Transcriptome of the NTS in exercise-trained spontaneously hypertensive rats: implications for NTS function and plasticity in regulating blood pressure

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The nucleus tractus solitarii (NTS) controls the cardiovascular system during exercise, and alteration of its function may underlie exercise-induced cardiovascular adaptation. To understand the molecular basis of the NTS’s plasticity in regulating blood pressure (BP) and its potential contribution to the antihypertensive effects, we characterized the gene expression profiles at the level of the NTS after long-term daily wheel running in spontaneously hypertensive rats (SHRs). Genome-wide microarray analysis was performed to screen for differentially expressed genes in the NTS between exercise-trained (12 wk) and control SHRs. Pathway analysis using the Kyoto Encyclopedia of Genes and Genomes database revealed that daily exercise altered the expression levels of NTS genes that are functionally associated with metabolic pathways (5 genes), neuroactive ligand-receptor interactions (4 genes), cell adhesion molecules (3 genes), and cytokine-cytokine receptor interactions (3 genes). One of the genes that belonged to the neuroactive ligand-receptor interactions category was histamine receptor H1. Since we confirmed that the pressor response induced by activation of this receptor is increased after long-term daily exercise, it is suggested that functional plasticity in the histaminergic system may mediate the facilitation of blood pressure control in response to exercise but may not be involved in the lowered basal BP level found in exercise-trained SHRs. Since abnormal inflammatory states in the NTS are known to be prohypertensive in SHRs, altered gene expression of the inflammatory molecules identified in this study may be related to the antihypertensive effects in exercise-trained SHRs, although such speculation awaits functional validation.

blood pressure; exercise; hypertension; nucleus tractus solitarii; histamine

Since hypertension is a risk factor for numerous cardiovascular-related diseases, such as heart attacks, end-stage renal failure, and stroke, it is crucial to maintain the basal blood pressure (BP) level within the normal range. It is generally accepted that regular aerobic exercise with moderate intensity lowers and prevents the incidence of hypertension in patients with high normal and stage 1 hypertension (20). This antihypertensive effect of exercise therapy is not limited to humans; an animal model of human essential hypertension, the spontaneously hypertensive rat (SHR), has been widely used to investigate the effect of exercise training on the progression of hypertension. Schlüter et al. (25) recently summarized the exercise training-induced effects on the basal BP level in SHRs and revealed that a significant fall in BP was observed in SHRs that started an exercise program in the prehypertensive or early hypertensive state (i.e., 4–6 wk), but not in older SHRs with established hypertension. The mechanisms underlying these exercise training-induced antihypertensive effects may be polygenic; however, accumulating evidence suggests that improvement of the attenuated baroreceptor reflex plays an important role in this process (15). This is thought to contribute to the reduction of the basal activity of muscle sympathetic nerves, which has been identified in never-treated hypertensive patients after aerobic exercise training (15). Similarly, a reduction in peripheral sympathetic outflow has been identified in SHRs after daily treadmill running (14, 18). These findings indicate that exercise training induces remarkable neuroplasticity in the cardiovascular centers, and this contributes to reducing the basal BP level and, hence, results in an improvement of hypertension.

The central baroreflex arc, which regulates sympathetic outflow, includes the nucleus of the solitary tract (NTS) and the caudal/rostral ventrolateral medulla. Among these brain sites, the NTS reportedly exerts an important role in regulating the baroreflex during exercise (6, 17, 19, 21, 22, 28). The NTS is the central termination site of baroreceptor input and receives direct projections from spinal dorsal horn neurons, which transmit inputs from skeletal muscle receptors (21, 22). The NTS also receives numerous inputs from other brain sites including the dorsomedial hypothalamus or hypothalamic paraventricular nucleus (6, 17); these pathways are often referred to as the “central command” (8, 16). Therefore, the NTS is considered a central site that integrates the descending and ascending inputs while regulating baroreceptor function during exercise. This suggests that the NTS is likely one of the cardiovascular centers where functional and neuroanatomical plasticity could occur.

Since we previously reported that the abnormal expression of some genes in the NTS is one factor associated with neurogenic hypertension in SHRs (9, 33, 34, 35), it is also of interest to know whether exercise training would affect the expression profiles of hypertension-related genes in the NTS of SHRs. In the present study, the objectives were twofold. First, we investigated the molecular basis of the NTS plasticity induced by daily exercise training. Second, we assessed whether the abnormal expression of hypertension-related genes in the NTS would be improved by exercise training. To this end, we performed genome-wide microarray expression analysis using the NTS tissue of SHRs after long-term daily exercise.

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MATERIALS AND METHODS

Animals and animal care. Three-week-old male SHRs and their normotensive controls, Wistar Kyoto (WKY) rats, obtained from Japan SLC, (Shizuoka, Japan) were used in this study. The animals were housed in a temperature-controlled room with a fixed 12:12-h light-dark cycle (08:00–20:00 and 20:00–08:00). Food and tap water were provided ad libitum. All experiments were approved by the Ethics Committee for Animal Experiments at Wakayama Medical University and complied with the guidelines of the Physiological Society of Japan.

Grouping. SHRs and WKY rats were assigned to either a daily exercise-trained group or a sedentary untrained group. All animals were housed individually in conventional plastic cages measuring 28 (w) × 44 (l) × 18 (h) cm. The exercise treatment consisted of daily voluntary exercise using a rotatory wheel (circumference: 1 m) attached to a half side of the lid (SWY-30; Melquest, Toyama, Japan). The exercise treatment was performed from 4 to 16 wk of age. The total running distance covered during the daily exercise was measured with a counter display (CNT-10; Melquest). Body weight was also measured every week in both groups.

NTS transcriptomic analysis and data handling. After the treatment period, SHRs (exercise-trained group, n = 6; untrained group, n = 6) were killed by ether inhalation, followed by decapitation. The brain was rapidly removed from the cranium and the NTS between 1.0 mm rostral and 0.5 mm caudal to the calamus scriptorius was dissected from the medulla oblongata in each animal. NTS samples were homogenized in 400 μl TRIzol reagent (Invitrogen, Carlsbad, CA) as described previously (9). Transcriptomic services were provided by Takara Bio (Shiga, Japan). RNA was quantified using a NanoDrop-1000 spectrophotometer, and quality was monitored with an Agilent 2100 Bioanalyzer (Agilent Technologies, Santa Clara, CA). Cyanine-3 (Cy3)-labeled cRNA was prepared from 0.5 μg total RNA using the Quick Amp Labeling Kit (Agilent) according to the manufacturer’s instructions, followed by RNeasy column purification (QIAGEN, Valencia, CA). We fragmented 1.65 μg of Cy3-labeled cRNA at 60°C for 30 min in a reaction volume of 55 μl containing 1× Agilent Fragmentation Buffer and 2× Agilent Blocking Agent, according to the manufacturer’s instructions. On completion of the fragmentation reaction, 55 μl of 2× Agilent Hybridization Buffer was added to the fragmentation mixture and hybridized to Agilent Whole Rat Genome Oligo Microarrays for 17 h at 65°C in a rotating Agilent hybridization oven. After hybridization, the microarrays were washed for 1 min at room temperature with GE Wash Buffer 1 (Agilent) and 1 min with 37°C GE Wash Buffer 2 (Agilent) and then dried immediately by brief centrifugation. The slides were scanned immediately after washing on an Agilent DNA Microarray Scanner (G2565CA) using the two-color scan setting for 4 × 44 k array slides (scan area, 61 × 21.6 mm; scan resolution, 5 μm; dye channel set to Green and Red PMT at 100%). For data processing, Agilent Feature Extraction Software (v 10.10.1.1) was used. The calibrated data from each microarray were used, and the ratio of each probe between the two groups was calculated. When the data obtained from WKY rats did not show the same trend as the NTS transcriptome in SHRs, they were annotated as SHR specific.

Finally, to evaluate whether the genes that were differentially expressed between the exercise-trained and untrained SHRs were NTS specific, total RNA from the medulla oblongata without the NTS was extracted (exercise-trained and untrained SHRs, n = 6 each). Transcriptomic analysis using the two-color method was also performed in duplicate as described above. When the data obtained from the medulla oblongata did not show the same trend as the NTS transcriptome in SHRs, they were annotated as NTS specific.

BP measurement. BP was measured in different sets of animals. After the 12-wk treatment period, SHRs (exercise-trained SHRs, n = 8; untrained SHRs, n = 8) were anesthetized with pentobarbital sodium (50 mg/kg) given intraperitoneally (ip). A radio transmitter (TA11PA-C40; Data Sciences International, St. Paul, MN) was implanted to record BP from the abdominal aorta as described previously (35). The animals were returned to their home cages and each treatment was resumed. Seven days after implantation, the BP in conscious animals was measured continuously for 5 min every hour from 09:00 until 08:05 on the following day with each treatment. HR was derived from the interpulse interval.

Microinjection study. To assess the functional significance of the identified molecules in exercise-trained animals, we investigated the cardiovascular effects of the histamine receptor H1 and the serotonin (5-hydroxytryptamine, 5-HT) 1A (5-HT1A) receptor, whose genes were both downregulated in the NTS of exercise-trained SHRs (see RESULTS). We chose these molecules to be tested since they are known to directly affect neuronal functions as they are neuroactive ligand-receptor interactions in the KEGG database. The other identified molecules, which are neuroactive ligand-receptor interactions (i.e., galanin receptor 3 and peptide YY; see RESULTS), were not tested in this study as their specific agonists have not been fully established.

Microinjection studies were performed in different sets of animals as described previously (4). After the 12-wk treatment period, the animals (exercise-trained and untrained SHRs, n = 12 each; exercise-trained and untrained WKY rats, n = 6 each) were anesthetized with urethane (1.45 g/kg ip). We monitored the level of anesthesia regularly by assessing the limb withdrawal response to a noxious pinch throughout the experiment, and an additional dose of urethane (0.145 g/kg ip) was administered when necessary. Mean BP (MBP) and HR were monitored and recorded as described previously (4).

The experimental procedures were modified from those reported previously (4). The urethane-anesthetized rats were placed in a stereotactic head holder (SR-5; Narishige Scientific Instrument Lab, Tokyo, Japan), and the caudal dorsal medulla was exposed through a midline incision in the dorsal neck. Microinjections of drug solution or vehicle were made into the NTS using either single- or multibarreled glass micropipettes (GC200F-10; Harvard Apparatus, Edenbridge, UK) with an outer diameter of 20–30 μm. For bilateral microinjections into the NTS, the tip of the micropipette was posi-
toned at 0.5 mm rostral to the calamus scriptorius, 0.4 mm lateral from the midline, and 0.5 mm deep from the dorsal surface of the brainstem. A Hamilton microsyringe and a syringe pump (CVF 3200, Nihon Kohden) were used for microinjections. First, 5-HT1A agonist H1 receptor agonist, 8-OH-DPAT (1 or 10 nmol/50 nl; both from Sigma-Aldrich, MO), into the NTS in different sets of SHRs.

All drugs were dissolved in artificial cerebrospinal fluid. After completion of the experiment, the microinjection sites were marked with 50 nl of India ink for histological verification as described previously (4). Only those rats with microinjection sites wholly within the NTS were used for analysis.

**Immunohistochemistry for histamine receptor H1 in the NTS.** Immunohistochemistry was performed as described previously (4). Native SHRs \( n = 3 \) were fixed, and the brain stem was removed as described previously (4). Serial sections (40 μm thick) of the NTS were obtained using a freezing microtome. A H1 receptor antibody (sc-20633; Santa Cruz Biotechnology, Santa Cruz, CA) and neuron marker (anti-NeuN; Millipore, Temecula, CA) were used as described previously (4).

**Statistical analysis.** All values are expressed as means ± SE for each group, and an unpaired t-test was used for comparisons between two groups. Differences were considered significant for \( P < 0.05 \).

**RESULTS**

**Basal physiological parameters in exercise-trained and untrained rats.** There was no significant difference in the averaged total running distance between exercise-trained SHRs and WKY rats (637 ± 30 km and 611 ± 41 km, respectively; Table 1). The body weight in exercise-trained SHRs was significantly lower than that in untrained SHRs (297 ± 4 vs. 342 ± 5 g, respectively, \( P < 0.001 \)), while no significant difference was found between exercise-trained WKY and untrained WKY rats (Table 1). BP was significantly lower in exercise-trained SHRs than untrained SHRs (e.g., 24-h average MBP: 134 ± 1.5 vs. 139 ± 1.8 mmHg, respectively, \( P < 0.05 \)), while no significant difference was found in HR between the two groups of SHRs (Table 2).

**Gene expression profiles in the NTS of exercise-trained SHRs.** Transcriptomic analysis revealed that 154 genes in the NTS were significantly differentially expressed between exercise-trained and untrained SHRs (Supplemental Data 1). A total of 47 genes were upregulated, while 107 were downregulated in exercise-trained SHRs compared with the control group. Of them, 88 genes were found to be NTS specific. Moreover, 56 genes were found to be SHR specific, whereas 40 were validated as not strain dependent (Supplemental Data 1).

Pathway analysis using the KEGG database revealed that exercise training in the NTS altered the expression levels of NTS genes that are functionally associated with metabolic pathways (5 genes), neuroactive ligand-receptor interactions (4 genes), cell adhesion molecules (3 genes), and cytokine-cytokine receptor interactions (3 genes, Table 3). The differentially expressed genes belonging to neuroactive ligand-receptor interactions were the histamine receptor H1 gene (Hrh1), serotonin receptor 1A gene (Htr1a), and galanin receptor 3 gene (Galr3), which were all downregulated in exercise-trained SHRs, while the peptide YY gene (Pyy) was upregulated in exercise-trained SHRs (Table 3 and Fig. 1). The gene expression profile of Hrh1 and Pyy was also found in the NTS of WKY rats, while that of Galr3 was found only in the NTS of SHR (Table 3). Interestingly, the genes for cell adhesion molecules, which were the protein tyrosine phosphatase, receptor type, C gene (Ptprc), selectin, platelet (p-selectin) ligand gene (Selplg), and similar to B7-like protein GL50-B (predicted) gene (Icoslg), were all found to be downregulated in exercise-trained SHRs (Table 3 and Fig. 2). In this regard, although the difference was >0.75-fold, it should be noted that the claudin 19 gene (Cldn19) and claudin 11 gene (Cldn11), which play roles in cell adhesion and leukocyte transendothelial migration, were also significantly downregulated in exercise-trained SHRs compared with the control group (Table 3 and Fig. 2), suggesting an alteration of the adherence ability of leukocytes in the NTS of exercise-trained animals. Moreover, the changes in Cldn19 expression were found to be SHR specific.

Since we previously reported that the altered gene expression profiles of some inflammatory related molecules at the level of the NTS are strongly related to the hypertension

<table>
<thead>
<tr>
<th>SHR</th>
<th>Total Running Distance, km</th>
</tr>
</thead>
<tbody>
<tr>
<td>CTR ( n = 14 )</td>
<td>342 ± 5</td>
</tr>
<tr>
<td>EXE ( n = 14 )</td>
<td>297 ± 4*</td>
</tr>
<tr>
<td>WKY</td>
<td>637 ± 30</td>
</tr>
</tbody>
</table>

<table>
<thead>
<tr>
<th>SHR</th>
<th>Total Running Distance, km</th>
</tr>
</thead>
<tbody>
<tr>
<td>CTR ( n = 6 )</td>
<td>391 ± 6</td>
</tr>
<tr>
<td>EXE ( n = 6 )</td>
<td>384 ± 6</td>
</tr>
</tbody>
</table>

Data were obtained from animals for microarray study and 24-h blood pressure (BP) recording. SHRs, spontaneously hypertensive rat; WKY, Wistar-Kyoto rat; CTR, untrained animals; EXE, exercise-trained animals. \(* P < 0.001\) compared with CTR.

<table>
<thead>
<tr>
<th>SBP</th>
<th>MBP</th>
<th>DBP</th>
<th>HR</th>
</tr>
</thead>
<tbody>
<tr>
<td>CTR ( n = 8 )</td>
<td>24 h</td>
<td>176 ± 2.5</td>
<td>139 ± 1.8</td>
</tr>
<tr>
<td>light</td>
<td>172 ± 2.4</td>
<td>135 ± 1.7</td>
<td>117 ± 1.5</td>
</tr>
<tr>
<td>dark</td>
<td>180 ± 2.9</td>
<td>143 ± 2.2</td>
<td>124 ± 2.0</td>
</tr>
</tbody>
</table>

**EXE \( n = 8 \)**

<table>
<thead>
<tr>
<th>SBP</th>
<th>MBP</th>
<th>DBP</th>
<th>HR</th>
</tr>
</thead>
<tbody>
<tr>
<td>24 h</td>
<td>171 ± 1.9</td>
<td>134 ± 1.5*</td>
<td>115 ± 1.7*</td>
</tr>
<tr>
<td>light</td>
<td>169 ± 2.2</td>
<td>133 ± 1.8</td>
<td>115 ± 1.9</td>
</tr>
<tr>
<td>dark</td>
<td>173 ± 1.9</td>
<td>135 ± 1.6*</td>
<td>117 ± 1.8*</td>
</tr>
</tbody>
</table>

HR, heart rate; CTR, untrained SHRs; EXE, exercise-trained SHRs; SBP, systolic blood pressure; MBP, mean blood pressure; DBP, diastolic blood pressure; 24-h average values for 24 h; light, average value for 12 h during the light phase; night, average value for 12 h during the night phase. \(* P < 0.05\) and \(+ P < 0.01\) compared with CTR.

The online version of this article contains supplemental material.
phenotype of SHRs (11, 38, 39, 41), we examined the effects of exercise training on the expression profiles of these genes. These molecules include junctional adhesion molecule-A (JAM-A), leukotriene B4–12-hydroxydehydrogenase (LBTB4–12HD), chemokine (C-C motif) ligand 5 (Ccl5), chemokine (C-C motif) receptor 1 (Ccr1), chemokine (C-C motif) receptor 3 (Ccr3), chemokine binding protein 2 (Ccbp2), and tumor necrosis factor (ligand) superfamily member 4 (Tnfsf4). However, our transcriptomic analysis failed to find a significant alteration of the expression levels of these genes in exercise-trained SHRs (Fig. 3).

Effect of histamine-induced cardiovascular actions in the NTS of exercise-trained SHRs. When the histamine receptor H1 agonist was bilaterally microinjected into the NTS, MBP and HR were found to be increased in both groups of SHRs. However, the maximal response in MBP was significantly higher in the exercise group compared with the control group (52.3 ± 6.9 vs. 12.8 ± 7.1 mmHg, respectively, P < 0.01, Fig. 4). HR responses were not significantly different between the two groups of rats [25.0 ± 14.7 vs. 16.5 ± 8.0 beats/min (bpm), respectively, Fig. 4]. It should be noted that the increased pressor responses in MBP were also found in exercise-trained WKY rats (19.3 ± 1.3 vs. 12.0 ± 3.0 mmHg, respectively, P < 0.05), suggesting that this exercise-induced effect was not strain specific. As found in SHR, HR responses were not significantly different between exercise-trained and control WKY rats (27.1 ± 3.0 vs. 34.3 ± 3.0 bpm, respectively).

Effect of serotonin-induced cardiovascular actions in the NTS of exercise-trained SHRs. When serotonin was bilaterally microinjected into the NTS, MBP and HR were decreased in both exercise-trained and control SHRs. However, there were no significant intergroup differences for maximal response in MBP (−34.8 ± 9.2 vs. −35.1 ± 12.7 mmHg, respectively, Fig. 5) or HR (−23.5 ± 3.0 vs. −22.5 ± 5.2 bpm, respectively, Fig. 5). The cardiovascular effects of 8-OH-DPAT (1 and 10 nmol/50 nl) were minor, and there were also no significant intergroup differences in the maximal response in MBP (10 nmol, 2.4 ± 3.7 vs. 0.7 ± 0.9 bpm, respectively; Fig. 5) or HR (4.6 ± 4.0 vs. 1.4 ± 3.4 bpm, respectively; Fig. 5).

H1-receptor expression and microinjection sites in the NTS. The H1 receptor was widely distributed in the brain stem area of SHRs, including the dorsal vagal nucleus, hypoglossal nucleus, and NTS. As we previously reported, the H1 receptor is extensively colocalized with NeuN, a neuronal marker, indicating that this receptor is expressed predominantly in neurons (Fig. 6A–C). We confirmed that microinjection sites were wholly within the restricted area of the NTS in all rats used for data analysis (Fig. 6D).

DISCUSSION

In the present study, we investigated whether alterations of genetic characteristics could occur specifically at the level of the NTS after daily voluntary wheel running in SHRs. We performed a microarray study to identify genes in this nucleus that are differentially expressed between SHRs assigned to a daily voluntary exercise group and those assigned to a sedentary untrained group. Pathway analysis using the KEGG database revealed that daily wheel running altered the expression levels of NTS genes that are functionally associated with metabolic pathways, neuroactive ligand-receptor interactions, cell adhesion molecules, and cytokine-cytokine receptor interactions. One of the genes that belonged to neuroactive ligand-receptor interactions was Hrh1, which was downregulated in the NTS of exercise-trained rats. Since we confirmed that the activation of this receptor induces pressor and tachycardiac responses at the level of the NTS and the pressor response exhibits functional plasticity after long-term daily exercise, it is suggested that the histaminergic system may play an important role in regulating the cardiovascular system during exercise and its functional plasticity may be involved in exercise training-induced cardiovascular adaptations. Although we have not yet functionally identified specific gene(s) in the NTS that contribute to the antihypertension effects of daily exercise in SHRs, we believe that our data provide a direction for its further investigation.

NTS plasticity after exercise training. Our data from the microarray study revealed that daily wheel running altered the expression levels of NTS genes that are functionally associated with neuroactive ligand-receptor interactions. One of the genes in the neuroactive ligand-receptor interactions category was Hrh1, which was downregulated in the NTS of exercise-trained rats. This gene was also found in exercise-trained WKY rats, suggesting a nonstrain-specific response. Surprisingly, the pressor response to the H1 receptor-specific agonist was significantly higher in exercise-trained SHRs/WKYS than in control untrained groups, indicating the neuroplasticity of the histaminergic system in the NTS. Therefore, the downregulation of the H1 histamine receptor mRNA found in the NTS of exercise-trained rats did not represent desensitization of the receptors. This might be secondary to the facilitation of the histaminergic system as a compensatory event, although such speculation requires verification. Whatever the mechanism, the functional plasticity in the histaminergic system after exercise training is unlikely to be involved in the exercise-induced antihypertensive effects in SHRs.

Questions arise as to what physiological significance may be attributed to the functional facilitation of the histaminergic system in the NTS. We speculate that the histaminergic system may play a role in regulating the cardiovascular system during exercise, but not in controlling BP and HR resting levels. Central histamine has been found to possess neurotransmitter properties (2, 4, 26, 27, 31). Histamine-immunoreactive neuronal cell bodies are found exclusively in the tuberomammillary nucleus (TMN) of the posterior hypothalamus (10, 26, 31), but immunoreactive fibers are observed throughout the cerebral cortex and in other parts of the brain, including the NTS (36, 37). With regard to histamine receptors, four distinct subtypes, designated H1, H2, H3, and H4, have been identified in the brain (5, 12, 26), and we previously found that the H1 receptor in the NTS is expressed more predominantly than the other subtypes and is localized to neurons (4). This was further confirmed in this study. With regard to its function, central histamine is an important regulator of the sleep-wake cycle, arousal level, pain sensation, stress response, and food intake (5, 11, 12, 26). In addition to these functions, central histamine is known to be involved in cardiovascular regulation (1, 2, 3). In this regard, we previously confirmed that histamine within the NTS acutely increases BP and HR via the activation of the H1 receptor (4). Taken together with our present data, we speculate that the H1 receptor in the NTS may be involved in increasing BP and HR during exercise. Moreover, the neuroplasticity of the histamin-
### Table 3. Representative NTS genes differentially expressed between exercise-trained and untrained SHRs by transcriptomic analysis

<table>
<thead>
<tr>
<th>Feature Number</th>
<th>Description</th>
<th>Gene Symbol</th>
<th>Fold Differences</th>
<th>P Value</th>
<th>NTS Specificity</th>
<th>SHR Specificity</th>
<th>KEGG Pathway</th>
</tr>
</thead>
<tbody>
<tr>
<td>230</td>
<td>Rattus norvegicus 5-hydroxytryptamine (serotonin) receptor 1A (Htr1a), mRNA [NM_0125855]</td>
<td>Htr1a</td>
<td>0.72</td>
<td>0.0348</td>
<td>yes</td>
<td>yes</td>
<td>Neuroactive ligand-receptor interaction</td>
</tr>
<tr>
<td>2843</td>
<td>Rattus norvegicus similar to cystinosin (LOC287478), mRNA [XM_220649]</td>
<td>Ctns</td>
<td>0.71</td>
<td>0.0220</td>
<td>yes</td>
<td>yes</td>
<td>Lysosome</td>
</tr>
<tr>
<td>5225*</td>
<td>Rattus norvegicus claudin 19 (Cldn19), mRNA [NM_001008514]</td>
<td>Cldn19</td>
<td>0.77</td>
<td>0.0005</td>
<td>yes</td>
<td>yes</td>
<td>Celladhesion molecules (CAMs) // Tight junction // Leukocyte transendothelial migration</td>
</tr>
<tr>
<td>7341</td>
<td>Rattus norvegicus cytidine monophosphate-N-acetylneuraminic acid hydroxylase (CMAh), mRNA [NM_001024273]</td>
<td>Cmah</td>
<td>1.44</td>
<td>0.0137</td>
<td>yes</td>
<td>yes</td>
<td>Amino sugar and nucleotide sugar metabolism</td>
</tr>
<tr>
<td>7770</td>
<td>Rattus norvegicus transducin (beta)-like 1X-linked receptor 1 (Tb1xr1), mRNA [NM_001108941]</td>
<td>Tb1xr1</td>
<td>0.71</td>
<td>0.0318</td>
<td>yes</td>
<td>yes</td>
<td>Wnt signaling pathway</td>
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<tr>
<td>8760</td>
<td>Rattus norvegicus gamma-glutamyl transferase 6 (Ggt6), mRNA [NM_001002820]</td>
<td>Ggt6</td>
<td>0.62</td>
<td>0.0011</td>
<td>yes</td>
<td>yes</td>
<td>Taurine and hypotaurine metabolism // Selenoamino acid metabolism // Cyanoamino acid metabolism // Glutathione metabolism // Arachidonic acid metabolism // Metabolic pathways</td>
</tr>
<tr>
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<td>Rattus norvegicus olfactory receptor 745 (Olr745), mRNA [NM_001000578]</td>
<td>Olr745</td>
<td>0.69</td>
<td>0.0160</td>
<td>yes</td>
<td>yes</td>
<td>Olfactory transduction</td>
</tr>
<tr>
<td>10936</td>
<td>Rattus norvegicus protein tyrosine phosphatase, receptor type, C (Ptpcr), transcript variant 4, mRNA [NM_0138507]</td>
<td>Ptpcr</td>
<td>0.58</td>
<td>0.0407</td>
<td>yes</td>
<td>yes</td>
<td>Celladhesion molecules (CAMs) // T cell receptor signaling pathway // Fc gamma R-mediated phagocytosis // Primary immunodeficiency</td>
</tr>
<tr>
<td>12910</td>
<td>Rattus norvegicus histamine receptor H1 (Hrh1), mRNA [NM_017018]</td>
<td>Hrh1</td>
<td>0.75</td>
<td>0.0109</td>
<td>no</td>
<td>no</td>
<td>Calcium signaling pathway // Neuroactive ligand-receptor interaction</td>
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<tr>
<td>15826</td>
<td>Rattus norvegicus interleukin 20 receptor, alpha (Il20ra), mRNA [NM_00107521]</td>
<td>Il20ra</td>
<td>0.68</td>
<td>0.0471</td>
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<td>yes</td>
<td>Cytokine-cytokine receptor interaction // Jak-STAT signaling pathway</td>
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<tr>
<td>18068</td>
<td>Rattus norvegicus chemokine (C-C motif) ligand 17 (Ccl17), mRNA [NM_057151]</td>
<td>Ccl17</td>
<td>1.39</td>
<td>0.0082</td>
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<td>Cytokine-cytokine receptor interaction // Chemokine signaling pathway</td>
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<tr>
<td>18407</td>
<td>Rattus norvegicus vesicle-associated membrane protein 1 (Vamp1), mRNA [NM_013090]</td>
<td>Vamp1</td>
<td>0.75</td>
<td>0.0307</td>
<td>yes</td>
<td>yes</td>
<td>SNARE interactions in vesicular transport</td>
</tr>
<tr>
<td>18570</td>
<td>Rattus norvegicus period homolog 3 (Drosophila) (Per3), mRNA [NM_023978]</td>
<td>Per3</td>
<td>0.70</td>
<td>0.0077</td>
<td>yes</td>
<td></td>
<td>Circadian rhythm-mammal</td>
</tr>
<tr>
<td>19876</td>
<td>Rattus norvegicus phosphoinositide-3-kinase, catalytic, gamma polypeptide ( Pik3cg), mRNA [NM_001106723]</td>
<td>Pik3cg</td>
<td>0.68</td>
<td>0.0062</td>
<td>yes</td>
<td></td>
<td>Inositol phosphate metabolism // ErbB signaling pathway // Chemokine signaling pathway // Phosphatidylinositol signaling system // mTOR signaling pathway // Apoptosis // VEGF signaling pathway // Focal adhesion // Toll-like receptor signaling pathway // Jak-STAT signaling pathway // Natural killer cell mediated cytotoxicity // T cell receptor signaling pathway // B cell receptor signaling pathway // Fc epsilon RI signaling pathway // Fc gamma R-mediated phagocytosis // Leukocyte transendothelial migration // Neurotrophin signaling pathway // Regulation of actin cytoskeleton</td>
</tr>
<tr>
<td>20010</td>
<td>AI171206 EST217160 Normalized rat muscle, Bento Soares Rattus sp. cDNA clone RMUBH55 3' end, mRNA sequence [AI171206]</td>
<td></td>
<td>0.72</td>
<td>0.0093</td>
<td>yes</td>
<td>yes</td>
<td>Continued</td>
</tr>
</tbody>
</table>
ergic system in the NTS may explain one of the adaptation mechanisms that regulate the cardiovascular system during exercise. The facilitation of the histaminergic system may contribute to the efficient adjustment of blood supply to those organs with high metabolic demands, thereby increasing exercise capacity. Further investigations are required to identify the facilitatory mechanisms of histamine transmission via the H₁ receptor in the NTS. It would also be important to know whether the TMN-NTS pathway (i.e., histaminergic system) has a role in the mechanisms that underlie central command-mediated cardiovascular control during exercise (8, 16).

Our finding of other neuroactive receptor genes such as Htr1a exhibiting altered expression patterns after daily exercise is also of note, since its physiological agonist, serotonin in the brain including the NTS, is known to regulate the cardiovascular system during exercise (8, 16).
Does exercise affect hypertension-related genes in the NTS?

The NTS is one of the brain sites that are expected to contribute to the elevated basal BP level in hypertensive patients. We previously reported that altered gene expression profiles at the level of the NTS are strongly related to the hypertension phenotype of SHRs (9, 33, 34, 35). Such molecules include serotonin microinjected into the NTS decreases BP and HR as previously reported (23), but no differences in the cardiovascular response were found between the exercise-trained SHR and the control group. Since the depressor and bradycardiac responses are known to be mediated mainly by the 5-HT2A receptor in the NTS (23), such results can be expected. Consistent with findings of other reports, we also found that stimulation of the 5-HT1A receptor in the NTS exhibited only a minor effect on cardiovascular regulation. However, these observations do not rule out the role of the 5-HT1A receptor expressed in the NTS in regulating the cardiovascular system. The reason is that the 5-HT1A receptor may act as an autoreceptor (13). If this is the case, the cardiovascular effect of the 5-HT1A receptor in response to manipulation of endogenous serotoninergic tone needs to be determined. Moreover, the functional significance of the altered expression of Htr1a, as well as other identified genes such as Galr3 and Ppy, in the NTS of exercise-trained animals also needs to be elucidated.

Fig. 2. Altered expression levels of NTS genes for cell adhesion molecules in exercise-trained SHRs. Transcriptomic analysis revealed significant differential expression of the protein tyrosine phosphatase, receptor type, C gene (Ptprc), selectin, platelet (p-selectin) ligand gene (Selplg), similar to B7-like protein GL50-B (predicted) gene (RGD1562791_predicted), claudin 19 gene (Cldn19), and claudin 11 gene (Cldn11). *p < 0.05, **p < 0.01.

Fig. 3. Gene expression profiles of hypertension-related molecules in the NTS of exercise-trained and untrained SHRs. Transcriptomic analysis revealed no significant differential expression of the genes for junctional adhesion molecule-A (JAML), leukotriene B4–12-hydroxydehydrogenase [prostaglandin reductase 1 (Pgrr1)], chemokine (C-C motif) ligand 5 (Ccl5), chemokine (C-C motif) receptor 1 (Ccr1), chemokine (C-C motif) receptor 3 (Ccr3), and chemokine binding protein 2 (Ccbp2). The tumor necrosis factor (ligand) superfamily member 4 gene (Tnfsf4) was found to be significantly downregulated in exercise-trained SHRs (*p < 0.05). However, the difference was minor (1.18-fold), and therefore the gene was not considered as differentially expressed.

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JAM-A and LTB4–12HD, which directly or indirectly induce leukocyte adhesion to endothelial cells, and cytokine/chemokine-related molecules, such as Ccl5, Ccr1, Ccr3, Cebp2, and Tnfsf4 (9, 33, 34, 35). In this regard, questions arise as to whether exercise training/exercise therapy can reverse the detrimental properties of the NTS in SHRs. If it does, this could be reflected by the normalization of the expression profiles of hypertension-related genes after exercise therapy; however, we failed to see significant changes in the expression levels of these genes. This may be due to the fact that the gene expression profiles found in the NTS of SHRs are likely to be genetically programmed (i.e., a genetic predisposition to hypertension) and not affected by daily exercise, although this hypothesis awaits further experiments with different exercise protocols such as forced aerobic exercise. For example, Felix and Michelini (7) reported that the expression of angiotensinogen mRNA in the NTS, which is also known to be associated with hypertension, decreased in the NTS of SHRs after treadmill exercise training; however, we failed to see a reduction in its expression after voluntary wheel running exercise, suggesting that the type of exercise may affect the gene expression profiles in the NTS.

Although exercise training may not normalize the abnormally expressed genes in the NTS of SHRs, it should be noted that mRNA of some leukocyte adhesion molecules such as Selplg, Cldn19, and Cldn11 was decreased in the NTS of exercise-trained animals, suggesting that exercise training could attenuate leukocyte adherence within the capillaries of the NTS. We have previously shown the accumulation of endogenous leukocytes inside the capillaries of the NTS in SHRs (35), and this may induce an increase in vascular resistance and hypoperfusion within the NTS; the latter may affect central neural cardiovascular activity conducive to neurogenic hypertension (32). Considering these findings, it would be of interest to know whether the downregulation of the mRNA for adhesion molecules in exercise-trained SHRs improves leukocyte accumulation in the NTS and if this reduces...
their high basal BP level. Moreover, on the basis of recent findings, including our own, that some cytokines/chemokines and their receptors are involved in regulating neuronal homeostasis (9, 24, 29, 34, 38), it needs to be determined whether the inflammatory molecules identified by microarray analysis have effects on neuronal functions and, if so, whether the altered gene expression of such molecules contributes to the antihypertensive effects in exercise-trained SHRs.

In summary, we found that daily wheel running altered gene expression profiles at the level of the NTS in SHRs. Some of those genes were related to neuroactive ligand-receptor interactions, suggesting that neuronal functions within the NTS were altered after daily exercise training. We postulate that this plastic capacity of the NTS neuronal circuit is one of the mechanisms that facilitate cardiovascular control during exercise, thereby increasing exercise capacity. Moreover, we found that the expression levels of some inflammatory related genes were altered in the NTS of exercise-trained SHRs. Although we have not yet identified the NTS mechanisms underlying the exercise training-induced antihypertensive effects, we believe that our data provide a direction for further investigations.

Limitations

The changes in BP found in this study (~5 mmHg) were smaller than those of previous studies which used treadmills (~12 mmHg) or a wheel running exercise (~9 mmHg) (25). This may be due to the methodological difference in measuring BP. We used radio telemetry to record BP, while other studies have measured BP by a tail-cuff method. The main advantage of radio telemetry is that data can be obtained continuously from freely moving animals over 24 h, whereas the tail-cuff method requires animals to be sedentary and "restrained" and is therefore not suitable for repeated measurements. Since we did not limit physical activity when BP was measured, our BP levels might be high. Another reason might be that since a recovery period over 1 wk is normally required after the implantation of transmitters, the reduced physical activity due to surgery might affect the BP level in exercise-trained SHRs. Nevertheless, it should be noted that the NTS RNAs for microarray analysis were obtained from intact animals, and the expression levels might be high. Another reason might be that since a decrease in baroreceptor sensitivity is associated with reduced angiotensinogen mRNA expression within the nucleus tractus solitarii (Hypertension 50: 780–785, 2007). Nevertheless, it should be noted that the NTS RNAs for microarray analysis were obtained from intact animals, and the expression levels might be high. Another reason might be that since a decrease in baroreceptor sensitivity is associated with reduced angiotensinogen mRNA expression within the nucleus tractus solitarii (Hypertension 50: 780–785, 2007).

DISCLOSURES

No conflicts of interest, financial or otherwise, are declared by the author(s).

AUTHOR CONTRIBUTIONS


REFERENCES


