Transforming growth factor-β in the brain regulates fat metabolism during endurance exercise

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Inoue, and Tohru Fushiki. Transforming growth factor-β in the brain regulates fat metabolism during endurance exercise. Am J Physiol Endocrinol Metab 291: E1151–E1159, 2006.—We have previously reported that the concentration of transforming growth factor-β (TGF-β) increases in the cerebrospinal fluid of rats during exercise and that there is an increase in whole body fat oxidation following the intracisternal administration of TGF-β. These results led us to postulate that TGF-β in the brain regulates the enhancement of fatty acid oxidation during exercise. To test this hypothesis, we carried out respiratory gas analysis during treadmill running following the inhibition of TGF-β activity in rat brain by intracisternal administration of anti-TGF-β antibody or SB-431542, an inhibitor of the type 1 TGF-β receptor. We found that each reagent partially blocked the increase in the fatty acid oxidation. We also compared the plasma concentrations of energy substrates in the group administered anti-TGF-β antibody and the control group during running. We found that the plasma concentrations of nonesterified fatty acids and ketone bodies in the group administered anti-TGF-β antibody were lower than in the control group at the end of running. In the same way, we carried out respiratory gas analysis during treadmill running after depressing corticotropin-releasing factor activity in the brain using intracisternal administration of astressin, an inhibitor of the type 1 TGF-β receptor. However, there were no significant differences in respiratory exchange ratio or oxygen consumption in moderate running (60% maximum oxygen consumption). These results suggest that brain TGF-β has a role in enhancing fatty acid oxidation during endurance exercise and that this regulation is executed at least partly via the type 1 TGF-β receptor signal transduction system.

Energy substrates during exercise are determined by the intensity and duration of the exercise. The main fuel sources for exercise are carbohydrates (CHO) and fat (32). To exercise properly, the appropriate proportions of CHO and fat should be mobilized during the exercise. The preferential use of fat is considered advantageous for endurance exercise, because it is usually a more abundant source of energy than CHO. For example, in endurance exercise, such as the marathon, the prior utilization of fat stores enables glycogen to be spared, which can be very useful for the finishing spurt in the event, and leads to enhanced performance (15, 21). Therefore, it is important to elucidate the mechanism that determines the proportion of each substrate utilized in response to the intensity of the exercise and the recruitment of total energy substrates in fulfilling the demand for energy during exercise.

There have been numerous attempts to elucidate this mechanism. Randle et al. (28, 29) found that an increase in the concentration of nonesterified fatty acids (NEFA) in the perfusate enhanced NEFA transport while depressing CHO uptake into isolated skeletal muscles. However, glycogenolysis and CHO oxidation during exercise are enhanced by the prior augmentation of the glycogen content in muscle (14, 35, 38). A decline in hepatic glycogen because of starvation or a low-CHO diet inversely decreases CHO oxidation and increases fat oxidation during endurance exercise (8, 9, 26, 35). As described above, many studies have shown that peripheral factors such as glycogen, plasma NEFA, insulin, and glucagon influence the selection of energy substrates. On the other hand, some studies have demonstrated the involvement of the central nervous system (CNS) in the regulation of metabolism in the peripheral tissues (7, 12, 23, 25, 27, 31), although they have not examined the role of the CNS during exercise. Therefore, it is most likely that the CNS participates in the regulation of energy metabolism during exercise. However, to the best of our knowledge, few studies have investigated the regulatory role of the CNS in energy source selection during exercise.

Our laboratory has previously reported that the concentration of transforming growth factor-β (TGF-β) increases in the cerebrospinal fluid of rats during exercise and that the intracisternal administration of TGF-β enhances fat oxidation and increases the concentration of both NEFA and ketone bodies in plasma (1, 18, 39). These results led us to postulate that TGF-β in the brain regulates the enhancement of fatty acid oxidation during exercise.

To test this hypothesis, we analyzed metabolic variance during exercise following the inhibition of TGF-β activity in rat brain. As we were particularly interested in the regulation of fatty acid oxidation, we subjected rats to moderate exercise (~60% of maximal oxygen consumption, 60% VO2max) so as not to exceed the lactate threshold (36, 37). Generally, during moderate exercise, CHO is thought to be used first, with fatty acid oxidation gradually increasing as the exercise continues (36).

Increased expression of corticotropin-releasing factor (CRF) is also caused by exercise. Timofeeva et al. (33) showed that CRF mRNA increases in many brain regions after exercise. Recently, the increase in CRF after exercise was reported to be relevant to postexercise anorexia (20). CRF has not, however, been studied in relation to its effect on metabolism during the exercise.
exercise. Therefore, we analyzed metabolic changes during exercise with concomitant depression of brain CRF activity to elucidate its contribution to energy metabolism and determine whether it has any relation to the effect of TGF-β.

**EXPERIMENTAL PROCEDURES**

**Animals**

Male Sprague-Dawley rats (~200 g) were purchased from Charles River Japan (Yokohama, Japan). All animals were acclimatized to an inverse 12:12-h light-dark cycle (1800 light on/0600 light off) for 1 wk to become active during the experimental time. They were housed individually in plastic cages (33 × 23 × 12 cm). Room temperature was maintained at 22 ± 0.5°C and humidity at 50 ± 5%. Rats could access food and water ad libitum. The care and treatment of the experimental animals conformed to the Kyoto University guidelines for the ethical treatment of laboratory animals. All protocols were approved by the Animal Experimentation Committee of the Graduate School of Agriculture, Kyoto University.

**Reagents**

Pan-specific anti-TGF-β antibody, which binds to TGF-β1, -β2, -β3, and -β5, and preimmune antibody, used as control for immunonabulation, were purchased from R&D Systems (Minneapolis, MN). To exclude the possibility of contamination that might cause artifacts in the experiment, the antibody vehicle was replaced with artificial cerebrospinal fluid by gel filtration (NAP-10 Column; Amersham Biosciences, Piscataway, NJ). SB-431542 (Torcis Bioscience, Avonmouth, UK), a specific inhibitor of type 1 TGF-β receptor (TβR1) kinase activity (4, 16), was dissolved in a mixture of DMSO and ultra-purified water (1:2 vol/vol). Astressin (Sigma, St. Louis, MO), which specifically antagonizes CRF receptors 1 and 2 (13), was dissolved in ultra-purified water.

**Apparatus**

A rat treadmill (Columbus Instruments, Columbus, OH) was used for running exercise. This apparatus consisted of three identical running lanes, each enclosed in a metabolic chamber (54.6 × 116.8 × 203.2 cm). The slope of the running belts was 10° throughout the training and test sessions.

The gas analysis system consisted of three treadmill chambers (as described above), CO₂ and O₂ analyzers (Arco System, Tokyo, Japan), and an air-lane switching system (Arco System). Details of the methods have been described previously (19). Briefly, room air was pumped through the chambers at a rate of 2.0 l/min, while expired air was dried in a thin cotton column and then directed to an O₂/CO₂ analyzer, which used mass spectrometry. The respiratory exchange ratio (RER), amount of oxidation of CHO per body weight (CHO/w), and amount of fatty acid per body weight (Fat/w) were calculated from O₂ consumption (VO₂) and CO₂ production. Details of the equations involved have been described elsewhere (19). Before the test sessions began, the analyzer was calibrated using highly purified standard gas with an accurately known composition.

**Treadmill Training and Surgery**

All rats were trained to run for 30 min/day on a treadmill for 6 days, so that they were well accustomed to experimental handling and running on the treadmill at 21 m/min (~60% VO₂ max). Two days after the last training, they were anesthetized with 2.5% isoflurane (Abbott Japan, Osaka, Japan), clamped into a stereotactic apparatus, and implanted with a permanent 23-gauge guide cannula for sample injection into the cisterna magna. Each cannula was inserted 3.0 mm posterior to the lambda and 8.7 mm deep and inclined anteriorly at an angle of 60° to the horizontal plane. These coordinates were modified from those used in our laboratory’s previous study (39). After implantation of the guide cannula, the rats were allowed to recover for 3 days.

**Metabolic Analyses During Exercise: Depression of CRF Activity in Brain**

Anti-TGF-β antibody (5 μg) and SB-431542 (0.1 μmol), dissolved in 10 μl of vehicle, were injected into the cisterna magna through a 50-μl microsyringe and a polyethylene tube. Fifteen minutes after injection, rats were moved to the metabolic chambers, and the measurement of RER and VO₂ per body weight (VO₂/w) was started, using the gas analysis system. For the first 20 min, the rats were kept at rest; they were then made to run at 21 m/min for 30 min. After running, they were kept sedentary in the same chamber for another 30 min. Metabolic data were recorded throughout the entire test session. However, because data near the commencement of the gas analysis were unstable, data covering the first 10 min were discarded.

Energy substrates and hormones in the blood were measured under the same conditions as for gas analysis described above. Blood samples were collected by decapitation. Rats were divided into six groups, treated as follows: after injection with anti-TGF-β or preimmune antibody, rats were kept at rest for 20 min and then decapitated (designated as prerunning; 0 min); after injection with anti-TGF-β or preimmune antibody, rats were kept at rest for 20 min and then forced to run for 30 min at 21 m/min before being decapitated (at the end of running: 30 min); and after injection with anti-TGF-β or preimmune antibody, rats were kept at rest for 20 min with a further 30-min rest period and then decapitated (nonrunning). Plasma samples were isolated by centrifugation and stored at −20°C until analysis. Plasma glucose, NEFA, ketone bodies, and lactic acid were measured using appropriate assay kits (Glucose AR2: Wako Pure Chemical Industries, Osaka, Japan; NEFA C Test Wako: Wako Pure Chemical Industries; Ketone Test: Sanwa Chemical Institute, Nagoya, Japan; and Determiner LA: Kyowa Medex, Tokyo, Japan, respectively). Plasma insulin and glucagon were measured using appropriate ELISA kits (Ultral仓 sensitive Rat Insulin Kit, Morinaga, Yokohama, Japan, and Glucagon EIA kit, Yanaihara Institute, Shizuoka, Japan, respectively).

**Metabolic Analyses During Exercise: Depression of CRF Activity in Brain**

Astrassin (10 μg, dissolved in 10 μl of vehicle) was injected into the cisterna magna of rats, and gas analyses were carried out during an exercise session using the same procedures described above in relation to inhibition of TGF-β activity in the brain. To evaluate the degree of depression of CRF activity, we compared the plasma concentrations of ACTH among three groups of rats treated as follows: untreated (control group); 30 min after the injection of ultra-purified water (10 μl); rats were injected with CRF (4.97 μg, dissolved in 10 μl of vehicle) and kept at rest for a further 30 min and then decapitated (vehicle-CRF group); and 30 min after the injection of astressin (10 μg), rats were injected with CRF (4.97 μg) and kept at rest for a further 30 min and then decapitated (astressin-CRF group). Plasma samples were isolated by centrifugation and stored at −20°C until analysis. The concentration of plasma ACTH was determined by radioimmunoassay using 125I (ACTH-RIA Kit; Bachem Peninsula Laboratories).

**Metabolic Analyses in the Sedentary State**

Anti-TGF-β antibody (5 μg), SB-431542 (0.1 μmol), and astressin (10 μg) were injected into the cisterna magna. After injection, rats were moved to the metabolic chambers, and measurement of RER and VO₂/w was started. This measurement was conducted for 70 min while the rats remained sedentary.

**Statistical Analysis**

Data are presented as means ± SE. Data from gas analyses were evaluated using two-way (time × treatment) repeated-measures
ANOVA during and after running periods. When statistical significance existed, an unpaired t-test was performed at each measurement time. Data from measurements of plasma NEFA, ketone bodies, glucose, lactic acid, insulin, and glucagon were evaluated using an unpaired t-test. P < 0.05 was considered significant. Plasma ACTH concentrations of energy substrates and hormones in the anti-TGF-β group with those in the control group. Before exercise (0 min), there were no significant differences in plasma glucose or lactic acid concentrations between the two groups (Fig. 3, A–D, respectively). At the end of treadmill running (30 min), the plasma concentrations of NEFA and ketone bodies in the anti-TGF-β group (n = 5) were lower than those in the control group (n = 6) (Fig. 3, A and B, respectively). However, there were no significant differences in plasma glucose or lactic acid concentrations between the two groups (Fig. 3, C and D, respectively). The administration of anti-TGF-β antibody did not affect the concentrations of plasma insulin or glucagon, and there were no significant differences from the control group before or after exercise (Fig. 3, E and F, respectively).

We then studied the involvement of TGF-β in regulating fat metabolism by blocking its signal transduction with a specific...
inhibitor, SB-431542, which is an inhibitor of TβR1 [also known as activin receptor-like kinase 5 (ALK5)] that functions by inhibiting the kinase activity of this receptor. This inhibitor was injected into the cisterna magna of rats to block the signal transduction system of TβR1 in the brain. Figure 4 shows changes in the metabolic rates of rats to which SB-431542 (SB-431542 group; n = 9) or vehicle (vehicle group; n = 8) was intracisternally administered. The RER of the SB-431542 group was significantly higher than that of the vehicle group over 30 min of treadmill running (Fig. 4A: \( P = 0.0128 \)). Changes in \( \text{VO}_2/\text{w} \) during exercise were almost the same in the two groups (Fig. 4B: \( P = 0.105 \)). \( \text{CHO}/\text{w} \) in the SB-431542 group was higher than in the vehicle group (Fig. 4C: \( P = 0.0019 \)). No significant difference in Fat/w was observed between the two groups during treadmill running (Fig. 4D: \( P = 0.333 \)). Although total fat oxidation during 30 min of treadmill
running tended to be lower in the SB-431542 group than the vehicle group, there was no statistical difference (vehicle group, 318.7 ± 17.0 μg/g; SB-431542 group, 276.9 ± 13.8 μg/g; \( P = 0.073 \), unpaired t-test).

Over the 30 min after the end of running, CHO/w in the SB-431542 group was higher than in the vehicle group (Fig. 4C: \( P < 0.0062 \)). However, there were no significant differences in RER (Fig. 4A: \( P = 0.486 \)), \( \dot{V}O_2 \)/w (Fig. 4B: \( P = 0.362 \)), or Fat/w (Fig. 4D: \( P = 0.804 \)) from the vehicle group.

The RER and \( \dot{V}O_2 \) of rats maintained in a sedentary state after the administration of vehicle or SB-431542 showed that SB-431542 had no significant effect on metabolic rate compared with vehicle (Fig. 5, A and B: \( P = 0.8675 \) and 0.8928, respectively).

### Metabolic Analyses During Exercise: Depression of CRF Activity in Brain

The activity of CRF was depressed by the injection of astressin into the cisterna magna of rats. Astressin is a potent inhibitor of CRF receptors 1 and 2. The changes in metabolic rate in rats to which vehicle (vehicle group; \( n = 5 \)) or astressin (astressin group; \( n = 7 \)) was administered intracisternally are shown in Fig. 6, A and B. There were no significant differences in RER or \( \dot{V}O_2 \)/w between the two groups during running (Fig. 6, A and B: \( P = 0.0722 \) and 0.9842, respectively) or over the 30 min after the end of running (Fig. 6, A and B: \( P = 0.9346 \) and 0.9214, respectively). Although RER in the astressin group tended to be lower than in the control group before running...
there was no significant difference between the groups (control group, 0.833 ± 0.017; astressin group, 0.811 ± 0.012; \(P = 0.297\), unpaired \(t\)-test). The numbers on the histogram bars represent the number of animals per group. \(^{a}P < 0.05\) vs. control group determined by one-way ANOVA and post hoc Fisher’s paired least significant difference (PLSD). \(^{b}P < 0.05\) vs. vehicle-CRF group determined by one-way ANOVA and post hoc Fisher’s PLSD.

Additionally, we carried out gas analyses of rats kept sedentary after intracisternal administration of vehicle or astressin and found no significant differences in RER or \(V_{O2}/w\) between the two groups (Fig. 7, A and B, respectively).

DISCUSSION

Inhibition of TGF-\(\beta\) activity in the brain by intracisternal administration of anti-TGF-\(\beta\) antibody blocked the decrease in RER and increase in fatty acid oxidation (Fat/w) during treadmill running, as determined by respiratory gas analysis (Fig. 1, A and D). This suggests that TGF-\(\beta\) in the brain has the physiological function of facilitating the overall oxidation of fatty acids during exercise. In addition, the increased concentrations of NEFA and ketone bodies in plasma that normally occur during exercise were lessened by the same treatment (Fig. 3, A and B); in contrast, intracisternal administration of TGF-\(\beta\) increases the concentrations of NEFA and ketone bodies in plasma (39). Because increased plasma NEFA during
endurance exercise results from an excess supply of NEFA from lipolysis beyond the actual requirements of the peripheral tissues (22), it is presumed that the inhibition of TGF-β activity in the brain prevented lipolysis in adipose tissue and attenuated the increase in plasma NEFA concentration. A tendency toward an increased plasma glycerol concentration (an index of lipolysis) has been reported following intracisternal administration of TGF-β (39), and this supports the speculation described above.

In the liver, the enhancement of fat metabolism produces more acetyl-CoA from fatty acids and, consequently, more ketone bodies from the conversion of acetyl-CoA. Therefore, there may be two explanations for abrogation of the increase in the concentration of plasma ketone bodies in this study. One is the decrease in plasma NEFA, which is the source of ketone body production; the other is the possibility that the oxidation of fatty acids in the liver itself was suppressed by inhibition of TGF-β activity in the brain. Because ketone bodies, unlike fatty acids, are water soluble, they can be transported more easily than fatty acids, incorporated into the heart and skeletal muscles, and used as an energy source. Although no reported studies have examined the role of ketone bodies during endurance exercise, it is likely that ketone bodies are also utilized as an energy source during exercise.

Rats administered anti-TGF-β antibody retained a higher CHO/w than control rats (Fig. 1C). As our laboratory has already demonstrated that the intracisternal administration of TGF-β to sedentary rats has no effect on CHO/w (39), inhibition of TGF-β in the brain is unlikely to directly enhance CHO oxidation. Accordingly, the observed increase may indicate the compensatory mobilization of CHO for exercise maintenance because the acquisition of energy from fatty acid oxidation had been impeded by inhibiting TGF-β activity in the brain.

\( \dot{V}_{\text{O}_2}/w \) was not affected by inhibiting brain TGF-β activity (Fig. 1B). Our laboratory has previously reported that the intracisternal administration of TGF-β does not change \( \dot{V}_{\text{O}_2}/w \) in sedentary rats (39). These findings indicate that the energy requirement for exercise is solely determined by the intensity of the exercise and that TGF-β in the brain regulates fatty acid metabolism without altering total energy expenditure.

Blockade of TGF-β signal transduction by the intracisternal administration of SB-431542, an inhibitor of TβR1 (ALK5), lessened the decrease in RER during treadmill running (Fig. 4A), increased CHO/w (Fig. 4C), and tended to depress Fat/w (Fig. 4D). \( \dot{V}_{\text{O}_2}/w \) was unaffected (Fig. 4B). These changes are essentially the same as occurred following immunoneutralization of the ligand (i.e., TGF-β inhibition by anti-TGF-β antibody) and confirm the significance of TGF-β activity in energy metabolism during exercise. The changes in Fat/w were not statistically significant compared with the control group, unlike the changes following treatment with anti-TGF-β antibody. We also found that the total amount of fat oxidation over 30 min of exercise tended to be less in the SB-431542 group than in the vehicle group, but the difference was not significant. This lack of significance was partly because of the gradual decrease in Fat/w in the SB-431542 group before the exercise commenced; however, we cannot explain the cause of this decrease because no change was observed between the groups when sedentary (Fig. 5).

In the experiment with anti-TGF-β antibody, the difference in RER between the control and anti-TGF-β groups became apparent in the latter half of the period of treadmill running. In contrast, in the experiment with SB-431542, the difference was clear almost from the beginning of the exercise. This might reflect differences in the efficacy of TGF-β inhibition as a result of the dissimilar modes of action of the two agents. The immunoneutralization of TGF-β with anti-TGF-β antibody depletes ligand concentration, probably in the cerebrospinal fluid, and blocks signal generation, whereas SB-431542 is incorporated in neurons and prevents TβR1 (ALK5) from transmitting the TGF-β signal. Because the increase in active TGF-β concentration during exercise (18) occurs as a consequence of the activation of latent TGF-β in the cerebrospinal fluid and membrane matrix (30), and because there will be a delay before TGF-β is bound by its specific antibody, there may be “leakage” of the signal and a lag in the manifestation of immunoneutralization. On the other hand, SB-431542 may block TβR1 function before the start of exercise. This may explain the difference in time course changes in RER between treatment with anti-TGF-β antibody and treatment with SB-431542. In addition, it is possible that SB-431542 inhibits other ALK homologues (17, 40), which may further help to explain the different results observed with the two agents.

Exercise has been reported to induce an increase in CRF mRNA expression in many parts of the brain (33), while intracerebroventricular administration of CRF increases \( \dot{V}_{\text{O}_2} \) (2, 3), and intracerebroventricular administration of urocortin, a member of the CRF family, induces an increase in \( \dot{V}_{\text{O}_2} \) and facilitates fatty acid oxidation (10). These findings led us to postulate that CRF might have a regulatory role in energy metabolism during exercise. CRF activity was inhibited by the intracisternal administration of astressin, as shown by a 50% reduction of the increase in ACTH concentration (Fig. 6C). However, changes in RER (Fig. 6A) and \( \dot{V}_{\text{O}_2}/w \) (Fig. 6B) during exercise were unaffected by CRF activity. It is thus likely that CRF does not influence the regulation of energy metabolism in moderate-intensity running at 60% \( \dot{V}_{\text{O}_2\max} \). On the other hand, the possibility of its involvement in more intense and stressful exercise remains (11). Although Timofeeva et al. (33) showed an increase in the expression of CRF mRNA during exercise at 60%–70% \( \dot{V}_{\text{O}_2\max} \), they did not examine the CRF peptide. To determine the role of CRF, changes in its biodegradable expression should be examined at various intensities of exercise.

How does the increment in TGF-β in the brain affect the peripheral tissues and enhance the utilization of fat? Because the plasma concentrations of insulin and glucagon at 0 and 30 min were not influenced by the inhibition of TGF-β activity in the brain via intracisternal administration of anti-TGF-β antibody (Fig. 3, E and F), the effect of TGF-β in the brain does not appear to be transduced by insulin or glucagon. It is well known that catecholamines act through β-adrenoceptors in adipose tissue and induce lipolysis during endurance exercise (5, 6, 11, 34). Our laboratory previously demonstrated that intracisternal administration of TGF-β does not result in significant changes in the concentrations of adrenaline and noradrenaline in serum in the sedentary state (39). Although the level of catecholamines was not determined in this study, glucagon, which has similar effects to catecholamines, showed no change. These data suggest the action of TGF-β in the brain is transduced via the autonomic nervous system and not by humoral factors.
Inhibition of TGF-β activity in the brain failed to completely suppress the increase in fatty acid oxidation. As far as the regulation of hormone-sensitive lipase activity in skeletal muscle is concerned, Langfort et al. (24) showed an increase in hormone-sensitive lipase activity in response to muscle contraction induced by electrical stimulation in isolated preparations. This indicates that self-regulation of fatty acid mobilization occurs in skeletal muscle, independent of both hormonal and nervous systems. There appear to be at least two regulatory mechanisms for fat metabolism in peripheral tissues: one dependent on, and the other independent of, TGF-β in the brain. These are likely to influence each other in actual endurance exercise.

In conclusion, we have now demonstrated the following: 1) TGF-β in the brain enhances fatty acid oxidation throughout the whole body during endurance exercise, and this regulation is executed at least partly via the TβR1 (ALK5) signal transduction system. This is the first report demonstrating the involvement of the cerebral factor in the regulation of energy metabolism during exercise. 2) During moderate exercise that does not exceed the lactate threshold, CRF may not be related to the regulation of energy metabolism.

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REFERENCES


