Physical exercise decreases neuronal activity in the posterior hypothalamic area of spontaneously hypertensive rats

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Physical exercise decreases neuronal activity in the posterior hypothalamic area of spontaneously hypertensive rats. J Appl Physiol 98: 572–578, 2005. First published October 8, 2004; doi:10.1152/japplphysiol.00184.2004.—Recently, physical exercise has been shown to significantly alter neurochemistry and neuronal function and to increase neurogenesis in discrete brain regions. Although we have documented that physical exercise leads to molecular changes in the posterior hypothalamic area (PHA), the impact on neuronal activity is unknown. The purpose of the present study was to determine whether neuronal activity in the PHA is altered by physical exercise. Spontaneously hypertensive rats (SHR) were allowed free access to running wheels for a period of 10 wk (exercised group) or no wheel access at all (nonexercised group). Single-unit extracellular recordings were made in anesthetized in vivo whole animal preparations or in vitro brain slice preparations. The spontaneous firing rates of PHA neurons in exercised SHR in vivo were significantly lower (8.5 ± 1.6 Hz, n = 31 neurons) compared with that of nonexercised SHR in vivo (13.7 ± 1.8 Hz, n = 38 neurons; P < 0.05). In addition, PHA neurons that possessed a cardiac-related rhythm in exercised SHR fired significantly lower (6.0 ± 1.8 Hz, n = 11 neurons) compared with nonexercised SHR (12.1 ± 2.4 Hz, n = 18 neurons; P < 0.05). Similarly, the spontaneous in vitro firing rates of PHA neurons from exercised SHR were significantly lower (3.5 ± 0.3 Hz, n = 67 neurons) compared with those of nonexercised SHR (5.6 ± 0.5 Hz, n = 58 neurons; P < 0.001). Both the in vivo and in vitro findings support the hypothesis that physical exercise can lower spontaneous activity of neurons in a cardiovascular regulatory region of the brain. Thus physical exercise may alter central neural control of cardiovascular function by inducing lasting changes in neuronal activity.

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period, and before experimentation, the wheels were locked for 24 h before experimentation to avoid immediate postexercise effects on blood pressure (29). Final resting weights and blood pressures were then taken, at which point animals were prepped for either in vivo or in vitro electrophysiological experiments. In a subset of animals, hearts were harvested, rinsed clean of blood with saline, and weighed. Heart weight-to-body weight ratio (g/kg) was calculated for a peripheral training indicator (11). All rats were kept on a 12-h light-dark cycle and given free access to standard rat chow and water for the duration of the study.

Resting systolic blood pressure measurements. Resting systolic blood pressures were measured for exercised and nonexercised groups via tail-cuff plethysmography (ITTC Life Science) at the beginning and after the 10-wk exercise period. Animals were placed in a small transparent tube located in a heated enclosure (27–30°C) and allowed to acclimate to the chamber for ~10 min before resting systolic blood pressure was recorded. Animals were visually monitored during the measurement period to ensure that motion artifact was not introduced into the readings. This indirect method has been previously shown to provide reliable and valid systolic blood pressure values (10). A recent study has evaluated the tail cuff method vs. intraarterial pressure and found that tail cuff produces a higher systolic pressure vs. intraarterial; however, the within-observer differences for the tail cuff method were very low (25). Three clear and repeatable measurements were used to obtain average resting systolic blood pressure values.

In vivo extracellular recordings. After the 10-wk exercise period, the animals were anesthetized by intraperitoneal injection of a single unit. A rate monitor (Frederick Haer) was used to quantify the firing rate of a neuron, and a window discriminator (Frederick Haer) was used to isolate single units. A window discriminator and rate monitor (Frederick Haer) were used to quantify the firing rate of single neurons. The spontaneous neuronal activity was recorded for a minimum of 5 min. Recording sites were documented on pictures of Paxinos and Watson (40) with a camera reflex to noxious paw pinch.

A common carotid artery was cannulated (PE-50) to measure mean arterial pressure with a pressure transducer (Gould), and heart rate was determined from the pulsatile arterial pressure signal with a biotachometer (Gould). A tracheotomy was performed, and the animal was allowed to spontaneously breathe room air supplemented with 100% O2 throughout the experiment. Body temperature was monitored with a rectal temperature probe and maintained between 36.5 and 38.0°C with a heating pad and radiant heat lamp.

Animals were fixed in a horizontal skull position with a stereotaxic apparatus (Kopf), such that the cranial landmarks, bregma, and lambda were within 300 μm of each other in the horizontal plane. A parietal craniotomy was performed bilaterally to allow stereotaxic placement of a microelectrode into the PHA according to the coordinates of Paxinos and Watson (40).

Animals were allowed to stabilize after the surgery for ~30 min. Sharpened tungsten recording electrodes (3–5 MΩ, Frederick Haer) were vertically positioned into the PHA and advanced with a motorized micromanipulator (Frederick Haer) until the action potentials of an individual, spontaneously active neuron were isolated. Single units were isolated in the same manner that were done in previous studies (5, 6, 13, 45). Raw activity was amplified (100,000 ×, Grass, P511), filtered (band pass 300–3,000 Hz), and visualized on an oscilloscope. A window discriminator (Frederick Haer) was used to isolate single neuronal activity. The waveform of the neuron was monitored on an oscilloscope to ensure a constant waveform indicative of a single unit. A rate monitor (Frederick Haer) was used to quantify the firing rate of single neurons. Spontaneous neuronal activity and cardiovascular variables were recorded for a minimum of 5 min. The neuronal responses to increased mean arterial blood pressure were then tested and recorded. Elevations in mean arterial blood pressure were elicited by intravenous injection of 3–5 μg of the vasoconstrictor phenylephrine (Sigma). Up to six tracts (three on each side) were made bilaterally in the PHA of each rat separated by no less than 500 μm. At the last recording site in the brain, an electrolytic lesion was created by passing direct current (300 μA, 10 s; Grass D.C. Constant Current Lesion Maker LMSA) through the electrode to mark the recording site.

At the end of the experiment, animals were administered a lethal intravenous injection of pentobarbital sodium (325 mg/ml). Brains were removed and placed in 10% formalin solution for 7 days. After fixation, the brains were dehydrated in 20% sucrose and the area of the brain containing the hypothalamus was blocked and sliced (50-μm thickness) in coronal sections on a freezing microtome (American Optical Instruments). Alternate sections were stained with cresyl violet. Recording sites were reconstructed from electrolytic lesions, and locations were verified by comparison to a rat brain stereotaxic atlas (40).

In vitro extracellular recordings. After the 10-wk exercise period, the rats were rapidly decapitated and their brains were carefully removed. Brains were bathed with chilled artificial cerebral spinal fluid (pH = 7.3–7.4, 295–305 mosmol/kgH2O) containing Earle’s balanced salts solution (Sigma; 116 mM NaCl, 5.4 mM KCl, 1.8 mM CaCl2·2H2O, 0.81 mM MgSO4, 0.88 mM NaH2PO4, 5.5 mM glucose, 4.45 mM), sodium bicarbonate (26 mM) and HEPES (15 mM). Brains were cut into ~500 μm coronal slices on a tissue chopper. Slices containing PHA were transferred to an inclined plane interface-style recording chamber, warmed (36–37°C), humidified, and perfused with oxygenated (95% O2-5% CO2) artificial cerebrospinal fluid. Also, a humidified gas mixture (95% O2-5% CO2) was blown continuously over the surface of the slices.

The tissue was allowed to acclimatize for at least 1 h after placement in the recording chamber before data collection. Recording pipettes were constructed by pulling capillary glass (World Precision Instruments, 1.0-mm inner diameter) with a two-stage pipette puller and filled with 4 M NaCl (resistance 3–5 MΩ). The recording electrodes were coupled via a high-impedance probe (Grass, HIP5) to a differential amplifier (Grass, P511). A micromanipulator and hydraulic microdrive (Narishige, MO-8) were used to place the recording electrode into the PHA with the aid of a dissecting scope (Nikon) by using the wall of the third ventricle, the mammillothalamic tracts, and fornix as neuroanatomical landmarks. The electrode was advanced from the surface of the slice to a maximal depth of 250 μm, until the action potentials of a spontaneously active neuron could be isolated. Raw activity was amplified (100,000 ×, Grass, P511), filtered (band pass 300–3,000 Hz), and visualized on an oscilloscope. Neuronal activity was also sent to an audio monitor and storage oscilloscope to help isolate single units. A window discriminator and rate monitor (Frederick Haer) were used to quantify the firing rate of single neurons. The spontaneous neuronal activity was recorded for a minimum of 5 min. Recording sites were documented on pictures adapted from Paxinos and Watson (40) by observing the placement of the electrode tip in the brain slice through a microscope (Nikon).

Data analysis. Data were recorded on a chart recorder (Gould), computer acquisition equipment (ADI Power Lab Chart 3.4.2 with a 200-Hz sampling rate), and videotape (Vetter Instruments) for storage and offline analyses. Systolic blood pressure and body weight values of exercised and nonexercised groups were calculated and statistically analyzed with a two-way ANOVA (one-factor replication). Mean neuronal firing rates were determined from the average firing rate throughout the 5-min collection time (both in vivo and in vitro experiments). Differences between mean neuronal firing rates, heart rate, and arterial blood pressure responses to phenylephrine in exercised vs. nonexercised groups were tested with Student’s t-tests. Computer averaging (Run Technologies DataPac III 1.61) coupled with double-blind analysis were used in the in vivo preparation to determine whether neurons displayed cardiovascular rhythms (45). Data are presented as means ± SE. In all cases, statistical significance was reached at P < 0.05.

RESULTS

A total of 41 SHR were used in this study. Twenty-one SHR were allowed access to running wheels for 10 wk, and 20...
served as nonexercised controls. Of the 21 exercised SHR, 8 were used in the in vivo experiments and the remaining 13 exercised SHR were used in the in vitro experiment. Of the 20 nonexercised SHR, 8 were used in the in vivo experiments and the remaining 12 were used for in vitro experiments.

Before rats were allowed access to running wheels, there was no difference in body weights between the exercise (96 ± 4 g, n = 21) and nonexercise groups (94 ± 5 g, n = 20; Table 1). After the 10-wk wheel access, body weights still did not differ between groups (321 ± 6 g, n = 21 for exercised and 332 ± 5 g, n = 20 for nonexercised; Table 1). The exercised rats gradually increased running distances for ~5–6 wk, at which time they reached peak levels (~7,000–9,000 m/day; Fig. 1). Rats did show a peripheral cardiovascular response to wheel running. In a subsection of the groups, the effects of physical exercise on heart weights were determined. Total heart weights from exercised rats had a significantly lower final resting systolic blood pressure (141 ± 20; Table 1). After the 10-wk exercise period, the exercised group reached peak levels (~110 mmHg, Table 1). Blood pressure did not differ between the exercise group (141 ± 20 for nonexercised and 137 ± 3 mmHg, P < 0.02; Table 1). Both groups demonstrated significant elevations of arterial blood pressure from 4–5 wk of age until final measurements at 14–15 wk, which is typical for this model of hypertension (36, 37, 52).

Before wheel access was provided to the rats, resting systolic blood pressure did not differ between the exercise group (141 ± 3 mmHg, n = 21) and nonexercise group (137 ± 3 mmHg, n = 20; Table 1). After the 10-wk exercise period, the exercised group had a significantly lower final resting systolic blood pressure (197 ± 5 mmHg, n = 21) compared with the nonexercised group (209 ± 4 mmHg, n = 20, P < 0.02; Table 1). Both groups demonstrated significant elevations of arterial blood pressure from 4–5 wk of age until final measurements at 14–15 wk, which is typical for this model of hypertension (36, 37, 52).

In vivo extracellular recordings were obtained from a total of 31 PHA neurons in exercised SHR (n = 8 animals) and 38 neurons in nonexercised SHR (n = 8 animals). The mean spontaneous firing rate of PHA neurons in exercised SHR was 8.5 ± 1.6 Hz, which was significantly lower than that of neurons in nonexercised SHR (13.7 ± 1.8 Hz, P < 0.05; Fig. 2A). As a control, the spontaneous firing rates of 16 thalamic neurons in exercised SHR and 10 thalamic neurons in nonexercised SHR were recorded along with 8 dorsal medial hypothalamic neurons in exercised SHR and 9 dorsal medial neurons in nonexercised SHR. The mean spontaneous firing rate of the thalamic neurons showed no significant difference between the two groups (12.4 ± 2.6 Hz for the exercise group vs. 6.8 ± 2.3 Hz for the nonexercise group). In addition, the mean spontaneous firing rate of the dorsal medial hypothalamic neurons also showed no significant difference between the two groups (12.2 ± 2.9 Hz for the exercise group vs. 9.5 ± 2.9 Hz for the nonexercise group).

Neurons were then tested for responsiveness to increases in mean arterial blood pressure. In nonexercised SHR, injection of phenylephrine produced a significant rise in mean arterial blood pressure (185 ± 5 vs. 223 ± 3 mmHg, P < 0.001). A change in spontaneous firing rate did not accompany the increase in mean arterial blood pressure (13.9 ± 2.3 vs. 15.0 ± 4 g/kg, P < 0.05).

Table 1. Differences between exercised and nonexercised rats

<table>
<thead>
<tr>
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<th>Before Exercise</th>
<th>After Exercise</th>
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<tr>
<td></td>
<td>Exercised SHR</td>
<td>Nonexercised SHR</td>
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<tr>
<td>Body weight, g</td>
<td>96±4</td>
<td>94±5</td>
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<td>Heart weight-to-body</td>
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<td>weight ratio, g/kg</td>
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<tr>
<td>Systolic blood pressure, mmHg</td>
<td>141±3</td>
<td>137±3</td>
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Values are means ± SE. Significant difference between exercised and nonexercised spontaneously hypertensive rats (SHR) after exercise. *P < 0.02 and †P < 0.005.
2.5 Hz, \( P > 0.05 \)). Similar results were obtained in the exercised SHR. The mean arterial blood pressure was significantly increased by phenylephrine injections (179 ± 6 vs. 223 ± 5 mmHg, \( P < 0.001 \)), whereas spontaneous firing rates did not change (9.1 ± 2.0 vs. 9.3 ± 2.2 Hz, \( P > 0.05 \)).

Computer averaging demonstrated that 35% (11/31) of PHA neurons recorded in exercised SHR displayed a discharge rhythm temporally related to the cardiac cycle, whereas 47% (18/38) of PHA neurons recorded in nonexercised SHR displayed a similar characteristic. The mean firing rate of the cardiac cycle-related neurons was significantly lower in exercised SHR (6.0 ± 1.8 Hz) in contrast to similar neurons in nonexercised SHR (12.1 ± 2.4 Hz, \( P < 0.05 \); Fig. 2C). Histological reconstruction of recording sites demonstrated a similar distribution between exercised and nonexercised groups (Fig. 3B).

In vitro extracellular recordings were obtained from a total of 67 PHA neurons from exercised SHR \((n = 13)\) and 58 PHA neurons from nonexercised SHR \((n = 12)\). The mean spontaneous firing rate of neurons in exercised SHR was 3.5 ± 0.3 Hz, which was significantly lower than that of PHA neurons in nonexercised SHR (5.6 ± 0.5 Hz, \( P < 0.001 \); Fig. 4). The recording sites, again, showed similar distributions within the PHA between exercised and nonexercised SHR (Fig. 5).

**DISCUSSION**

The key finding of these experiments is that physical exercise can decrease neuronal activity in a brain region involved

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**Fig. 3.** A: photomicrograph of representative brain slice illustrating recording electrode placement. B: histological location of recording sites in exercised (■) and nonexercised SHR (●) for the in vivo preparation. Numbers to the right of the diencephalic diagrams are relative to bregma. 3V, third ventricle; mt, mammillothalamic tracts; f, fornix; DM, dorsomedial hypothalamus; PMV, ventral premammillary nuclei; VMH, ventromedial hypothalamic nucleus; Arc, arcuate hypothalamic nucleus; LH, lateral hypothalamus; STh, subthalamic nucleus; PHA, posterior hypothalamic area. Hypothalamic figures were adapted from Paxinos and Watson (40). C: example of an action potential from the storage oscilloscope taken from a nonexercised SHR. The location of this cell is marked with a ★ in B.
in cardiovascular regulation. Using both in vivo and in vitro extracellular single unit recordings, we have demonstrated that physical exercise decreases spontaneous activity of PHA neurons in SHR. The decreases in activity were parallel with a significant decrease in resting systolic blood pressure. The PHA projects to both brain stem presympathetic and spinal preganglionic neurons (3). We believe the reduced PHA activity results in decreased sympathetic tone via these efferent connections and thus decreasing resting systolic blood pressure. Importantly, this decrease in neuronal activity occurs in a model of spontaneous hypertension and is associated with a reduced resting systolic blood pressure. Specifically, our findings demonstrate that physical exercise can reduce the discharge rate of neurons located in the PHA of the SHR, and this change is present in both in vivo and in vitro preparations.

The reduced PHA neuronal discharge in response to physical exercise was observed in vivo, demonstrating that alterations are present in a fully intact organism. The decreases in neuronal activity were specific to the PHA, because control neurons located outside the PHA did not show similar decreases in activity in physically exercised animals. Interestingly, PHA neurons that showed a discharge rate temporally related to the cardiac cycle also showed significant decreases in discharge rate. The latter findings do not provide causative evidence of a relationship between the PHA discharge rate and resting systolic blood pressure but do show that a specific population of neurons within the PHA that are linked to the cardiovascular system are altered with physical exercise.

Similar findings of physical exercise-induced alterations in neuronal activity were observed in an in vitro brain slice preparation. The persistence of the exercise-induced decrease in PHA activity suggests that the alteration observed in the whole animal may be due to local diencephalic changes. At this point, we cannot conclude that alterations in activity are intrinsic properties of PHA neurons themselves. Rather, our findings suggest that at least some alterations occur in relatively isolated sections of the PHA. Further electrophysiological experiments are required to more fully understand alterations in synaptic function and/or membrane properties associated with the decreased activity.

To our knowledge these findings are the first to demonstrate alterations in neuronal activity in a cardiovascular-related brain region with physical exercise. Alterations in neuronal functioning after physical exercise are just now starting to be elucidated. Some laboratories have begun to demonstrate alterations in neurotransmitter and/or neurotransmitter metabolite levels, neurotransmitter binding, and upregulation or downregulation of genes involved in several neurotransmitter systems after physical exercise (2, 12, 15, 17, 20, 21, 30, 34). Other studies have demonstrated angiogenesis in the cerebellum and motor cortex of normotensive rats after running wheel activity and suggest that it occurs in parallel with increased neural activity (8, 46). Van Praag and colleagues (49) have found that physical exercise results in a significant increase in the number of newly born neurons in the dentate gyrus of adult normotensive rats. In addition, they have also demonstrated similar neurogenesis and an increase in long-term potentiation after physical exercise in the dentate gyrus of mice (48). Another study has documented electrophysiological alterations in basic motor neuron biophysical properties after physical exercise (7). Our findings are similar to the above-mentioned studies, suggesting that physical exercise is capable of eliciting alterations in brain function.
Similar to previous research in SHR, physical exercise reduced resting systolic blood pressure in the exercised group compared with the nonexercised group (11, 22, 26, 39). In the present study, SHR were given wheel access at 4–6 wk of age, a time when resting systolic blood pressure is not significantly elevated above normotensive control animals (36, 37, 52). During the period of wheel access (from 5–15 wk of age), rats still developed a significant rise in resting systolic blood pressure. Because wheel access was introduced before animals were hypertensive, the present experimental paradigm is investigating an interaction between wheel exposure and the development of hypertension in this animal. Although wheel running had a statistically significant reduction on the development of hypertension, rats still showed a high level of resting systolic blood pressure. It would be interesting to test other models of hypertension to see whether exercise such as wheel running can impact resting systolic blood pressure to a greater extent. It would also be beneficial to provide wheel access to hypertensive rats at a developmental state at which hypertension has already been developed, to test the impact of physical exercise apart from critical windows of hypertension development. Lastly, the duration of the positive effects of physical exercise on resting systolic blood pressure remains unexplored. Therefore, detraining studies may provide important information on the mechanisms of adaptation as well as provide information on how to sustain the positive effects.

We have shown that physical exercise can significantly reduce PHA neuronal activity in SHR. The PHA is a heterogeneous brain nucleus (1). However, we believe that the neurons we have recorded from are the glutamatergic projection neurons of the PHA. We came to this conclusion on the basis of the firing patterns of the neurons and the fact that the majority of PHA neurons are glutamatergic with a smaller population of GABA-positive neurons (1). We have previously shown a marked increase in glutamic acid decarboxylase gene transcription in the PHA after physical exercise (32). Our working hypothesis is that physical exercise increases GABAergic input on to PHA projection neurons, thus lowering their activity. This change in PHA activity reduces sympathetic input onto brain stem presympathetic neurons and thus spinal preganglionic neurons, resulting in reduced sympathetic tone and resting blood pressure. The underlying cellular mechanisms of the reduced firing rates remain unknown, although possibilities range from a direct alteration in intrinsic neuronal properties to changes in afferent synaptic activity. Future studies will be required to more conclusively determine the cellular mechanisms altered by physical exercise. Important issues to address include 1) determining the cellular mechanisms by which physical exercise can induce alterations in central neural regulation of cardiovascular function and 2) elucidating systemic mechanisms that induce changes in neurons involved with autonomic regulation. Doing so will enable the use of physical exercise and stimuli evoked during activity as a model for determining strategies by which to improve cardiovascular health.

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