Increased Noradrenergic Activity in the Ventromedial Hypothalamus during Treadmill Running in Rats

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Summary  Physical exercise dramatically increases the energy expenditure of animals. In terms of energy substrate, at the onset of exercise, the contribution of carbohydrates to the energy expenditure is relatively predominant, and decreases gradually with the progression of exercise, while fat consumption increases progressively. The ventromedial hypothalamus (VMH) is a nucleus in the hypothalamus that regulates whole body energy metabolism via the sympathetic nervous system. Some reports have indicated that noradrenergic projections to the VMH are involved in energy metabolism during exercise. However, it is not clear whether exercise influences the activity of noradrenergic projections to the VMH. We hypothesize that during exercise, noradrenergic neurons projecting to the VMH are activated, and play an important part in enhancing fat oxidation. To test this hypothesis, we used in vivo microdialysis to investigate the effect of exercise on the activity of monoaminergic (noradrenaline: NA, dopamine: DA, serotonin: 5-HT) neurons projecting to the VMH of rats. Rats were subjected to running at 15 m/min (incline 3°) for 60 min. During treadmill running, noradrenergic and dopaminergic activities increased significantly in the VMH. Extracellular 5-HT concentrations in the VMH did not change during treadmill running at the exercise intensity. Given the known effects of NA in the VMH on energy metabolism, our results suggest that the increase in noradrenergic activity in the VMH is related to the enhancement of fat oxidation during exercise.

Key Words  ventromedial hypothalamus, exercise, noradrenaline, microdialysis, fat oxidation

Physical exercise dramatically increases the energy expenditure of animals. In terms of energy substrate, at the onset of exercise, the contribution of carbohydrates to the energy expenditure is relatively predominant, and decreases gradually with the progression of exercise, while fat consumption increases progressively. Carbohydrate is the more versatile energy substrate, as it can contribute towards both aerobic and anabolic energy production. However, the carbohydrate stores in the body are comparatively little relative to the fat stores. Thus, sparing carbohydrate by enhancing fat oxidation would be advantageous for performance during endurance exercise.

Neural activity in the central nervous system (CNS) during exercise has been examined in several brain areas to date (1). During treadmill running, noradrenergic and dopaminergic activities in the striatum (2), dopaminergic activity in the nucleus accumbens (3), and serotonergic activity in the hippocampus and frontal cortex (4) increase significantly, as measured by in vivo microdialysis. However, neural activity during exercise in the hypothalamus, which is closely related to energy metabolism, has not been elucidated to date.

The ventromedial hypothalamus (VMH) is a nucleus in the hypothalamus that regulates whole body energy metabolism via the sympathetic nervous system (SNS) (5–8). Furthermore, electrical stimulation of the VMH increases blood glycerol and non-esterified fatty acids (NEFA) (9) and decreases the respiratory exchange ratio (RER) (10). Hence, the VMH appears to be the pivotal site in the CNS for fat metabolism (9–11).

Some reports have also indicated that the VMH is involved in energy metabolism during exercise (12–16). Electrolytic lesions of the VMH attenuate the increase in blood NEFA and noradrenaline (NA) during swimming (14–16). Microinjection of a β-adrenoceptor antagonist into the VMH attenuates the increase in blood glucose and NEFA (12), and NA (13) during swimming. Hence, noradrenergic neurons projecting to the VMH appear to be involved in the regulation of energy metabolism during exercise. However, it is not clear whether exercise influences the activity of noradrenergic projections to the VMH.

Based on the above findings, we hypothesize that during exercise, noradrenergic neurons projecting to the VMH are activated, and play an important part in enhancing fat oxidation. To test this hypothesis, we used in vivo microdialysis to investigate the effect of exercise on the activity of monoaminergic (NA, dopa-
Table 1. Effect of the treadmill running on plasma energy substrates and hormones.

<table>
<thead>
<tr>
<th></th>
<th>0 min</th>
<th>30 min</th>
</tr>
</thead>
<tbody>
<tr>
<td>Glucose (mg/dL)</td>
<td>153.8±8.005</td>
<td>154.4±9.686</td>
</tr>
<tr>
<td>Lactate (mM)</td>
<td>1.270±0.3421</td>
<td>0.794±0.3153</td>
</tr>
<tr>
<td>NEFA (mEq/L)</td>
<td>0.335±0.0532</td>
<td>0.643±0.04110</td>
</tr>
<tr>
<td>3-Hydroxybutyric acid (mM)</td>
<td>115.2±24.00</td>
<td>211.7±31.41*</td>
</tr>
<tr>
<td>TG (mg/dL)</td>
<td>91.32±16.71</td>
<td>70.86±9.538</td>
</tr>
<tr>
<td>Insulin (ng/mL)</td>
<td>1.068±0.1796</td>
<td>0.793±0.08164</td>
</tr>
<tr>
<td>Glucagon (ng/mL)</td>
<td>0.7178±0.02314</td>
<td>0.623±0.03342</td>
</tr>
<tr>
<td>Glucocorticoid (ng/mL)</td>
<td>481.8±112.8</td>
<td>1.658±229.2**</td>
</tr>
<tr>
<td>Adrenaline (pg/mL)</td>
<td>14.70±1.359</td>
<td>14.97±4.467</td>
</tr>
<tr>
<td>Noradrenaline (pg/mL)</td>
<td>2.850±0.1441</td>
<td>4.285±0.6335</td>
</tr>
</tbody>
</table>

Effect of treadmill running on blood energy substrates and hormones of the rats. 0 min, values immediately before exercise; 30 min, values at the end of exercise. Running speed was 15 m/min, and the slope of the running belts was 3°. The values are expressed as means±SE (n=4–6). **p<0.01, *p<0.05.

MATERIALS AND METHODS

Animals. This study was conducted in accordance with the ethical guidelines of the Kyoto University Animal Experimentation Committee and in complete compliance with the National Institutes of Health Guide for the Care and Use of Laboratory Animals, and was approved by the above-mentioned committee. Male Sprague-Dawley rats (5 wk old for respiratory gas analysis during exercise, 7.5 wk old for microdialysis; Japan Charles River, Tokyo, Japan) were used. All animals were maintained under controlled environmental conditions (22±0.5°C, 12-h light-dark cycle), and fed ad libitum with commercial chow (MF, Oriental Yeast Co., Ltd., Tokyo, Japan) and water. The rats were housed individually in plastic cages (33×23×12 cm).

Microdialysis procedure. The animals were anesthetized with isoflurane (Forane, Abbott Japan, Tokyo, Japan) and placed in a stereotaxic frame adapted for rat surgery. Subsequently, the skull was exposed, and holes for microdialysis were drilled. The coordinates for the VMH guide cannula (AG-12, Eicom, Kyoto, Japan) and microdialysis probe (AI-12-01, Eicom, membrane length 1.0 mm) were AP: -2.3, ML: 0.5, DV: -9.0 from the bregma. All coordinates were determined according to the stereotaxic atlas of Paxinos and Watson (17). Cannulas were secured to the skull with an adhesive bond (LOCTITE 454, Henkel Japan, Yokohama, Japan). A dummy cannula (AD-12, Eicom) was inserted into the guide cannula and secured with cap nut (AC-1, Eicom).

Rats were allowed 5–7 d to recover from the surgery, and only animals with body weights greater than those before surgery were used. To habituate the rats to running on the treadmill, on the day before the experiment the rats (~9 wk of age) were made to run at 8 m/min (incline 3°) for 15 min. On the experimental day, the dummy cannula was removed, and the microdialysis probe (membrane length 1.0 mm) was inserted into the VMH via the guide cannula. The rats were allowed to remain sedentary for 120 min on the treadmill (MK-680, Muramachi Kikai, Tokyo, Japan) without food and water, before being subjected to running at 15 m/min (incline 3°) for 60 min. After running, they were allowed to remain sedentary for 70 min. Ringer’s solution, containing 147 mM Na+, 4 mM K+, 2.3 mM Ca++, and 155.6 mM Cl− was perfused at 2 µL/min using a micro syringe pump (ESP-64, Eicom) and dialysate was collected every 10 min for 180 min from 50 min before the start of running using an automated fraction collector (EFC-82, Eicom). Samples were cooled to 4°C by an electronic cooler (EFR-82, Eicom) during this procedure, and then stored at −80°C until determination.

To quantify the monoamine levels in the dialysate, samples were analyzed by reversed-phase HPLC with an electrochemical detector using an EICOMPAK CAX column (2.0×, 200 mm long; Eicom). The applied voltage was set at 450 mV (relative to an Ag/AgCl reference electrode). The mobile phase was comprised of 70% (v/v) 0.1 M ammonium acetate buffer (pH 6.0), 30% (v/v) methanol, 50 mM sodium sulfate, and 50 mg/L 2Na-EDTA (flow rate, 0.25 mL/min). The mean of the values obtained from 3 samples before the treadmill running was set at 100% (baseline levels), and all subsequent sample values were expressed as a percentage of this baseline value.

Histological analysis. After completion of the experiment, the rats used for microdialysis were deeply anesthetized with pentobarbital sodium. The brain was removed from the skull, frozen, and cut into 30 µm sections. The placement of the microdialysis probe was verified by thionine blue staining. Data obtained from the rats with inappropriate probe placement were excluded from the analysis.

Respiratory gas analysis during exercise. Rats were trained to run for 60 min/d on a treadmill for 5 d/wk. The initial treadmill speed was 8 m/min (incline 3°). The speed was gradually increased so that after 2 wk, these rats could run at 25 m/min (incline 3°) for 60 min. After 2 wk of training, rats (~9 wk of age) underwent respiratory gas analysis during treadmill running by indirect calorimetry as described previously (18).

All rats were randomly assigned to three RER measurements at each exercise intensity (5, 15, 25 m/min...
The experiments were conducted on 3 consecutive days. Rats were deprived of food for 90 min, transferred to the treadmill chamber (Treadmill Simplex II, Columbus Instruments, Columbus, OH), and the respiratory gas analysis was immediately started. Rats were allowed to remain sedentary for 30 min in the chamber, made to run at the designated intensity described above, and again allowed to remain sedentary for an additional 30 min.
Analysis of plasma energy substrates and hormones. Blood samples were collected by decapitation. To habituate the rats to running on the treadmill, on the day before the experiment, rats were made to run at 8 m/min (incline 3°) for 15 min. Rats were deprived of food for 90 min, and samples were collected before and after treadmill running at 15 m/min (incline 3°) for 30 min. Plasma samples were isolated by centrifugation and stored at −20°C until analysis. Plasma glucose, lactate, NEFA, 3-hydroxybutyric acid, and triacylglycerol (TG) were measured using appropriate assay kits (Glucose AR2: Wako Pure Chemical Industries, Ltd., Osaka, Japan; Determiner LA: Kyowa Medex, Tokyo, Japan; NEFA C Test Wako: Wako Pure Chemical Industries; Ketone Test: Sanwa Chemical Institute, Nagoya, Japan; and TG E Test Wako: Wako Pure Chemical Industries, respectively). Plasma insulin, glucagon and glucocorticoid were measured using appropriate ELISA kits (Ultradsensitive Rat Insulin Kit: Morinaga, Yokohama, Japan; Glucagon ELISA Kit Wako: Wako Pure Chemical Industries; and ACTIVE Rat Corticosterone ELIA: Diagnostic Systems Laboratories, Webster, Texas, respectively). Plasma adrenaline and noradrenaline were measured as previously described (19).

Statistics. Data are expressed as the mean ± SE. Data from experiments of microdialysis and energy metabolism during exercise were analyzed by one-way ANOVA and Bonferroni’s multiple comparison test. Data from measurements of plasma samples were analyzed using an unpaired t-test. p-values of 5% or less were considered statistically significant. Statistical analysis was conducted using the GraphPad Prism 4 software package (GraphPad, San Diego, CA).

RESULTS

Respiratory gas analysis during exercise

Respiratory exchange ratio (RER; Fig. 1A), oxygen consumption (VO₂; Fig. 1B), carbohydrate oxidation (CHO; Fig. 1C), and fat oxidation (FAT; Fig. 1D) during treadmill running at 5, 15, 25 m/min (incline 3°) for 60 min are shown in Fig. 1. As the exercise load intensified, oxygen consumption in the rats increased significantly. Similarly, oxidation of carbohydrate and fat was augmented depending on the exercise intensity. However, there were no significant differences between carbohydrate oxidation at 5 m/min and that at 15 m/min, or between fat oxidation at 15 m/min and that at 25 m/min. The proportions of energy derived from fat oxidation during treadmill running at 5, 15, 25 m/min were 42.9±4.71, 43.9±2.67, 34.9±2.11%, respectively, and decreased significantly at 25 m/min compared with those at 5 and 15 m/min.

Effect of treadmill running on plasma energy substrates and hormones

Plasma concentrations of glucose, lactate, NEFA, 3-hydroxybutyric acid, TG, insulin, glucagon, glucocorticoid, adrenaline, and noradrenaline before and after the treadmill running at 15 m/min for 30 min are shown in Table 1. Plasma NEFA, 3-hydroxybutyric acid, and glucocorticoid concentrations were significantly higher after the treadmill running than those before running (NEFA: p<0.01; 3-hydroxybutyric acid: p<0.05; glucocorticoid: p<0.01). Treadmill running at this intensity caused no significant changes in the plasma concentrations of other substances or hormones. Effect of treadmill running on extracellular monoamine levels in the ventromedial hypothalamus

As shown in Fig. 2, NA and DA concentrations increased significantly during the treadmill running (NA: p<0.001; DA: p<0.001, by repeated measures ANOVA). The NA levels were significantly higher at each time point from 15 to 75 min compared with the NA level just before the onset of running (−5 min (p<0.001, by Bonferroni’s multiple comparison test). Significantly higher levels of DA were observed at 45 and 65 min compared with that just before running (−5 min (45 min: p<0.01; 65 min: p<0.05, by Bonferroni’s multiple comparison test). The levels of 5-HT did not change significantly during or after running compared with that at −5 min (p=0.8374, by repeated measures ANOVA). The baseline values of NA, DA, and 5-HT were 21.95±1.602, 13.63±3.091, and 19.56±2.456 pg/mL, respectively.

DISCUSSION

Fat oxidation in the rats during treadmill running at 15 m/min (incline 3°) for 60 min was more likely to be effective than running at other velocities, as determined by respiratory gas analysis (The percentage of energy derived from fat oxidation: 5 m/min, 42.9%; 15 m/min, 43.9%; 25 m/min, 34.9%). Fat oxidation rates have been reported to increase with increasing exercise intensity, peak at intensities between 45 and 65% of maximum oxygen consumption, and then decrease at higher intensities (20–22). With respect to the efficiency of fat oxidation, the exercise conditions employed
in this experiment were regarded as between low and moderate intensity. As we focused on fat metabolism during exercise in the present study, this exercise condition was used in the subsequent studies.

To the best of our knowledge, this study represents the first time that changes in monoaminergic activity in the VMH during treadmill running were determined using in vivo microdialysis (Fig. 2).

Extracellular NA concentrations in the VMH increased significantly during the treadmill running, increasing immediately after the onset of running, and reverting to the basal level after the completion of the running exercise. Microinjection of NA into the VMH has been shown to elicit an increase in the firing rate of sympathetic nerves (6), and an increase in blood NEFA (11). In addition, microinjection of a β-adrenoceptor antagonist into the VMH attenuated the increase in blood NEFA during swimming (12). The increase in noradrenergic activity in the VMH during treadmill running was expected to accelerate lipolysis in adipose tissues via the SNS, and increase the blood NEFA. Randle et al. found that an increase in the concentration of NEFA in the perfusate enhanced NEFA transport into isolated muscle cells (23, 24). Hence, the increase in noradrenergic activity in the VMH could be related to the mechanism of enhancing fat oxidation during exercise.

The VMH regulates energy homeostasis via the SNS (5–8). The adrenal medulla is intimately connected with the SNS, and activation of sympathetic nervous activity elicits the secretion of adrenaline and noradrenaline into the blood from the adrenal medulla, which influences energy metabolism. In the present study, there was a significant increase in plasma concentrations of NEFA and 3-hydroxybutyric acid during exercise, but no differences in plasma concentrations of catecholamines compared with before running. This suggested that the changes in the secretion of catecholamines from the adrenal medulla had no effect on the metabolic changes during exercise at the exercise intensity employed in this study.

White adipose tissue (WAT) is innervated by the SNS, and sympathetic activation promotes lipolysis and the secretion of NEFA and glycerol into the blood (25, 26). Noradrenergic neurons projecting to the VMH increase energy metabolism via the SNS (6, 11). However, few projections from the VMH to WAT have been reported (26–28). Meanwhile, neurons in the VMH project to several brain areas, especially to the periaqueductal gray (PAG) (29). Sympathetic innervation to WAT has been demonstrated using a viral transneuronal tracer, and SNS projections to WAT appear to involve many neurons from the PAG (26–28). Therefore, we suggest that the increase in noradrenergic activity in the VMH during treadmill running stimulates the SNS innervation of WAT through synaptic transmission from the PAG, and results in the enhancement of fat oxidation during exercise.

Extracellular DA concentrations in the VMH increased significantly during treadmill running, increasing from 25 min after the onset of running, and reverting to the basal level after completion of the running exercise. As the fat oxidation was evoked immediately after the onset of running (Fig. 1D), there was a time lag between the onset of running and the increase in the VMH DA levels. Therefore, it is likely that dopaminergic neurons projecting to the VMH are not involved in the enhancement of fat oxidation during exercise. In addition, there have been many reports that dopaminergic neurons projecting to the VMH are related to energy metabolism to date. This may support our hypothesis described above.

Extracellular 5-HT concentrations in the VMH did not change during treadmill running at the exercise intensity employed in this microdialysis study. Because microinjection of 5-HT into the VMH produces an increase in the firing rate of sympathetic nerves (6), it would appear that serotonergic neurons projecting to the VMH play a regulatory role in energy metabolism. However, the present study shows that serotonergic neurons projecting to the VMH are not involved in energy metabolism during moderate exercise.

Taking these data into consideration, it is most likely that the activity of noradrenergic neurons projecting to the VMH is involved in the enhancement of fat oxidation during exercise.

The cell bodies of noradrenergic neurons projecting to the CNS are localized in the locus cereuleus and the brain stem. The brain stem receives metabolism-related neuronal and humoral input from peripheral tissues. As noradrenergic neurons originating from the A1 and the A2 regions of the brain stem appear to be activated during treadmill running (32), we expect that the same neurons were also activated under our experimental conditions. Noradrenergic neurons projecting to the VMH are activated during insulin-induced hypoglycemia (33) or 2-DG-induced inhibition of glucose oxidation (34). These observations indicate that the activities of noradrenergic neurons projecting to the VMH play a critical role in glucose homeostasis. In addition, microinjection of a β-adrenoceptor antagonist into the VMH attenuates the increase in blood glucose and NEFA during swimming (12). At the onset of exercise, we observed acute increases in energy expenditure and carbohydrate oxidation, as indicated by the increments in oxygen consumption and RER (Fig. 1). These changes may lead to a relative shortage of blood glucose. Noradrenergic neurons projecting to the VMH might subsequently detect this low energy level and transmit the information to the VMH. Thus, this activation would then lead to: (1) activation of the VMH, (2) an increase in SNS activity, partly through the PAG, (3) increased lipolysis in adipose tissues, (4) recruitment of NEFA into the blood, and (5) enhancement of FA oxidation.

REFERENCES


