>
<tbody>
<tr>
<td><strong>Bilateral muscimol (n = 6 each group)</strong></td>
</tr>
<tr>
<td>Control conditions</td>
</tr>
<tr>
<td>Sedentary control</td>
</tr>
<tr>
<td></td>
</tr>
<tr>
<td>Exercise trained</td>
</tr>
<tr>
<td></td>
</tr>
<tr>
<td><strong>Bilateral kynurenate (n = 8 each group)</strong></td>
</tr>
<tr>
<td>Control conditions</td>
</tr>
<tr>
<td>Sedentary control</td>
</tr>
<tr>
<td></td>
</tr>
<tr>
<td>Exercise trained</td>
</tr>
<tr>
<td></td>
</tr>
</tbody>
</table>

Values are means ± SE. LSNA, lumbar sympathetic nerve activity. *P < 0.05 main effect of injection.
are other possible explanations for the lack of difference in Sed and ExTr rats. First, on the basis of studies from DiCarlo and colleagues (8, 11, 39), an increased tonic influence from cardiopulmonary receptor input may contribute to blunted sympathoexcitation produced by withdrawal of arterial baroreceptor-mediated excitation of NTS neurons. Similar to arterial baroreceptor afferents, cardiopulmonary receptor afferents also utilize glutamate as their primary neurotransmitter at the level of the NTS (16). Thus similar responses to kynurenate in the present study could be due to combined blockade of arterial baroreceptor input and increased cardiopulmonary receptor influence in ExTr animals (11). A second possibility is that ExTr equally blunts both tonic GABAergic inhibition and glutamatergic excitation of NTS neurons. Such a concomitant reduction in both inhibitory and excitatory mechanisms could offset potential differences in sympathoexcitation produced by kynurenate or bicuculline in ExTr animals. Additional studies are necessary to determine whether overall tonic EAA-mediated excitation is perfectly balanced by offsetting mechanisms (e.g., cardiopulmonary receptor influence or GABA transmission) or is simply unchanged by ExTr.

Unlike responses to kynurenate, pressor and sympathoexcitatory responses to generalized inhibition of the NTS with muscimol were blunted in ExTr animals. At a dose used in previous studies (6, 38), we expected that muscimol would inhibit the majority of neuronal activity in the NTS in both groups. In support of this, muscimol suppressed baroreflex-mediated sympathoinhibition similarly in Sed and ExTr animals and was similar in this effect compared with kynurenate. In Sed animals, muscimol and kynurenate appeared to produce similar pressor and sympathoexcitatory responses. In contrast, in ExTr rats, the pressor and sympathoexcitatory responses to muscimol were reduced compared with kynurenate. Collectively, these findings support the hypothesis that exercise training alters the balance of excitatory and inhibitory transmission in the NTS.
tively, these differential sympathoexcitatory responses suggest that ExTr produces additional alterations in NTS neurotransmission that are inhibited by muscimol and independent of EAA receptors.

A reduced response to inhibition of the NTS could indicate that resting activity of the NTS is reduced by ExTr. Mechanistically, a reduction in NTS activity would require a reduction in excitatory neurotransmission, an enhancement of inhibitory neurotransmission, or a reduction in the excitability of the neurons themselves. We did not observe any evidence consistent with reduced glutamatergic excitation or enhanced GABAergic inhibition. Thus, if ExTr reduces resting outflow of the NTS, it must be because of other receptor systems. In this regard, catecholaminergic or peptidergic systems in the NTS have been reported to be altered by ExTr (50). However, the effect of these alterations on resting NTS output is unknown.

Another important point is that, if ExTr reduced the sympathoinhibitory influence of the NTS, we might have expected higher resting MAP and HR in ExTr animals. We did not observe differences in resting MAP and HR in our anesthetized preparations. In addition, ExTr has been associated with no change or a decrease in resting MAP (19, 23, 35). Thus, if ExTr reduces the sympathoinhibitory influence of the NTS, it is likely that it also produces other central alterations that influence regulation of arterial pressure and sympathetic outflow. In fact, an alternative explanation to reduced sympathoexcitation produced by muscimol is that output from the NTS is normal and that alterations in other brain regions are actually responsible for blunted sympathoexcitation. In either case, given what is known about the mechanisms regulating NTS neuronal activity and the physiological effects of ExTr on resting arterial pressure, it seems reasonable to hypothesize that ExTr produces alterations in neurotransmission in a number of brain regions, including the NTS, that collectively result in a reduced ability to raise sympathetic outflow. Certainly, changes in neurotransmitter systems in other cardiovascular brain regions such as the hypothalamus have been reported in ExTr animals (12, 21, 52) and may contribute to responses observed in these studies.

GABA\textsubscript{A} receptor blockade with bicuculline produced depressor, bradycardic, and sympathoinhibitory responses in both

Fig. 7. Group data for changes in MAP, HR, and LSNA to blockade of GABA\textsubscript{A} receptors in the NTS with bilateral microinjections of bicuculline methiodide (0.1 mM, 90 nl, or 9 pmol/side) in Sed (n = 8) and ExTr (n = 9) rats. Bicuculline produced depressor, bradycardic and sympathoinhibitory responses that were significant in both groups. HR responses to bicuculline were significantly reduced in ExTr rats. *P < 0.05, main effect of bicuculline. #P < 0.05, main effect of ExTr.

Fig. 8. Histological analysis of microinjection sites marked with pontamine sky blue (2%, 30 nl) in the NTS of a subset of Sed rats (○, n = 7) and ExTr rats (●, n = 7; 5 bilateral and 2 unilateral). Pipette tips were located within the intermediate NTS, lateral to the area postrema ~500 μm rostral to calamus scriptorius. AP, area postrema; CC, central canal; DMV, dorsal motor nucleus of the vagus; TS, tractus solitarius; XII, hypoglossal nucleus.
groups. HR but not MAP or LSNA responses were blunted by ExTr, suggesting that ExTr may selectively blunt GABA_A-mediated inhibition of NTS neurons involved in control of HR. Although MAP responses were similar, it is possible that we would have observed an effect of ExTr on sympathetic nervous system activity if we had recorded from different sympathetic outflows (i.e., renal or splanchnic). Alternatively, ExTr may selectively alter GABAergic control of sympathetic or parasympathetic nervous system activity to the heart vs. sympathetic activity to the vasculature.

Given the reduced response to bicuculline and muscimol, it is possible that ExTr reduces the sensitivity of NTS neurons to GABA_A-mediated inhibition. A reduced sensitivity to GABA could be mediated by GABA_A receptor downregulation or desensitization. If this were the case, we would predict that muscimol would have had less of an effect on baroreflex function in ExTr rats. In addition, ExTr rats would be expected to have lower resting MAP and HR due to decreased inhibition of NTS neurons. Although these are indirect pieces of evidence, we did not observe either of these effects in the present study. Clearly, more in-depth studies are required, including dose-response curves to GABA compounds, to determine whether GABA receptors are functioning normally in the NTS of ExTr rats.

Limitations. Our study has certain limitations, the first of which is that these studies were performed in anesthetized animals. Although we observed significant changes in arterial pressure and sympathetic outflow, these responses, as well as control of resting variables in these animals, are likely to be influenced by anesthesia. Second, we normalized our sympathetic nerve recordings to 100% of the control level in each animal because technical limitations prevent us from directly comparing sympathetic activity across groups. By normalizing our sympathetic recordings to 100%, we may have overestimated the absolute level of change in nerve activity in one group if differences in absolute resting sympathetic nerve activity existed. On the basis of the literature, we would have predicted a similar or lower baseline level of sympathetic nerve activity in the ExTr animals (11, 33). Therefore, blunted sympathoexcitatory responses may have been even more evident in ExTr animals compared with Sed animals. Our conclusions are supported by the fact that MAP responses followed similar patterns compared with LSNA. Thus, we do not believe that our method of assessing sympathetic outflow or potential differences in resting sympathetic nerve activity affected our conclusions significantly.

Perspectives

There are interesting similarities and differences between the effects of ExTr observed in this study and those reported for other physiological and pathophysiological conditions. In particular, responses to manipulation of neurotransmission in nuclei involved in cardiovascular control, including the NTS, have been reported to be altered by dietary sodium (17, 18, 30) and in certain models of hypertension (32, 40, 45). Similar to ExTr, a low-salt diet has been shown to have no impact on responses to glutamate microinjected into the NTS (18) but diminishes responses to both muscimol and bicuculline (17, 30). In contrast, a high-salt diet enhances the increase in arterial pressure to muscimol and the decrease in arterial pressure to bicuculline (30). Therefore, the effect of ExTr or a low-salt diet appears to produce similar adaptations in brain stem control of sympathetic outflow and arterial blood pressure, and a high-salt diet appears to have the opposite effects. It will be of interest to determine in future studies whether similar mechanisms are involved and contribute to the blood pressure-lowering effects of ExTr in normotensive and hypertensive individuals (19, 35).

ACKNOWLEDGMENTS

The authors thank Amy Stuck, Michele Watson, and Tracey Buschmann for assistance with the histological preparations. We also thank members of the Neurohumoral Control of the Circulation group at the University of Missouri-Columbia for helpful comments on the manuscript. Finally, we thank Dr. Lori Thombs, Director of the Social Sciences Statistics Center, for statistical consultation on this work.

GRANTS

This research was supported by a Beginning Grant-in-Aid from the Heartland Affiliate of the American Heart Association (P. J. Mueller) and by National Heart, Lung, and Blood Institute Grant HL-55306 (E. M. Hasser and P. J. Mueller). This investigation was conducted in a facility constructed with support from Research Facilities Improvement Program Grant C06 RR-16498 from the National Center for Research Resources.

REFERENCES


