Chronic exercise alters caudal hypothalamic regulation of the cardiovascular system in hypertensive rats

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Received 3 May 2000; accepted in final form 19 September 2000

Kramer, Jeffery M., Joseph A. Beatty, Hugh R. Little, Edward D. Plowe, and Tony G. Waldrop. Chronic exercise alters caudal hypothalamic regulation of the cardiovascular system in hypertensive rats. Am J Physiol Regulatory Integrative Comp Physiol 280: R389–R397, 2001.—Previous studies have documented a deficit in the GABA neurotransmitter system within the caudal hypothalamus (CH) of spontaneously hypertensive rats (SHR). The reduction in inhibitory influence on this cardiovascular excitatory brain region is associated with an increased neuronal activity and resting blood pressure. The purpose of this study was to determine if chronic treadmill and wheel-running activities alter the ability of the CH to regulate cardiovascular function. SHR were exercised on a treadmill (5 times/wk) at moderate intensity or allowed free access to running wheels (7 days/wk) for a period of 10 wk. Resting blood pressures were obtained before and after the exercise training periods. After the exercise period, rats were anesthetized and microinjection experiments were performed. Treadmill-trained SHR exhibited a significantly blunted developmental rise in resting blood pressure after 10 wk of exercise. A similar yet less marked effect was observed in wheel-run rats. Microinjection of the GABA synthesis inhibitor 3-mercaptopropionic acid (3-MP) into the CH of nonexercised SHR did not produce any change in arterial pressure. In contrast, microinjection of 3-MP into the CH produced significant increases in blood pressure and heart rate in exercised SHR. These results demonstrate that exercise training can alter CH cardiovascular regulation in hypertensive rats and therefore may play a role in increasing cardiovascular health.

hypertension; plasticity; blood pressure

CHRONIC HIGH BLOOD PRESSURE, or hypertension, is one of the leading risk factors contributing to pathologic disease states. Uncontrolled hypertension results in an increased risk for heart and kidney disease, stroke, and other end organ disorders. Most clinical cases of human hypertension (>90%) fall under the category of idiopathic or essential hypertension. Currently, the leading animal model for human essential hypertension is the spontaneously hypertensive rat (SHR) developed by Okamoto (24). This rat strain develops a dramatic increase in resting blood pressure over the course of life without any external manipulation such as salt loading, neurologic intervention (e.g., baroreceptor denervation), or kidney manipulation.

Studies of hypertension etiology in SHR have suggested that the central nervous system is critically involved in the abnormal rise in arterial pressure (25, 51). Decerebration below the level of the hypothalamus significantly lowered arterial pressure in SHR, while similar decerebration had little effect on resting arterial pressure in the normotensive Wistar-Kyoto (WKY) control strain (51). More recently, several investigators have implicated more caudal regions of the hypothalamus in the etiology of hypertension in SHR (32, 50).

The caudal hypothalamus (CH) is a powerful pressor region of the brain bordered by the fornix, mammillothalamic tracts, and the third ventricle in the caudal-most extent of the hypothalamus (29, 44). Anatomically, this region of the brain sends efferent output to brain stem nuclei involved in sympathetic nervous activity and cardiovascular regulation (29, 44). Injection of a γ-aminobutyric-acid A (GABA<sub>A</sub>) receptor antagonist such as bicuculline or picrotoxin also evokes large increases in blood pressure, heart rate, sympathetic nerve activity, and respiratory activity in cats and rats (8, 46). These findings demonstrate that GABA plays a key role in tonically inhibiting sympathetic and cardiovascular excitatory outflow, and thus resting blood pressure, from this brain region.

Injection of bicuculline into the CH of SHR elicits a reduced blood pressure response compared with WKY, suggesting that there is less withdrawal of GABA inhibition through blocking postsynaptic GABA<sub>A</sub> receptors (50). Furthermore, central injection of GABA or a GABA<sub>A</sub> receptor agonist such as muscimol lowers blood pressure to a greater extent in the SHR vs. WKY (30, 50). These findings are consistent with the idea that a functional deficit in the GABAergic neurotransmitter system in the CH of SHR is linked to the elevated levels of resting arterial pressure. Further studies have identified biochemical and molecular deficits in the GABA neurotransmitter system within the CH of...
SHR (3, 18) that likely contribute to elevated neuronal discharges and resting blood pressure (33).

Current methods of treating hypertension in humans involve various antihypertensive medications and changes in lifestyle, including dietary alterations and increasing physical activity (exercise programs). Several studies (39, 40) have demonstrated the ability of chronic exercise to lower resting arterial blood pressure in hypertensive humans and animals. Investigations focusing on hypertensive rats have shown that 6–10 wk of treadmill exercise is able to lower resting blood pressure (41–43). Although studies have been published that examined neurochemical changes in the brain after exercise training in hypertensive animals (17), there have been no direct studies demonstrating a functionally relevant change in the central nervous system that impacts cardiovascular regulation.

We chose to examine the involvement of the CH in mediating cardiovascular adaptations to chronic exercise for three reasons: 1) elevated blood pressure in the SHR has been linked to a GABAergic deficiency in the CH, 2) GABA has a dramatic impact on cardiovascular regulation in the CH, and 3) the CH is likely involved in cardiovascular regulation during exercise. Therefore, our basic hypothesis was that chronic exercise performed by hypertensive rats would be able to change brain function in a way to help lower resting blood pressure. Specifically, we tested if chronic exercise could restore GABAergic regulation of cardiovascular function in the CH of SHR.

The results from this study indicate that exercise training can upregulate GABAergic cardiovascular regulation in the CH of SHR and that this plastic change represents a novel mechanism by which exercise may increase cardiovascular health.

METHODS

Animals. All procedures outlined in this study conform to the National Institutes of Health's Guide for the Care and Use of Laboratory Animals. The University of Illinois Animal Care Committee approved all procedures.

Male SHR (Harlan) were obtained at 4–5 wk of age. Rats were allowed to recover from travel for 2–3 days before being assigned into experimental groups. For treadmill training, rats were randomly assigned into exercise (n = 8) and nonexercise (n = 8) groups and housed according to training group with four animals per cage. Wheel-running animals were also randomly assigned into exercise (n = 14) and nonexercise (n = 13) groups. Animals that were placed into the exercise group were housed individually in cages with running wheels attached and were allowed free access to the wheels 24 h/day, 7 days/wk. Animals were housed independently to quantify distances run. Rats placed in the nonexercise (wheel control) group were also housed independently in cages identical to the running group but without running wheels attached. All rats were kept on a 12:12-h light-dark cycle and were allowed water and food ad libitum for the duration of the study.

Resting blood pressure and heart rate measurement. Resting systolic blood pressures (SBP) were obtained before the beginning and at the end of the 10 wk of exercise training. SBP was obtained indirectly by the tail photoplethysmographic technique (IITC). Briefly, rats were placed in plastic chambers and heated to 30°C to help vasodilate the tail artery. Rats were allowed to acclimate to the recording chamber for a period of 5–15 min before blood pressure was recorded. At this time, animals were resting comfortably in the chambers. Pressure was applied to the tail to occlude blood flow and was slowly released until the photoplethysmographic unit detected a flow signal. Three to five clear measurements were obtained and averaged to obtain resting SBP. We were careful to examine the animal during the waveform collection to ensure that movement artifact was not affecting our readings. Heart rate data were only obtained for the wheel groups.

Exercise training protocols. Treadmill training consisted of placing the rats in random order on a four-lane motor-driven treadmill (Columbus Instruments). Training was conducted 5 days/wk during the dark phase of the light-dark cycle with 2 days rest on the weekend for a period of 10 wk. The speed and grade of the treadmill were gradually increased to a final setting of 25 m/min at 5° grade. This level of work represents a moderate intensity for a rat (31, 43) and has been shown to result in decreases in resting arterial blood pressure. This intensity of training was picked because previous studies utilizing higher workloads observed an exacerbation of the hypertensive state (41). In some cases, the rats were gently tapped to encourage running. Electric shock grids were not utilized to avoid noxious or painful stimuli during the exercise period. Rats had to complete at least 80% of the training period to be included in the group data.

Rats in the wheel running groups were allowed free access to running wheels during the course of the 10-wk period. Rotation counters were attached to the wheels to quantitate the distance each animal ran per day. The rats became accustomed immediately to the cages and actively utilized the wheels. Consistent with reports by other authors, animals primarily exercised during the dark phase of the daily cycle (34). Therefore, both treadmill and wheel-running exercise were conducted during the dark phase of the light cycle that represents the normal activity period for laboratory rats. Utilizing this exercise paradigm allowed us to avoid circadian-mediated differences between groups.

Acute microinjection experiments. After the chronic exercise phase of the experiments, animals were weighed and anesthetized with an intraperitoneal injection of an α-chloralose (65 mg/kg) and urethane (800 mg/kg) mixture. An external jugular vein was cannulated for additional injection of anesthetic. Supplemental anesthetic was given on evidence of foot withdrawal to pinch. A carotid artery was also cannulated for measurement of pulsatile blood pressure (Statham) and heart rate (Gould Biotachometer). A tracheotomy was performed to allow the animal to spontaneously breathe room air supplemented with 100% oxygen. Small stainless steel wires (A-M Systems) were placed into the diaphragm through a small needle puncture and connected to a high-impedance probe (Grass HIP5). Signals from the high-impedance probe were amplified (50–100 K), filtered (300–3000 Hz), full-wave rectified, and integrated (Gould Integrator; 0.1-s bins) to provide a quantifiable measure of diaphragmatic electromyographic (DEM) activity. DEM activity is correlated to tidal volume and thus represents a neuromuscular correlate of this respiratory variable (6). Respiratory frequency was derived from the integrated DEM signal (Gould Biotachometer) and multiplied with DEM to provide an index of minute ventilation defined as minute diaphragmatic activity (mDEM). Body temperature was measured with a rectal probe (YSI) and maintained between 36.5 and 38.0°C with a heating pad and heat lamp when necessary. All measurements were recorded to a digital data acquisition system (Pentium PC; PowerLab 800 for PC running Chart for Windows v3.4.4, ADInstruments) or chart...
After surgical preparation, rats were placed in a stereotoxic head holder (Kopf) and the cranium was fixed in a flat-skull orientation by placing bregma and lambda in the same horizontal plane. A parietal craniotomy was performed to allow insertion of the tip of a micropipette into the CH. Glass micropipettes (WPI) were constructed on an upright, one-stage pipette-puller (Narishige), and the tips were manually broken back to an opening diameter of 20–40 μm. Pipettes were back-filled, inserted into a sealed holder, and attached to a pneumatic pico-pump (PV800, WPI) for micro-injections. Micropipettes were subsequently placed into the CH with a micromanipulator (Kopf) from intra-aural coordinates determined from a rat brain atlas (28). A microscope fitted with a calibrated reticle was used to determine the amount of solution injected from the pipette into the brain.

**Experimental protocol.** Pipettes were filled with the GABA synthesis inhibitor 3-mercaptopropionic acid (3-MP) that is obtained as a neat liquid (Sigma). Prior studies (11, 14) have shown that 3-MP is able to reduce GABA levels and release synapses determined from the brain. Control injections of pH-matched saline (pH = 2.3) were performed to observe any effects nonspecific to the GABA synthesis-inhibition action of the drug. Injection sites were marked with Chicago blue dye; all chemicals were separated with mineral oil within the pipette.

Rats were allowed to recover from surgery for a period of ~30–60 min before experiments proceeded. Baseline data were recorded 2–3 min before injections were performed. After injections of control saline or 3-MP (180–200 nl, each), cardiorespiratory variables were recorded every 10 min for a total of 60 min. Dye injections (180–200 nl) were made in the same location as the saline and 3-MP injection sites. After injections, animals were euthanized and the brains were removed and placed in formalin for ~1 wk, at which time they were switched to a 20% sucrose solution. Brains were cut into 50-μm coronal sections on a freezing microtome (American Optical), mounted on slides, and nissl-stained with cresyl violet for histological determination of injection sites.

**Data analysis.** Peak changes in cardiorespiratory variables were recorded for both 3-MP and saline injections. Peak changes were calculated by averaging 60 s of data and determining a mean value for all cardiorespiratory variables at all time points before and after injections. Times at which peak responses occurred were also noted. Only minimal cardiovascular changes were evoked by saline control injections and were subsequently subtracted from the 3-MP responses to eliminate nonspecific injection effects. Changes in respiratory variables DEMG and mDEMG were calculated as percentages from baseline due to the relative nature of the measurements. Comparisons of data between exercised and nonexercised groups were completed using unpaired Student’s t-tests. Statistical significance was deemed to occur at P < 0.05.

**RESULTS**

**Exercise results.** Eight rats completed the treadmill training, and 16 rats completed the wheel exercise period. We had an attrition rate of 20% (2 of 10 animals trained) for rats that did not complete the treadmill-training period. Injections into the CH of wheel-run animals and controls were successful in 13 of 16 attempts. All injections in treadmill animals (both exercised and controls) were correctly positioned. The hypertensive rats in our lab needed minimal encouragement for treadmill training. All of the rats originally placed in the wheel cages ran during the course of the chronic exercise period. Figure 1 illustrates the amount of distance animals ran per day. Most rats began to ramp up running distance after ~2 wk with access to the wheels. At peak levels, SHR ran ~8,000–9,000 m/day in the wheels. These values are very consistent with previously published results (2, 34). Rats trained on a treadmill ran, on average, ~1,200 m/day (60 min/day × 20 m/min = 1,200 m/day).

At the beginning of the exercise protocols, body weights did not differ between control and exercise groups in both the treadmill-training (153 ± 4 g for control vs. 152 ± 6 g for exercised) and wheel-training (141 ± 3 g for control vs. 134 ± 5 g for exercised) protocols. After treadmill training, body weight was 346 ± 9 g for control rats and 351 ± 8 g for exercised rats (P > 0.05 between groups). In contrast, body weight was significantly different between wheel con-

![Fig. 1. Average distances run by animals allowed access to a running wheel. Individual data points are the group-averaged distance run during the course of 1 day. Distances are depicted in meters. Note gradual rise in running activity over 1st 3 wk, plateau, and fall-off. Values are means ± SE.](http://ajpregu.physiology.org)
trol (348 ± 5 g) and wheel-run (328 ± 7 g) rats (P < .05) after the 10-wk exercise period.

Figure 2 depicts the resting SBP of all groups at the beginning and end of the course of study. Before exercise began, nonexercising control animals had a resting SBP of 147 ± 6 mmHg (treadmill control) and 147 ± 6 (wheel control) mmHg. Similar preexercise levels of resting blood pressures were observed in treadmill and wheel exercise groups (144 ± 9 and 156 ± 6 mmHg, respectively). All groups demonstrated a significant elevation in SBP during the course of the exercise-training period. However, the rise in blood pressure was less in the treadmill exercise group compared with the treadmill control group (final SBP of 194 ± 10 vs. 220 ± 9 mmHg; Fig. 2). Similar, yet less marked differences were observed in the wheel-running group (408 ± 6 beats/min for trained vs. 407 ± 7 beats/min for control; P > 0.05) and wheel groups (406 ± 6 beats/min for trained vs. 406 ± 8 beats/min for control; P > 0.05). Latency to cardiovascular responses was 17.8 ± 5.1 min in the wheel-run group and 37.5 ± 2.9 min in the treadmill group. The time courses of the responses in trained rats are consistent with responses in normotensive animals (7, 32). No significant differences in the respiratory responses (respiratory frequency, DEMG, and mDEM) to 3-MP injections were noted.

Figure 5 shows the histological reconstruction of injection sites into the CH. Most injections were located medially and corresponded with the posterior hypothalamic nuclei as described by Paxinos and Watson (28). Most injections were also tightly contained to an area ~500 μm in rostral-caudal length and 300–500 μm in medial-lateral width as determined by the Chicago blue dye injections. Injections did not invade the arcuate, lateral, or dorsomedial hypothalamic nuclei.

DISCUSSION

Our results demonstrate for the first time that exercise training can have an impact on central GABAergic regulation of cardiovascular function in hypertensive rats. In addition, exercise training was associated with a blunted maturational rise in resting blood pressure. These findings are consistent with the hypothesis that changes in CH GABAergic inhibition may contribute to
the overall reduction in arterial pressure observed after exercise training.

Previous studies have provided neurochemical evidence to support the idea of a CH GABAergic deficiency in SHR. CH GABA levels as well as GABA receptor numbers are decreased in the CH of SHR compared with WKY (3, 16). Activity levels and amount of glutamic acid decarboxylase (GAD), the rate-limiting enzyme for GABA synthesis (22), are lower in the CH of SHR than those found in WKY (3, 18). Moreover, GAD mRNA levels are lower in the CH of SHR than WKY, indicating that the GABAergic deficit may partly originate at the level of GAD gene transcription (18). GAD activity can be blocked with a competitive inhibitor such as 3-MP. Injecting 3-MP into the CH increases blood pressure and heart rate in normotensive WKY but does not have an effect in SHR (32). These findings are in agreement with the biochemical and molecular data demonstrating a lowered amount of CH GAD protein and GAD mRNA transcripts. Moreover, these findings are consistent with an elevated basal discharge of CH neurons in SHR compared with WKY (33).

Our findings of an increased level of blood pressure response to 3-MP injection in exercised SHR point to an increased ability to block GAD production of GABA. Therefore, because of a greater tonic GABAergic suppression of arterial pressure, there is a greater arterial pressure response when GABA is disinhibited. Consequently, the increased level of blood pressure response in a strain of rat that normally does not have a cardiovascular response to 3-MP injection into the CH must have arisen, at least in part, from an increased level of GAD protein or enzymatic activity after chronic bouts of exercise.

In contrast to the treadmill-trained SHR, wheel-run animals did not show a statistically significant lower blood pressure than compared with controls. These
findings are consistent with previously published findings (15). However, other studies have documented a significantly blunted arterial pressure in response to wheel exercise (27). The inconsistent findings observed in different publications may stem from variables such as age at beginning of training or animal vendor and therefore may impact the ability of a training paradigm to affect resting arterial pressure. However, even without an alteration in resting blood pressure, central neural adaptations in the CH may be involved in reducing cardiovascular responses to stress or increased baroreflex buffering ability noted after exercise training (26, 35).

An advantage of using a neurochemical intervention that blocks the production of GABA is that the substance acts on the GABAergic neurons themselves and not on pre- or postjunctional receptors. Therefore, results are specific to the GABAergic neurotransmitter system in the CH and not GABAergic neurons in another part of the brain that send projection(s) to the CH. This idea is strengthened by the observation that the majority of GABAergic neurons in the CH is local interneurons (37).

Many investigators have documented peripheral changes in blood pressure regulation after exercise training (39). There are a few key reasons why we believe that our results are due to central neural adaptations. Previous studies (8, 45) have shown that disinhibition of the CH results in a sympathetically mediated elevation in blood pressure. Our results are not likely explained by a vascular adaptation whereby a given level of sympathetic outflow results in an increased vasoconstriction in resistance vessels and subsequent rise in arterial pressure. Studies in rats strongly indicate that vasculature becomes less sensitive to vasoconstrictor agents after exercise training (4, 13). However, despite reduced vascular responsiveness, CH disinhibition results in an increase in arterial pressure.

Reductions in baroreflex buffering of changes in arterial pressure resulting from 3-MP injections also cannot explain our findings. In SHR, exercise training increases the ability of the arterial baroreflex to regulate the cardiovascular system (35). An increased ability to buffer arterial pressure by baroreflexes should have resulted in a blunted ability of 3-MP to increase arterial pressure in trained animals. In this case, it appears that central neural alterations after chronic exercise were able to overcome any increased feedback buffering elicited by baroreflexes.

We considered the possibility that a significantly altered ability of the CH to regulate cardiovascular function in the wheel-trained animals may be explained by the exercise-induced reduction in body weight. However, it is unlikely that this explains our results. The rats that ran on a treadmill did not show any difference in body weight compared with control. Therefore, changes in body weight cannot be considered a factor in the central changes and reductions in resting arterial pressure observed in this group.

Several neurotransmitter systems are altered after exercise training including serotonin (5, 17), dopamine (9, 17), norepinephrine (12, 20), and GABA (10, 23, 38). In particular, Dishman et al. (10) demonstrated that exercise training increases the amount of GABA present in the corpus striatum but lowers GABA receptor density after wheel running. These findings demonstrate that chronic exercise is able to increase GABA levels in the brain. Molecular changes in GAD mRNA levels have also been shown in the hypothalamus after an experimental paradigm including swim exercise (1). Preliminary data from this lab have verified these findings with treadmill training (21). However, further studies examining GAD enzyme and transcript levels as well as enzymatic activity in the hypothalamus after exercise training (especially wheel running) are warranted given the current functional data.

We did not determine the mechanism responsible for the exercise-induced alterations in the CH. However, several possibilities can be considered. Neurons in the CH exhibit sensitivity to baroreflex stimulation and display a pulse synchronous discharge as determined by spike-triggered averaging techniques (33, 48). The increase in blood pressure during exercise could activate neurons in the CH via baroreceptor afferents. In turn, the baroreceptor-driven increased activity may be a stimulus for increasing the GABAergic cardiovascular regulatory ability of this brain region in an activity-dependent manner. Several other neural mechanisms may be involved in activating the CH during exercise. In particular, exercise drives, including cen-
tral command (47) and muscle reflexes (49), are activators of the CH. As such, these stimuli also represent potential modulators of GABAergic gene transcription and after long-term exposure to repeated bouts of exercise might be able to alter CH regulation of cardiovascular function.

Studies utilizing c-fos gene product immunohistochemistry have shown a significantly elevated level of Fos protein in the caudal hypothalamus following treadmill exercise in rats (19). Fos-like immunostaining is a neuronal marker of increased activity and therefore indicates that neurons in the CH are activated during exercise in conscious animals. The findings also demonstrate increases in a gene transcription activator in an area of the brain after exercise. Increases in Fos protein may be a stimulator of GAD gene transcription, as at least one of the GAD genes have been shown to contain base sequences similar to the AP-binding regions (36). As a result, this molecular event may be a trigger for increasing GABAergic activity in the CH after chronic bouts of exercise.

In conclusion, this study has identified a novel mechanism by which chronic exercise can affect resting blood pressure and/or cardiovascular regulation. The
CH, a brain region with a GABAergic deficiency, involved in regulating blood pressure and activated during exercise is altered after chronic bouts of treadmill and wheel exercise. This functional plasticity has implications for cardiovascular regulation and suggests that the brain may play a bigger role in maintaining cardiovascular health than previously thought.

Perspectives

Our findings indicating a GABAergic upregulation in the CH are one of the first functional evidence of changes in a neurotransmitter system affecting central cardiovascular regulation. The findings are compelling in that they suggest that the central nervous system may play a larger role in how exercise can affect cardiovascular health than previously demonstrated. Furthermore, our findings may not be simply confined in the context of blood pressure regulation in hypertension. Exercise-induced plasticity of GABA neurotransmitter systems in the brain may also positively affect cognitive and motor functions. This may be particularly important in the elderly population. Often, movement (i.e., exercise) is used as therapy for recovery from debilitating neural trauma resulting from stroke and other disorders. Currently, there is little information as to how exercise is able to help individuals recover from neural trauma. Clearly, more work needs to be completed to advance our understanding of these important topics. Moreover, by studying how exercise exerts a positive influence on GABAergic and other neurotransmitter systems in the brain, we will begin to more fully understand the central neural mechanisms involved in helping to acquire and maintain health.

This study was supported by National Institutes of Health Grants HL-06296 and T32GM07143 and an American Heart Association Grant-in-Aid. J. M. Kramer was supported by an American Heart Association Fellowship.

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