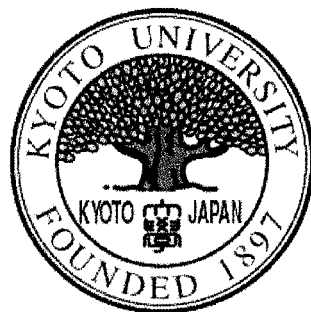


**Studies on physiological role of TGF- β
in infection-induced sickness behavior**

Shigenobu Matsumura

2007

Studies on physiological role of TGF- β in infection-induced sickness behavior



Graduate School of Kyoto University
Degree of Doctor of Philosophy
(Agriculture)

Shigenobu Matsumura

2007

GENERAL INTRODUCTION

It has been investigated the mechanism underlying the manifestation of fatigue in detail focusing on fatigue of skeletal muscle. On the other hand, although the importance is recognized for the elucidation of the cause of feeling of fatigue (central fatigue), or the mechanism which generates feeling of fatigue, there is still little research. In central nervous system, it remained unclear how feeling of fatigue is generated. Feeling of fatigue is recoverable by taking rest unlike the illness. For this reason, this research field is behind compared with other illnesses.

In a previous study, intracisternal administration of cerebrospinal fluid (CSF) collected from rats exhausted by swimming exercise to sedentary mice resulted in a significant decrease in the spontaneous motor activity (SMA) of the mice, as though they were tired (Inoue et al., 1998). The substance responsible for this suppression of spontaneous motor activity of animals was demonstrated to be transforming growth factor- β (TGF- β), suggesting that TGF- β in the brain is involved in the manifestation of feeling of fatigue (Inoue et al., 1999).

Even if it feels fatigue by exercise, exercise cannot be stopped when stopping exercise is connected to die, for example, while fighting with the enemy, escaping from the enemy. In this case, it is necessary to adapt the body metabolism as prolonged exercise can be continued as much as possible.

During prolonged exercise, utilization of energy substrates shows a gradual transition from carbohydrate to fat. Other studies have reported that there is a significant substrate shift toward fat oxidation after high-intensity exercise (Wolfe et al., 1990; Bahr et al., 1991). This is a common phenomenon in exercise physiology, but the complete mechanism of the switch in energy substrates has not been clarified. As compared with fat, carbohydrate is little storage in the body (peripheral tissue, such as skeletal muscle and liver) and glucose is a main energy source of brain. Depletion of glycogen, the storage form of carbohydrate in the skeletal muscle and liver, causes trouble to muscle contraction. A wild animal also needs to leave glycogen to escape from an enemy. Therefore, it becomes important to change energy substrate from carbohydrate to fat particularly in the prolonged exercise.

Feeling of fatigue is thought to be the defensive mechanism to prevent all out exhaustion. If so, TGF- β in the brain may have some positive effects for preventing peripheral exhaustion and recovery. Therefore we hypothesized that TGF- β in the brain changes metabolism by shifting energy substrate toward fat and adapts peripheral tissues to

prolonged exercise and recovery from exercise. In chapter 1, we found that intracranial administration of TGF- β increases fat oxidation, suggesting that feeling of fatigue is closely associated with energy metabolism (Yamazaki et al., 2002).

In addition to during and post-exercise, infection with, for example, an influenza virus causes a strong feeling of fatigue. Other various symptoms such as fever, chills, arthralgia (joint pain), drowsiness, and anorexia are also observed. While these symptoms are not usually considered beneficial, in the case of infection they constitute an important series of defensive responses. Indeed, once these symptoms occur, people recognize infection and try to cope with the sickness and to recover. A high body temperature (fever) suppresses viral proliferation and bacterial growth, and activates and induces the proliferation of immune cells (Kluger et al., 1991). During infection, the feeling of fatigue is a discomfort and makes animals inactive. This suggests that physical immobilization is advantageous for recovery from infection.

Some cytokines are secreted after exercise, and are considered to be related to certain physiological changes after exercise—e.g., anorexia, increase in body temperature and inflammation (Cannon et al., 1989; Pedersen and Hoffman-Goetz, 2000)—that are similar to the symptoms observed in infection. We postulated that TGF- β in the brain, which increases during exercise, plays a role in the response to infection. In chapter 2, we investigated the relationship between TGF- β in the brain and both fever and the feeling of fatigue as typical symptoms during infection. We found that TGF- β increases in the brain during infection, suggesting that TGF- β is related to feeling of fatigue induced by infection. Although we could not demonstrate that TGF- β is related to feeling of fatigue, we demonstrated that TGF- β is related to fever induced by infection (Matsumura et al., 2007). During infection, many factor causes fatigue. It is thought that TGF- β may not to be the main factor generating feeling of fatigue during infection. These results indicated that TGF- β causes fever and feeling of fatigue during infection and promotes recovery from infectious disease.

TGF- β was discovered by virtue of its capacity to induce anchorage-independent growth of normal rat kidney and fibroblast cell lines, i.e., its capacity to induce transformation (Moses et al., 1981; Roberts et al., 1981). Later research has identified TGF- β as one of the cytokines that has multifunctional effects on various cell types. TGF- β promotes cell survival or induces apoptosis, stimulates cell proliferation or induces differentiation (Massague and Chen, 2000; Dennler et al., 2002). TGF- β also regulates the immune system.

In the central nervous system, expressions of TGF- β are seen in various cell types, astrocyte, neuron, choroid plexus epithelial and meningeal cell. It has been reported that tissue trauma, HIV infection, ischemia, Alzheimer's disease, and multiple sclerosis increased TGF- β expression (Wyss-Coray, 2006). TGF- β has been shown to protect cultured neuron from injury. In vivo, administration of recombinant TGF- β potently protects animals against brain injury mediated by ischemic (McNeill 1994), excitotoxic (Ruocco et al., 1999) and oxidative stress (Henrich-Noack et al., 1996). TGF- β promotes synthesis of neurotrophins such as NGF (neuronal growth factor) and neurotrophin-3 and exhibit synergistic action with them (Unsicker et al., 2000). In addition to these functions, we demonstrated that TGF- β causes feeling of fatigue both during infection and exercise.

In the case of infection, the cause of feeling of fatigue apparently differs from exercise. By contrast, TGF- β is increased both by infection and exercise. Therefore TGF- β is thought to be the common factor generating feeling of fatigue. In the both cases, feeling of fatigue has a role for suppressing motivation of physical activity and promoting recovery from infection or exercise.

The evolutionary factor by which TGF- β came to have the function regulating behavior and metabolism is still unknown. TGF- β has a function repairing cell function from injury. Considering this function, it is natural that TGF- β regulates behavior and metabolism to repair the peripheral tissue exhausted by exercise or infection.

References

- Bahr, R., Hostmark, A. T., Newsholme, E. A., Gronnerod, O., and Sejersted, O. M. (1991). Effect of exercise on recovery changes in plasma levels of FFA, glycerol, glucose and catecholamines. *Acta Physiol Scand* **143**, 105-115.
- Cannon, J. G., Fielding, R. A., Fiatarone, M. A., Orencole, S. F., Dinarello, C. A., and Evans, W. J. (1989). Increased interleukin 1 beta in human skeletal muscle after exercise. *Am J Physiol* **257**, R451-455.
- Dennler, S., Goumans, M. J., and ten Dijke, P. (2002). Transforming growth factor beta signal transduction. *J Leukoc Biol* **71**, 731-740.
- Henrich-Noack, P., Prehn, J. H., and Kriegstein, J. (1996). TGF-beta 1 protects hippocampal neurons against degeneration caused by transient global ischemia.

Dose-response relationship and potential neuroprotective mechanisms. *Stroke* *27*, 1609-1614; discussion 1615.

Inoue, K., Yamazaki, H., Manabe, Y., Fukuda, C., Hanai, K., and Fushiki, T. (1999). Transforming growth factor-beta activated during exercise in brain depresses spontaneous motor activity of animals. Relevance To central fatigue. *Brain Res* *846*, 145-153.

Kluger, M. J. (1991). Fever: role of pyrogens and cryogens. *Physiol Rev* *71*, 93-127.

Massague, J., and Chen, Y. G. (2000). Controlling TGF-beta signaling. *Genes Dev* *14*, 627-644.

Matsumura, S., Shibakusa, T., Fujikawa, T., Yamada, H., Inoue, K., and Fushiki, T. (2007). Increase in transforming growth factor-beta in the brain during infection is related to fever, not depression of spontaneous motor activity. *Neuroscience* *144*, 1133-1140.

McNeill, H., Williams, C., Guan, J., Dragunow, M., Lawlor, P., Sirimanne, E., Nikolics, K., and Gluckman, P. (1994). Neuronal rescue with transforming growth factor-beta 1 after hypoxic-ischaemic brain injury. *Neuroreport* *5*, 901-904.

Moses, H. L., Branum, E. L., Proper, J. A., and Robinson, R. A. (1981). Transforming growth factor production by chemically transformed cells. *Cancer Res* *41*, 2842-2848.

Pedersen, B. K., and Hoffman-Goetz, L. (2000). Exercise and the immune system: regulation, integration, and adaptation. *Physiol Rev* *80*, 1055-1081.

Roberts, A. B., Anzano, M. A., Lamb, L. C., Smith, J. M., and Sporn, M. B. (1981). New class of transforming growth factors potentiated by epidermal growth factor: isolation from non-neoplastic tissues. *Proc Natl Acad Sci U S A* *78*, 5339-5343.

Ruocco, A., Nicole, O., Docagne, F., Ali, C., Chazalviel, L., Komesli, S., Yablonsky, F., Roussel, S., MacKenzie, E. T., Vivien, D., and Buisson, A. (1999). A transforming growth factor-beta antagonist unmasks the neuroprotective role of this endogenous cytokine in excitotoxic and ischemic brain injury. *J Cereb Blood Flow Metab* *19*, 1345-1353.

Unsicker, K., and Kriegstein, K. (2000). Co-activation of TGF-ss and cytokine signaling pathways are required for neurotrophic functions. *Cytokine Growth Factor Rev* *11*, 97-102.

Wolfe, R. R., Klein, S., Carraro, F., and Weber, J. M. (1990). Role of triglyceride-fatty acid cycle in controlling fat metabolism in humans during and after exercise. *Am J Physiol* *258*, E382-389.

Wyss-Coray, T. (2006). Tgf-Beta pathway as a potential target in neurodegeneration and Alzheimer's. *Curr Alzheimer Res* *3*, 191-195.

Yamazaki, H., Arai, M., Matsumura, S., Inoue, K., and Fushiki, T. (2002). Intracranial administration of transforming growth factor-beta3 increases fat oxidation in rats. *Am J Physiol Endocrinol Metab* *283*, E536-544.

Intracranial administration of transforming growth factor- β 3 increases fat oxidation in rats

Introduction

WE HAVE PREVIOUSLY REPORTED THAT INTRACISTERNAL ADMINISTRATION of cerebrospinal fluid (CSF) from exercise-exhausted rats produced a decrease in spontaneous motor activity, whereas CSF from sedentary rats had no such effect (19). The substance that regulates the urge for motion in response to exercise exhaustion was identified as transforming growth factor- β (TGF- β) (23, 24). Growing evidence indicates that accumulation of the active forms of TGF- β 2 and/or TGF- β 3 in the brain is involved in the fatigue induced by exercise (19, 20, 23).

The sensation of fatigue in the brain, however, may not merely be an inconvenience but may constitute a physiological defense mechanism against total exhaustion. If this is so, the active forms of TGF- β found in the brain may function positively to prevent peripheral exhaustion and to enhance recovery.

The brain may detect changes in the normal levels of the constituents of the blood, such as the concentration ratio of tryptophan to branched-chain amino acids (4, 6, 7, 11, 12, 26-29), which can then act as a specific signal to increase the organism's sensitivity to fatigue. However, little is known about the counteractive effects of the central nervous system (CNS) on peripheral energy metabolism.

After exercise exhaustion, the respiratory exchange ratio is usually lower for a more extended period than it is before exercise, although oxygen consumption returns readily to its preexercise level (5, 32). This suggests that the sensation of fatigue affects not only spontaneous motor activity but also energy metabolism, i.e., it enhances fat oxidation to conserve glucose. It seems reasonable to suppose that the substances released in the brain that accompany fatigue may regulate energy metabolism and induce the restoration of energy resources.

We have reported that the intracranial administration of TGF- β 3 suppresses spontaneous motor activity in mice without substantial exercise loading (20) and may affect peripheral energy metabolism. The TGF- β s represent a multifunctional family of cytokines with three closely related isoforms: TGF- β 1, TGF- β 2, and TGF- β 3. These isoforms are expressed in several cell types of the CNS, including neurons, astrocytes, and microglia (9). Unsicker et al.

(36) reported that TGF- β 2 and TGF- β 3 mRNAs are present in all brain areas, including the cerebral cortex, hippocampus, striatum, cerebellum, and brain stem. In this study, we used TGF- β 3 to represent the TGF- β isoforms for the following reasons. We have found that TGF- β 2 and TGF- β 3 suppress spontaneous motor activity equally, and a considerably higher dose of TGF- β 1 is required to exert a suppressive effect equal to that required for either TGF- β 2 or TGF- β 3 (20). TGF- β 2 and TGF- β 3 are ubiquitously abundant in the rat brain (36), and we have confirmed that TGF- β 1 and TGF- β 2 levels do not change in the brain, even when total TGF- β levels increase (unpublished data). In the present study, we demonstrate that injection of TGF- β 3 into the brain alters peripheral energy metabolism to resemble that induced during exercise exhaustion.

Materials and Methods

Animals and diets.

Male Sprague-Dawley rats (8 wk old, Japan Charles River, Yokohama, Japan) were used in the present study. All animals were maintained on an inverse 18:6-h light-dark cycle (light on for 18 h, and light off for 6 h) for 1 wk to make them active during the experimental time. They were individually housed in standard cages (25 $\times$ 38 $\times$ 17.5 cm; one rat per cage) under controlled conditions. Room temperature and humidity were regulated at 22 $\pm$ 0.5° C and 50%, respectively. During the study period, rats were given free access to water and a high-fat (30%) diet, containing 21 g/kg protein, 30 g/kg fat, and 49 g/kg carbohydrate. All animals were treated humanely as outlined in the National Research Council's Guide for the Care and Use of Laboratory Animals (Kyoto University Animal Care Committee according to NIH #86-23, revised 1985).

Intracranial injection (brain implantation of a guide cannula).

TGF- β 3 was purchased from R&D Systems (Minneapolis, MN). TGF- β 3 (40 ng), dissolved in 40 μ l of saline containing 0.5 mM HCl and 0.1% bovine serum albumin (BSA), was injected into the brains of rats. An equal volume of vehicle was used as a control. Soon after rats were purchased, they were anesthetized with 1 mg/kg pentobarbital sodium (Wako Pure Chemical Industries, Osaka, Japan), fixed onto a stereotaxic apparatus, and implanted with a permanent 23-gauge guide cannula for sample injection into the cisterna magna. Each cannula was inserted 2.5 mm posterior to the lambda and 8.5 mm deep, and it inclined anteriorly at an

angle of 45° to the skull surface. The cannula was secured to the skull with dental cement and then plugged with a cap. After implantation of the guide cannula, the rats were allowed to recover for 3 days before measurements were made of spontaneous motor activity, oxygen consumption (VO_2), and CO_2 production (VCO_2).

Determination of spontaneous motor activity.

The respiratory exchange ratio (RER) was measured by indirect calorimetry. Rats were fasted overnight on the day before the experiments, and food was provided for 1.5 h immediately before the experiments. Rats were placed in the chamber individually before the experiment for 1 h to maintain RER at a constant value. After the injection of TGF- β 3, RER was measured for ~1 h. To determine whether the effects of TGF- β 3 on the metabolic rate were specific, 1 μg of thyrotropin-releasing hormone (TRH) (Research Biochemicals Int, Natick, MA) dissolved in 40 μl of saline was also injected into the brains of rats as a positive control.

Assessment of metabolic rate.

The respiratory exchange ratio (RER) was measured by indirect calorimetry. Rats were fasted overnight on the day before the experiments, and food was provided for 1.5 h immediately before the experiments. Rats were placed in the chamber individually before the experiment for 1 h to maintain RER at a constant value. After the injection of TGF- β 3, RER was measured for ~1 h. To determine whether the effects of TGF- β 3 on the metabolic rate were specific, 1 μg of thyrotropin-releasing hormone (TRH) (Research Biochemicals Int, Natick, MA) dissolved in 40 μl of saline was also injected into the brains of rats as a positive control.

A specific laboratory-made open-circuit calorimeter was used. The gas analyzer consisted of six acrylic chambers, CO_2 and O_2 analyzers (model RL-600, AlcoSystem), and a switching system (model ANI6-A-S, AlcoSystem) to sample gas from each metabolic chamber. Each chamber had a 204-cm² floor and a height of 12.7 cm. Air flow (3 l/min) was circulated and monitored by a mass-sensitive flowmeter. Air from each chamber was sampled for 60 s. During the last second, the concentrations of O_2 and CO_2 were measured 100 times, and the mean values were used to calculate VO_2 and RER. The data were then processed mathematically using a specific algorithm. VO_2 and VCO_2 were calculated from changes in gas content (percent) and air flow (l/min) by use of differential gas analyzers. The RER was calculated as the ratio of VCO_2 to VO_2 . Carbohydrate and fat consumption rates were computed from VO_2 and the respiratory quotient according to the theory of Frayn (13).

Analysis of serum samples.

Blood was collected from severed neck veins, and serum was isolated by centrifugation and stored at -80°C until analysis. Serum glucose was measured using the glucose oxidase method combined with mutarotase by use of glucose AR-II and a commercial kit (Wako Pure Chemical Industries). Serum free fatty acids (FFA) were measured by an acyl-CoA synthetase and acyl-CoA oxidase method using NEFA C (Wako Pure Chemical Industries). Serum triglycerides were assayed by the glycerol-3-phosphate oxidase method with the triglyceride G test (Wako Pure Chemical Industries). Serum lactic acid was measured by the lactate oxidase method using Determiner LA (Kyowa Medics, Tokyo, Japan). Serum ketone bodies were measured using a ketone test (Sanwa Chemical Institute, Nagoya, Japan). For the catecholamine assay, serum samples were purified with aluminum oxide by the method of Anton and Sayre (1). Serum samples (100 μl) containing 10% $\text{Na}_2\text{S}_2\text{O}_5$ (50 $\mu\text{l}/\text{ml}$) and 3,4-dihydroxybenzylamide (40 ng/ml) as the internal standards were added to 100 μl of 2 M Tris $\cdot$ HCl buffer, pH 8.6, and aluminum oxide (100 mg/ml). The mixture was shaken in a microtube mixer for 10 min, the supernatant was removed, and the aluminum oxide was washed twice with methanol and distilled water. Epinephrine and norepinephrine were eluted with 60 μl of 0.5 N HCl. The eluate was assayed using an HPLC-electrochemical detector (37). Serum insulin and leptin were measured using a Mercodia Rat Insulin Kit (Mercodia, Uppsala, Sweden) and a Morinaga Rat Leptin Kit (Morinaga, Yokohama, Japan), respectively.

Measurements of muscle lipoprotein lipase activity and serum glycerol concentrations.

Gastrocnemius and soleus muscle samples were dissected away from visible fat. Samples (3-10 mg) of skeletal muscle (gastrocnemius) were ground in liquid nitrogen and incubated (in duplicate) in 200 μl of Krebs-Ringer solution, 0.1 M Tris $\cdot$ HCl buffer (pH 8.4) containing 1 g/100 ml of BSA and 2.5 U (50 mg/l) of heparin (35), with gentle shaking at 28°C . After 40 min, the tissue was removed from the medium by centrifugation for 5 min, and the supernatant was used for the measurement of lipoprotein lipase (LPL) activity with an LPL activity kit including a nonfluorescent substrate emulsion that becomes intensely fluorescent upon interaction with LPL (Roar Biomedical, New York, NY). The total protein content of each sample was measured by the Bradford method with Coomassie brilliant blue solution. LPL activity was assessed relative to the protein content of the tissue, and the values are expressed as the ratio of each group to the nontreatment group. Serum glycerol was determined by the

ultraviolet method by use of F-kit glycerol (J. K. International, Tokyo, Japan).

Statistics.

All data are expressed as means $\pm$ SE. Statistical analysis of differences between preinjection and postinjection measurements in the same group was performed with repeated-measures ANOVA test. Dunnett's test was used for post hoc analysis. The means of more than three groups measured at the same point in time were compared by one-way ANOVA followed by Dunnett's test.

Results

Effects of TGF- β 3 on spontaneous motor activity in rats.

The spontaneous motor activity of the rats was determined with Supermex for 1 h after the injection of TGF- β 3 by detecting the movement of infrared radiation emanating from the animal every 5 min. The spontaneous motor activity was gradually suppressed 20 min after injection in rats treated with TGF- β 3 (Fig. 1A). Figure 1B shows the total spontaneous motor activity for 1 h. Total counts were calculated by adding up all counts over 5-min periods. The administration of TGF- β 3 significantly suppressed the activity of the rat.

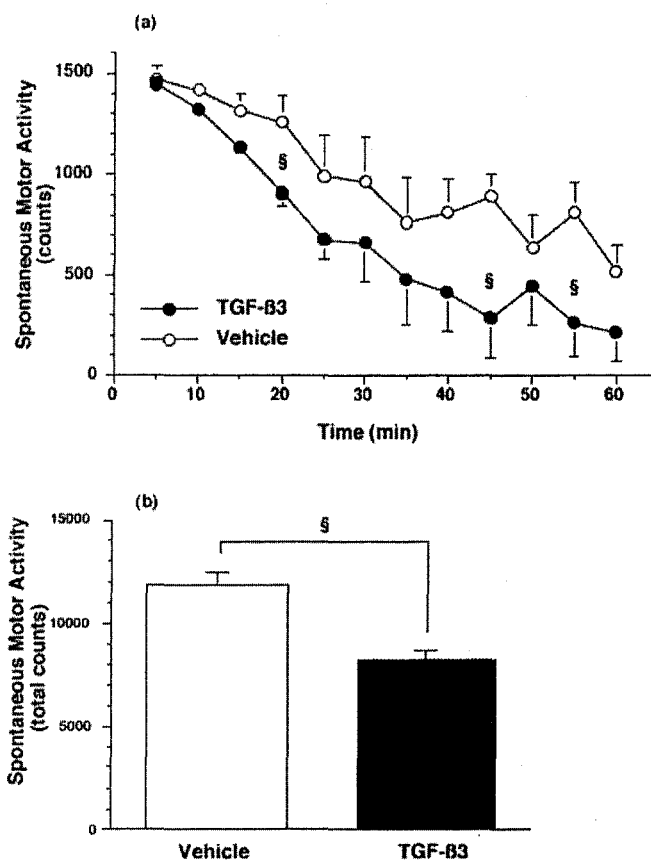


Fig. 1. Effects of intracranial injection of transforming growth factor (TGF)- β 3 on spontaneous motor activity in rats. TGF- β 3 (40 ng) or vehicle was injected into the cisterna magna of each rat ($n = 6-8$). Values are means $\pm$ SE. A: spontaneous motor activity assessed every 5 min as movement of animals detected by infrared radiation. B: total counts of spontaneous motor activity of rats during the hour after TGF- β 3 injection. § Significantly different from vehicle group ($P < 0.05$).

Effects of TGF- β 3 on metabolic rate.

Figure 2 shows the changes in metabolic rate in rats injected intracranially with vehicle, TGF- β 3, or TRH. The high-fat diet maintained RER at ~ 0.85 before sample injection. An injection of 40 ng of TGF- β 3 significantly lowered RER compared with that in the same rat before injection. TGF- β 3 significantly reduced the RER of the rat by 7 min after injection, and its effect was maintained for 1 h after injection. The same volume of vehicle injected as a control reduced the RER slightly. During the experiment, rats were deprived of access to food for 2 h and, therefore, RER was naturally reduced.

Intracranial injection of TRH elevated thermogenesis and O_2 in rats (14, 21). Food restriction before the experiment decreased RER, whereas TRH injection may increase RER. The decrease in RER was least in the TRH group.

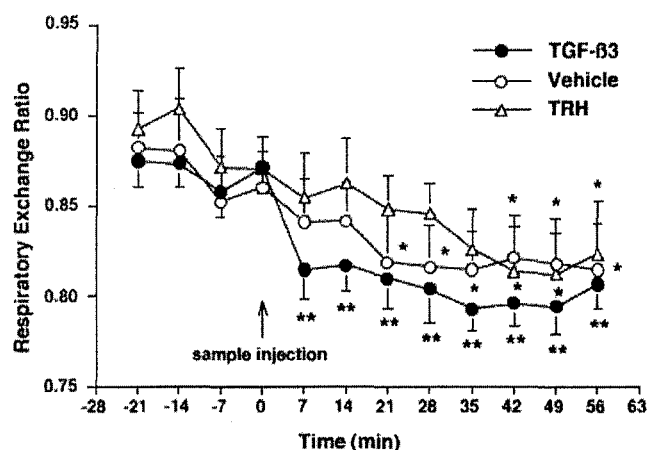


Fig. 2. Effects of intracranial injection of TGF- β 3 on the respiratory exchange ratio (RER) of rats. Each rat was housed in a metabolic chamber for 1 h before injection. TGF- β 3, vehicle, or thyrotropin-releasing hormone (TRH), dissolved in saline, was injected into the cisterna magna of each rat at time 0 under resting conditions ($n = 9-11$). RER was measured during the hour after injection of TGF- β 3. Values are means $\pm$ SE. * and **, Significantly different from same group before injection ($P < 0.05$ and $P < 0.01$, respectively).

VO₂ rose abruptly after the injection of each sample from time 0 to 7 min. Intracranial injection of TRH significantly increased VO₂, and the increase was maintained for ~40 min after injection. It has been reported previously that injection of TRH into the brain increases VO₂ (14). Therefore, this result demonstrates that the injected samples reached the intracerebral location responsible for the regulation of VO₂. VO₂ was similar in the TGF-β3 and vehicle groups (Fig. 3). These results suggest that intracranial TGF-β3 does not affect energy consumption.

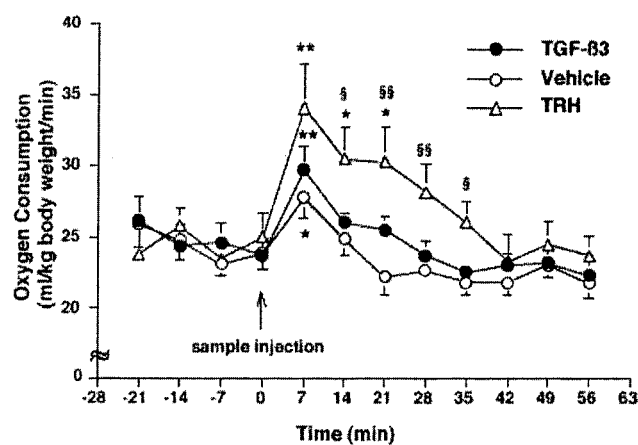


Fig. 3. Effects of intracranial injection of TGF-β3 on oxygen consumption in rats. Each rat was housed in a metabolic chamber, and TGF-β3 (40 ng), vehicle, or TRH (1 μg) was injected into the cisterna magna via a cannula under resting conditions (n = 9-11). Oxygen consumption was measured during the hour after injection of test solutions. Values are means ± SE. * and **, Significantly different from same group before injection (P < 0.05 and P < 0.01, respectively). § and §§, Significantly different from vehicle group (P < 0.05 and P < 0.01, respectively).

Carbohydrate oxidation decreased gradually after injection with both TGF-β3 and vehicle (Fig. 4A). We inferred that this reduction in carbohydrate oxidation was caused by food restriction before the experiment. Injection of TRH tended to increase carbohydrate oxidation for ~40 min. Intracranial TGF-β3 injection significantly increased fat oxidation for 1 h compared with preinjection values (Fig. 4B). Injection with either TRH or vehicle tended to increase fat oxidation, but not significantly.

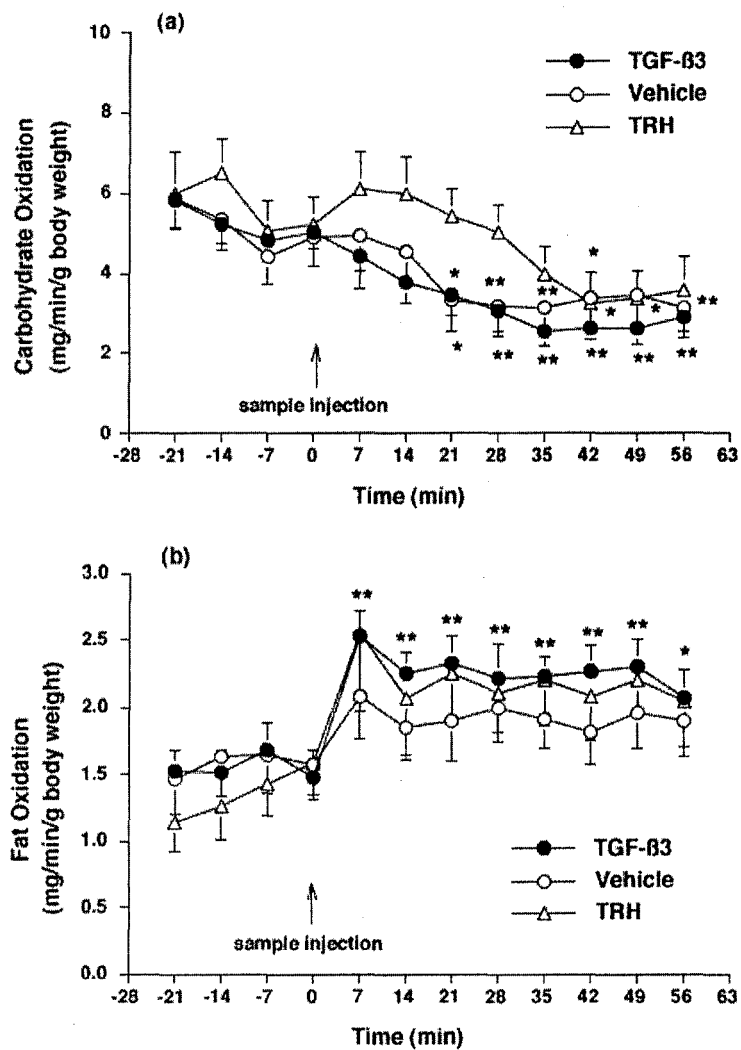


Fig. 4. Effects of intracranial injection of TGF- β 3 on carbohydrate (A) and lipid (B) oxidation in rats. Rats were treated as described in text and legend to Fig. 2. Carbohydrate and lipid oxidation values were calculated from the RER and oxygen consumption. Values are means $\pm$ SE. * and **, Significantly different from same group before injection ($P < 0.05$ and $P < 0.01$, respectively).

Figure 5 shows the rates of carbohydrate and fat oxidation from time 0 to 28 min and from 28 to 56 min, compared with each oxidation value for 28 min before injection. In the TGF- β 3 group, the rate of increase in fat oxidation was higher than in the vehicle group (Fig. 5B). However, the rate of change in carbohydrate oxidation was similar in the TGF- β 3 and vehicle groups.

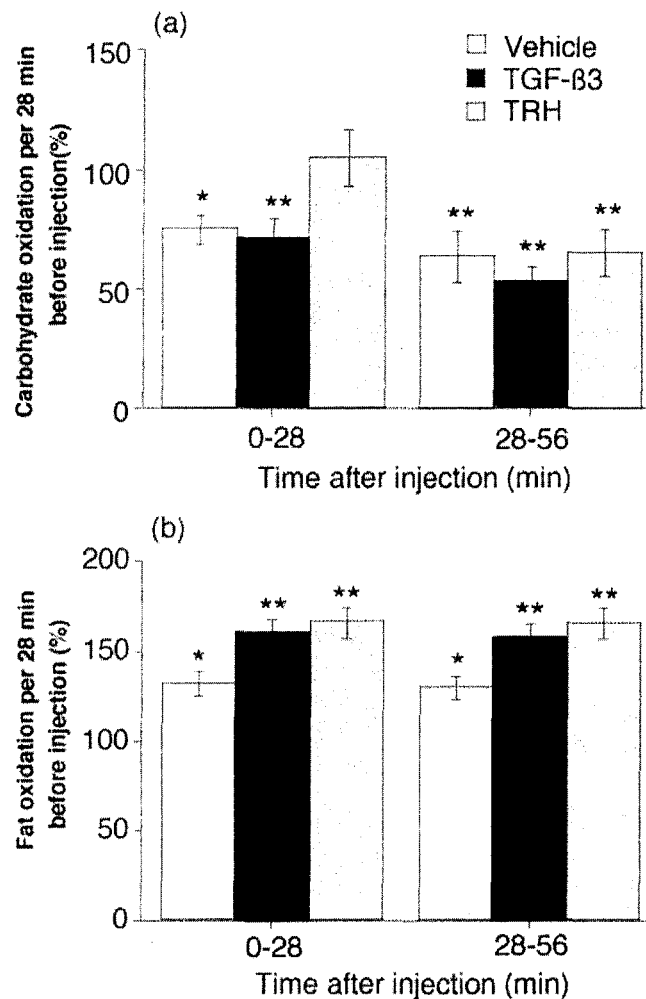


Fig. 5. Changes in carbohydrate (A) and fat (B) oxidation after vehicle (open bars), TGF-β3 (solid bars), or TRH (gray bars) injection into the rat brain. Data are expressed as a percentage of oxidation measured at 28 min before injection. Values are means $\pm$ SE. * and **, Significantly different from same group before injection ($P < 0.05$ and $P < 0.01$, respectively).

Changes in serum energy substrates and concentrations of hormones after injection with TGF-β3.

Serum concentrations of glucose and lactic acid, which are affected by carbohydrate oxidation, had not changed 14 and 28 min after injection in either the TGF-β3 or vehicle group (Fig. 6, A and B). The concentrations of the serum parameters associated with fat oxidation (FFA, triglycerides, and ketone bodies) were measured (Fig. 6, C-E). The concentrations of serum FFA in the vehicle group had decreased significantly at 28 min compared with those at 14 min, but no change was observed in the TGF-β3 group. Therefore, the serum FFA concentrations in the TGF-β3 group were apparently higher than those in the vehicle group. Triglyceride concentrations in the TGF-β3 group were lower than those in the vehicle group at 14 min. The

concentration of ketone bodies increased significantly in the TGF- β 3 group. These results suggest that intracranial injection of TGF- β 3 significantly facilitated fat oxidation and tended to restrict carbohydrate oxidation, which corresponds to the metabolic condition after exercise.

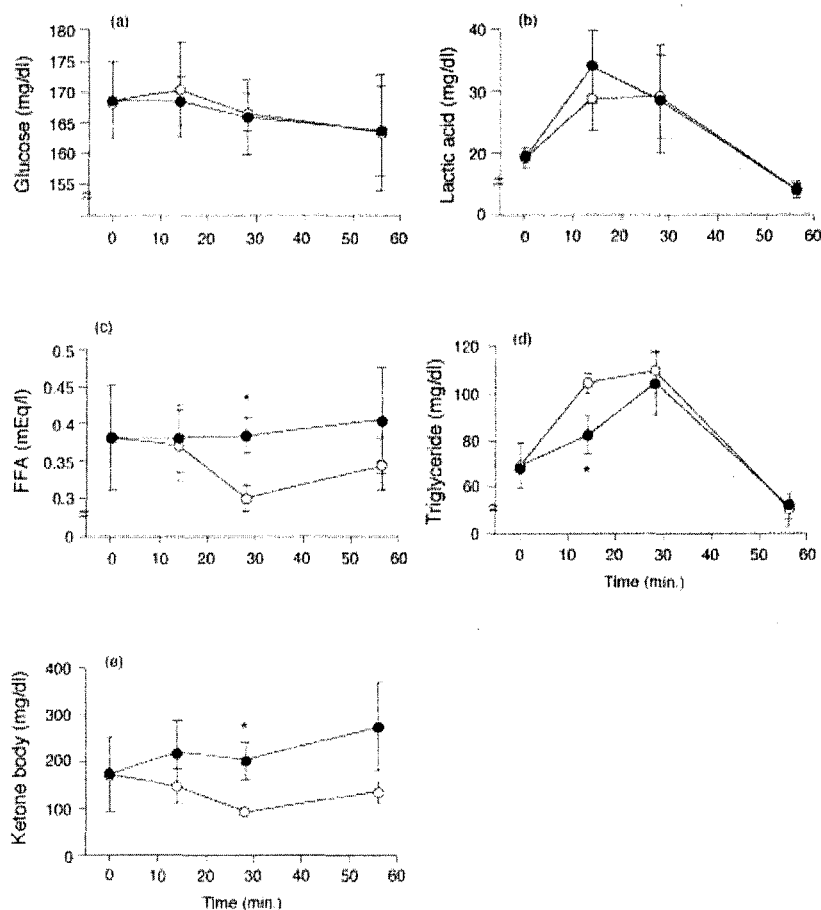


Fig. 6. Blood serum concentrations of glucose (A), lactic acid (B), free fatty acids (FFA, C), triglycerides (D), and ketone bodies (E) in rats 14, 28, and 56 min after injection into the cisterna magna of TGF- β 3 (●) or vehicle (○) (n = 5-8). Blood was collected from jugular veins; serum was isolated by centrifugation and tested for each constituent (details are described in text). Values are means $\pm$ SE. § Significantly different from control group (P < 0.05).

No significant changes in blood serum concentrations of catecholamines, insulin, or leptin were observed (Fig. 7, A-D). Epinephrine concentration at 14 min had increased in the TGF- β 3-treated rats compared with the vehicle group (Fig. 7A). Insulin concentration in the vehicle group increased at 14 min compared with the value before injection, but insulin levels in the TGF- β 3 group did not change (Fig. 7C). No significant change was observed in concentrations between the TGF- β 3 and vehicle groups (Fig. 7B). The concentration of leptin was similar in both groups (Fig. 7D).

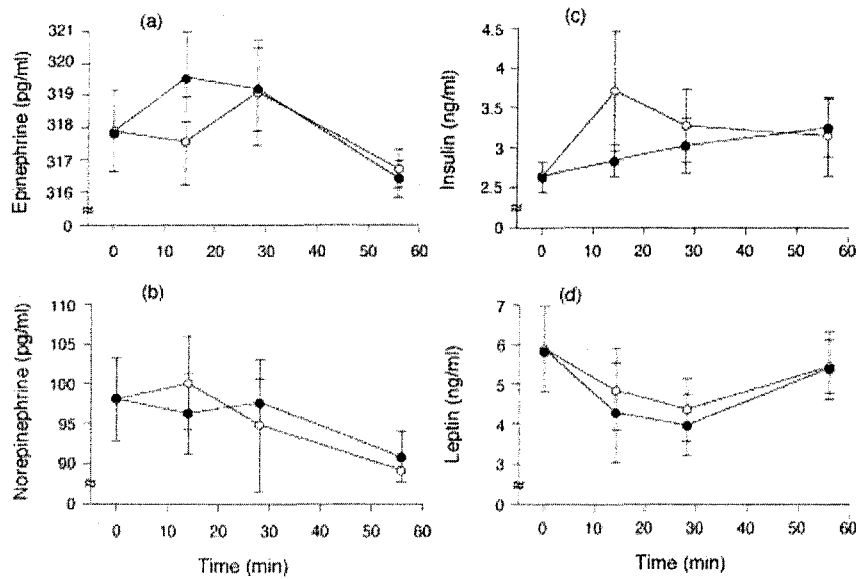


Fig. 7. Blood serum concentrations of epinephrine (A), norepinephrine (B), insulin (C), and leptin (D) in rats 14, 28, and 56 min after injection into the cisterna magna of TGF-β3 (●) or vehicle (○) (n = 5-8). Values are means ± SE. § Significantly different from vehicle group (P < 0.05).

Changes in muscle LPL activity and serum glycerol concentration after injection of TGF-β3.

To determine whether TGF-β3 caused an increase in fat oxidation in skeletal muscle, LPL activity was estimated. LPL activity in skeletal muscle, especially the gastrocnemius, is shown in Fig. 8A. In the gastrocnemius, LPL activity rose significantly 28 min after the injection of TGF-β3. In the vehicle group, there was no significant increase in LPL activity. Similar effects of TGF-β3 were also observed in the soleus muscle, although there was no significant difference compared with the vehicle group or before injection (data not shown).

Serum glycerol concentrations tended to increase after injection with TGF-β3, although the difference was not significant (Fig. 8B).

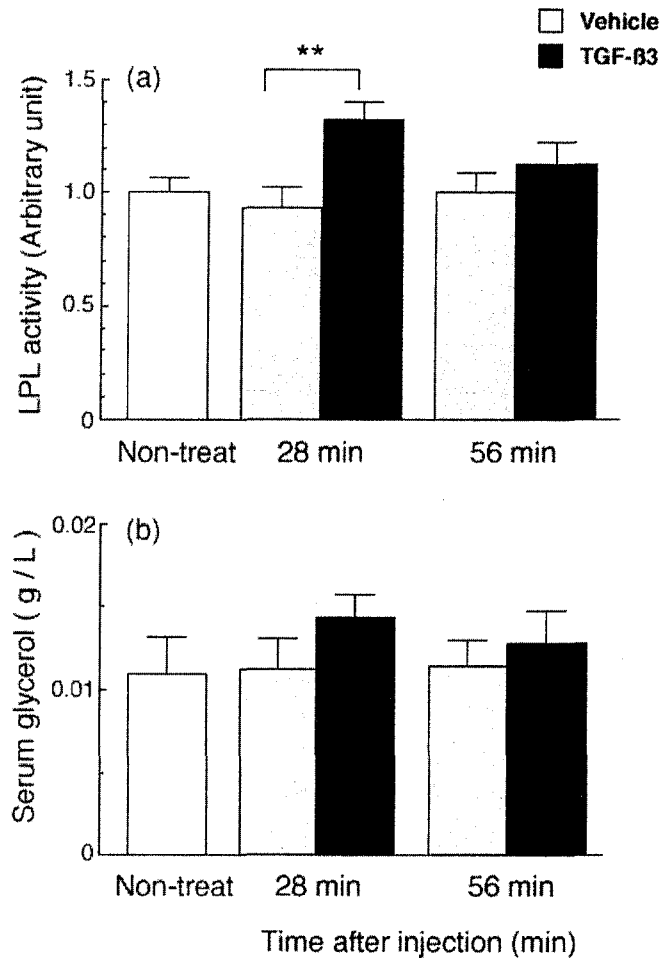


Fig. 8. Changes in muscle lipoprotein lipase (LPL) activity (A) and serum glycerol concentrations (B) after vehicle (gray bars) or TGF-β3 (solid bars) injection into rat brain (n = 5-6). Values are means $\pm$ SE. * and **, Significantly different from nontreatment or vehicle groups ($P < 0.05$ and $P < 0.01$, respectively).

Discussion

In previous studies, we have demonstrated that physical exercise causes an increase in TGF-β3 levels in the mouse brain and that intracranial TGF-β3 injection induces the suppression of spontaneous motor activity (19, 20). These studies indicated that TGF-β3 may be associated with the induction of central fatigue during exercise.

In the present study, we investigated the effects of intracranial injection of TGF-β3 on the peripheral metabolism in rats. Intracranial injection of TGF-β3 suppressed spontaneous motor activity in rats and decreased the RER (Figs. 1 and 2), similar to the corresponding experimentally induced effects in mice. The injection of TGF-β3 did not cause any toxicity or abnormal behavior in the rats. One day after injection of TGF-β3, both the spontaneous motor activity and the RER of every rat were restored to normal (data not shown).

Rats were fed a high-fat diet, which influences the RER. There was the dispersion in

RER value of each rat fed the commercial diet; however, the RER value stabilized between 0.8 and 0.9 by the high-fat diet. We used the high-fat diet because RER was more stable, and it facilitated the comparison between pre- and posttreatment. Also, we used it in previous experiments and wanted to be able to compare these data to those generated previously in our laboratory. We have been assured that there were no differences in the effect of TGF- β 3 on spontaneous motor activity and RER of rats between use of a high-fat diet and a commercial diet.

In general, the blood-brain barrier is highly permeable to water, carbon dioxide, oxygen, and most lipid-soluble substances and is almost impermeable to plasma proteins and most non-lipid-soluble large organic molecules. Accordingly, we speculated that the permeability of TGF- β into the blood-brain barrier might be very low or almost none. Therefore, we can derive from this premise that TGF- β 3 released from the brain directly affects spontaneous motor activity and the energy status of the animal. This implies that the effects of TGF- β 3 on peripheral tissues with energy are mediated through the CNS.

Overall, VO_2 was not altered by the injection of TGF- β 3 (Fig. 3). In this experiment, TRH was injected into the rat brain as a positive control on the basis of the report by Griffiths et al. (14) that acute or chronic injection of a TRH analog (RX-77368) and TRH itself stimulated VO_2 in rats (14). They demonstrated that intracranial TRH markedly increased the metabolic rate in rats without any apparent effect on physical activity. TRH is suggested to have a physiological role in the control of these phenomena via centralized activity. Hence, TRH injection is an appropriate procedure with which to verify cannula placement. The injection of either sample may cause a spike in VO_2 in both groups from immediately after injection to 14 min after injection. Therefore, it would appear that the injection of either sample may cause some stress to rats. However, any handling effects were apparent for only the first 14 min, as indicated by the fact that the VO_2 of vehicle animals returned to basal levels at this time. After this time, the differences observed should be strictly treatment related.

Short and Sedlock (32) reported that postexercise RER decreased for 1 h in humans. They reported that RER values were at or below baseline throughout much of the recovery period but that VO_2 immediately reverted to the baseline levels calculated before exercise. It has also been shown in rats that RER values decrease 10 min after exercise and that VO_2 is restored to basal levels (33). Intriguingly, the peripheral energy status induced by intracranial injection of TGF- β 3 appears to correspond to that induced by exercise. Our findings imply that the intracranial injection of TGF- β s may replicate energy metabolism after exercise.

Fat oxidation was facilitated by intracranial TGF- β 3 injection (Fig. 4B). Rats injected

with TGF- β 3 exhibited increased serum FFA and ketone body concentrations and decreased serum triglyceride concentrations compared with the vehicle group, which suggests that their lipid metabolism was elevated (Fig. 6, C-E). FFA concentrations in the TGF- β 3 group were higher than in the control group. Usually, fatty acids are seldom supplied from the triacylglycerol originally presented in blood plasma. Most FFAs oxidized during exercise are supplied from the triacylglycerol stored in adipose tissue and muscle (8, 16). Because the only product of hydrolysis that appears in the blood is glycerol, we examined whether TGF- β 3 actually induced hydrolysis by measuring serum glycerol. As shown in Fig. 8B, serum glycerol concentrations tended to increase after intracranial injection of TGF- β 3. From these results, it is reasonable to suppose that TGF- β 3 enhances FFA delivery to the muscles. Serum triglyceride concentrations had decreased by 15 min (Fig. 6D). It is inferred that an increase in LPL activity in skeletal muscle caused the decrease in serum triglycerides, which seems to be a consistent and reasonable proposition.

There were no differences in the metabolic parameters associated with carbohydrate oxidation between the rats injected with vehicle and those injected with TGF- β 3 (Fig. 6, A and B). However, the metabolic parameters associated with fat oxidation changed after injection of TGF- β 3. Furthermore, intracranial injection of TGF- β 3 significantly increased LPL activity in skeletal muscle at 28 min after injection (Fig. 8A). Serum ketone body concentrations increased, indicating that lipid oxidation was enhanced in the liver. This is because ketone bodies produced in the liver are more easily taken up by skeletal muscle as an energy resource. We anticipate that TGF- β 3 causes an increase in fat oxidation in both the liver and the muscle. It has been reported that exercise induces LPL activity in skeletal muscle (22, 31), which would be necessary to burn the fat efficiently. It is interesting that intracranial injection of TGF- β 3 caused LPL activity in skeletal muscle in the same way as exercise.

Leptin levels in rats injected with TGF- β 3 did not change. It has been reported that leptin production is not changed by short-term exercise (12, 30). We can suggest that our observation in this study is similar, because no changes in leptin were seen during short-term exercise.

Hwa and colleagues (17, 18) reported that a single intracerebroventricular injection of leptin increased energy expenditure while reducing the respiratory quotient in a dose-dependent manner. They demonstrated that leptin regulates the energy balance via multiple mechanisms, which was certainly mediated by the regulation of both food intake and energy metabolism (17, 18). Although both leptin and TGF- β augment fat oxidation, our results suggest that intracranial

injection of TGF- β 3 does not directly affect the peripheral leptin levels. How does intracranial TGF- β increase fat oxidation? Minokoshi et al. (25) showed that the intrahypothalamic injection of leptin increases the fatty acid oxidation by activating the 5'-AMP-activated protein kinase (25). Careful consideration of our results regarding LPL activity leads us to infer that intracranial injection of TGF- β 3 induced an increase in fat oxidation via the sympathetic nervous system, just as leptin works through the hypothalamic-sympathetic nervous system. However, at the present time, we cannot know whether both TGF- β and leptin modulate fat oxidation via the same neural pathway. Further studies are required to clarify whether there is an interaction between leptin and TGF- β s in the CNS.

As shown in Fig. 7A, epinephrine concentration tended to increase at 14 min after treatment with TGF- β 3. Serum epinephrine concentrations also increase after intense exercise. Tadjore et al. (34) reported that plasma epinephrine concentrations in rats increased after prolonged swimming. Cooper et al. (10) demonstrated that blood levels of epinephrine increase gradually after high- and low-intensity exercise in humans. In our study, however, the turnover rate of serum catecholamines was rapid; we suspected that catecholamines had already been recovered. Serum insulin levels increased in the vehicle group, whereas they did not change in the TGF- β 3 group (Fig. 7C). Injection of TGF- β 3 may suppress an increase in insulin to restrict fat storage. However, the reasons for the increase in insulin levels in the vehicle group are unclear.

The muscle LPL activity was increased by TGF- β 3; however, at this time there is no significant change in norepinephrine levels to explain this increase (Fig. 8A). Furthermore, despite the fact that LPL activity no longer increased after 56 min, fat oxidation kept increasing. This might suggest that the activation of LPL is not critical in this experiment after 56 min. Although we have not studied the fat oxidation in liver, we anticipated that the lipid oxidation also increased in liver because of the increase of ketone bodies, and that it might contribute to continuing fat oxidation after 56 min.

The metabolic changes induced by the injection of TGF- β 3 are very similar to the state of energy metabolism after physical exercise. Because energy expenditure did not change, intracranial injection of TGF- β 3 may cause a switch of energy substrates. During prolonged exercise, utilization of energy substrates shows a gradual transition from carbohydrate to fat. Other studies have reported that there is a significant substrate shift toward fat oxidation after high-intensity exercise (3, 38). This is a common phenomenon in exercise physiology, but the complete mechanism of the switch in energy substrates has not been clarified. TGF- β 3 that is

released in the brain during exercise may increase the rate of fat oxidation to conserve glucose. We also reported that the changes shown by electroencephalogram after intracranial injection of TGF- β were consistent with those after exercise (2). This suggested that the increase in TGF- β level in the brain is partly relevant to the change of neuronal activity after exercise. It seems reasonable that TGF- β released in the brain during exercise suppresses spontaneous motor activity to encourage rest and causes an alteration in the energy substrates of the peripheral system.

References

1. **Inoue, K., H. Yamazaki, Y. Manabe, C. Fukuda and T. Fushiki.** Release of a substance that suppresses spontaneous motor activity in the brain by physical exercise. *Physiol Behav* 64: 185-90, 1998.
2. **Manabe, Y., H. Yamazaki, C. Fukuda, K. Inoue, T. Fushiki and K. Hanai.** Hydra biological detection of biologically active peptides in rat cerebrospinal fluid. *Brain Res Brain Res Protoc* 5: 312-7, 2000.
3. **Manabe, Y., H. Yamazaki, C. Fukuda, K. Inoue, T. Fushiki and K. Hanai.** Suppression of S-methylglutathione-induced tentacle ball formation by peptides and nullification of the suppression by TGF-beta in Hydra. *Chem Senses* 25: 173-80, 2000.
4. **Inoue, K., H. Yamazaki, Y. Manabe, C. Fukuda, K. Hanai and T. Fushiki.** Transforming growth factor-beta activated during exercise in brain depresses spontaneous motor activity of animals. Relevance To central fatigue. *Brain Res* 846: 145-53, 1999.
5. **Davis, J. M., S. P. Bailey, J. A. Woods, F. J. Galiano, M. T. Hamilton and W. P. Bartoli.** Effects of carbohydrate feedings on plasma free tryptophan and branched-chain amino acids during prolonged cycling. *Eur J Appl Physiol* 65: 513-9, 1992.
6. **Davis, J. M. and S. P. Bailey.** Possible mechanisms of central nervous system fatigue during exercise. *Med Sci Sports Exerc* 29: 45-57, 1997.
7. **Newsholme, E. A., E. Blomstrand, P. Hassmen and B. Ekblom.** Physical and mental fatigue: do changes in plasma amino acids play a role? *Biochem Soc Trans* 19: 358-62, 1991.
8. **Newsholme, E. A., E. Blomstrand and B. Ekblom.** Physical and mental fatigue: metabolic mechanisms and importance of plasma amino acids. *Br Med Bull* 48: 477-95, 1992.
9. **Newsholme, E. A. and E. Blomstrand.** Tryptophan, 5- hydroxytryptamine and a possible explanation for central fatigue. *Adv Exp Med Biol* 384: 315-20, 1995.
10. **Newsholme, E. A. and E. Blomstrand.** The plasma level of some amino acids and

physical and mental fatigue. *Experientia* 52: 413-5, 1996.

11. **Blomstrand, E., P. Hassmen, B. Ekblom and E. A. Newsholme.** Administration of branched-chain amino acids during sustained exercise--effects on performance and on plasma concentration of some amino acids. *Eur J Appl Physiol* 63: 83-8, 1991.
12. **Blomstrand, E., F. Celsing and E. A. Newsholme.** Changes in plasma concentrations of aromatic and branched-chain amino acids during sustained exercise in man and their possible role in fatigue. *Acta Physiol Scand* 133: 115-21, 1988.
13. **Bailey, S. P., J. M. Davis and E. N. Ahlborn.** Effect of increased brain serotonergic activity on endurance performance in the rat. *Acta Physiol Scand* 145: 75-6, 1992.
14. **Bergman, B. C. and G. A. Brooks.** Respiratory gas-exchange ratios during graded exercise in fed and fasted trained and untrained men. *J Appl Physiol* 86: 479-87, 1999.
15. **Short, K. R. and D. A. Sedlock.** Excess postexercise oxygen consumption and recovery rate in trained and untrained subjects. *J Appl Physiol* 83: 153-9, 1997.
16. **Frayn, K. N.** Calculation of substrate oxidation rates in vivo from gaseous exchange. *J Appl Physiol* 55: 628-34, 1983.
17. **Anton, A. H. and D. F. Sayre.** A study of the factors affecting the aluminum oxide-trihydroxyindole procedure for the analysis of catecholamines. *J Pharmacol Exp Ther* 138: 360-375, 1962.
18. **Watanabe, T., T. Kawada and K. Iwai.** Enhancement by capsaicin of energy metabolism in rats through secretion of catecholamine from adrenal medulla. *Agric Biol Chem* 51: 75-79, 1987.
19. **Lin, M. T., A. Chandra, Y. F. Chern and B. L. Tsay.** Effects of thyrotrophic-releasing hormone (TRH) on thermoregulation in the rat. *Experientia* 36: 1077-8, 1980.
20. **Griffiths, E. C., N. J. Rothwell and M. J. Stock.** Thermogenic effects of thyrotrophin-releasing hormone and its analogues in the rat. *Experientia* 44: 40-2, 1988.
21. **Constam, D. B., J. Philipp, U. V. Malipiero, D. P. ten, M. Schachner and A. Fontana.** Differential expression of transforming growth factor-beta 1, -beta 2, and -beta 3 by glioblastoma cells, astrocytes, and microglia. *J Immunol* 148: 1404-10, 1992.
22. **Unsicker, K., K. C. Flanders, D. S. Cissel, R. Lafyatis and M. B. Sporn.** Transforming growth factor beta isoforms in the adult rat central and peripheral nervous system. *Neuroscience* 44: 613-25, 1991.
23. **McGregor, I. S., A. M. Lee and R. F. Westbrook.** Stress-induced changes in

respiratory quotient, energy expenditure and locomotor activity in rats: effects of midazolam. *Psychopharmacology (Berl)* 116: 475-82, 1994.

24. **Hwa, J. J., L. Ghibaudi, D. Compton, A. B. Fawzi and C. D. Strader.** Intracerebroventricular injection of leptin increases thermogenesis and mobilizes fat metabolism in ob/ob mice. *Horm Metab Res* 28: 659-63, 1996.

25. **Hwa, J. J., A. B. Fawzi, M. P. Graziano, L. Ghibaudi, P. Williams, H. M. Van, H. Davis, M. Rudinski, E. Sybertz and C. D. Strader.** Leptin increases energy expenditure and selectively promotes fat metabolism in ob/ob mice. *Am J Physiol* R1204-9, 1997.

26. **Tadjore, M., R. Bergeron, M. Latour, F. Desy, C. Warren and J. M. Lavoie.** Effects of dietary manipulations and glucose infusion on glucagon response during exercise in rats. *J Appl Physiol* 83: 148-52, 1997.

27. **Cooper, D. M., T. J. Barstow, A. Bergner and W. N. Lee.** Blood glucose turnover during high- and low-intensity exercise. *Am J Physiol* E405-12, 1989.

Increase in TGF- β in the brain during infection is related to fever, not depression of spontaneous motor activity

Introduction

TO ELUCIDATE THE MECHANISM UNDERLYING THE MANIFESTATION OF central fatigue, we screened for brain substances involved in generating the feeling of fatigue. In a previous study, intracisternal administration of cerebrospinal fluid (CSF) collected from rats exhausted by swimming exercise to sedentary mice resulted in a significant decrease in the spontaneous motor activity (SMA) of the mice, as though they were tired (14). The substance responsible for this suppression of spontaneous motor activity of animals was demonstrated to be transforming growth factor- β (TGF- β). This was inferred because the concentration of TGF- β in CSF increases in rats fatigued by exercise, the immunodepletion of TGF- β in CSF with an anti-TGF- β antibody eliminates the activity that decreased the SMA of mice, and the intracisternal administration of purified TGF- β results in a dose-dependent decrease in the SMA of mice and rats (15, 34). These observations suggest that TGF- β in the brain is involved in the manifestation of central fatigue.

Infection with, for example, an influenza virus causes a strong feeling of fatigue. Other various symptoms such as fever, chills, arthralgia (joint pain), drowsiness, and anorexia are also observed. The administration of a bacterial endotoxin, lipopolysaccharide (LPS), or a synthetic viral-like double-stranded RNA, polyinosinic-polycytidylic acid (poly I:C), to animals is often used as an experimental model of infection. By this procedure, the sickness symptoms described above are induced (13, 17, 21).

While these symptoms are not usually considered beneficial, in the case of infection they constitute an important series of defensive responses. Indeed, once these symptoms occur, people recognize infection and try to cope with the sickness and to recover. A high body temperature (fever) suppresses viral proliferation and bacterial growth, and activates and induces the proliferation of immune cells (19). During infection, the feeling of fatigue is a discomfort and makes animals inactive. This suggests that physical immobilization is advantageous for recovery from infection.

When monocytes and macrophages are activated by exogenous pyrogens, various

cytokines are secreted. Proinflammatory cytokines released in blood affect the brain, SMA, feeding, and modulation of the body temperature of animals (7, 9, 10, 30). In addition, proinflammatory cytokines administered for tumor therapy cause fever, anorexia, and the feeling of fatigue as adverse effects. These observations strongly indicate a relationship between the production of cytokines and the feeling of fatigue during infection.

Some cytokines are secreted after exercise, and are considered to be related to certain physiological changes after exercise—e.g., anorexia, increase in body temperature and inflammation (6, 27)—that are similar to the symptoms observed in infection. We postulated that TGF- β in the brain, which increases during exercise, plays a role in the response to infection. In this study we investigated the relationship between TGF- β in the brain and both fever and the feeling of fatigue as typical symptoms during infection.

Experimental Procedures

Animals

This study was conducted in accordance with the ethical guidelines of the Kyoto University Animal Experimentation Committee and the Japan Neuroscience Society and was in complete compliance with the National Institutes of Health Guide for the Care and Use of Laboratory Animals. All efforts were made to minimize the number of animals used and limit experimentation to what was necessary to produce reliable scientific information. Male Sprague-Dawley rats (8 weeks old) were used. All the animals were maintained in a 12-h light/12-h dark cycle (0600, lights on / 1800, lights off) for at least 1 week before surgery. They were allowed free access to water and standard lab chow in a room maintained at 22 $\pm$ 0.5°C and 50% humidity.

Surgery

A telemetry sensor/transmitter that measures core body temperature (model TA10-TA; Data Science Inc., St. Paul, MN) was intraperitoneally implanted in each rat anesthetized with pentobarbital sodium according to Ohnuki et al. (26). Following the telemetry sensor/transmitter implantation, a cannula for the intracisternal administration of IgG solution was implanted according to Yamazaki et al. (34). An anesthetized rat was fixed to a stereotaxic apparatus. A stainless guide cannula (23G) was introduced at 3.0 mm posterior to the lambda. It inclined anteriorly at an angle of 60° and was inserted 8.7 mm deep in this direction. The cannula was fixed with Loctite 454 (Loctite Japan, Yokohama, Japan). The rats were allowed to recover for 1

week after the surgery.

Experimental Protocol

Sample administration

All samples were administered at 20:00, 2 h after the onset of the dark period. Poly I:C (sodium salt; Sigma-Aldrich Japan K.K., Tokyo, Japan) was dissolved in pyrogen-free saline (Otsuka Pharmaceutical Co., Tokyo, Japan) and intraperitoneally injected into rats at 3 mg/kg body weight. An anti-TGF- β antibody (pan-specific anti-TGF- β antibody, rabbit polyclonal, IgG fraction purified by Protein A affinity chromatography, #AB-100-NA; R&D Systems, Minneapolis, MN) was dissolved in sterilized artificial cerebrospinal fluid (aCSF; 140 mM NaCl, 3 mM KCl, 1.5 mM Na₂HPO₄, 0.23 mM NaH₂PO₄, 1.5 mM CaCl₂, 1.26 mM MgCl₂, 3.4 mM D-glucose, 0.1% bovine serum albumin (BSA)) at a final concentration of 1 mg/ml, and 20 μ l (i.e., 20 μ g of the anti-TGF- β antibody) was intracisternally administered. As a control, the same amount of nonimmune IgG (immunoglobulin G, control normal rabbit IgG, #AB-105-C; R&D Systems) was administered. Recombinant human TGF- β (R&D Systems) was dissolved in sterilized artificial cerebrospinal fluid at a final concentration of 1 mg/ml, and 40 μ l (i.e., 40 ng of the TGF- β) was intracisternally administered.

SMA measurement

The SMA of the rats was measured from the onset of and throughout the dark period, which is the time of the day the rats are active. The SMA of each rat after intraperitoneal (i.p.) administration of poly I:C was examined using Supermex (Muromachi Kikai, Tokyo, Japan) for 16 h. This apparatus surveys the measurement area with multiple lenses that detect the infrared radiation emitted by animals. SMA was assessed as a single count when an animal moved from one region of the measurement area, which was optically divided by multiple lenses, to a neighboring region.

Body temperature measurement

Body temperature was measured according to Ohnuki et al. (26) with modifications. Measurement was started the day before the experiment. The rats were acclimated to the test environment and the baseline body temperature of each rat was recorded. Signals from the sensor/transmitter that measures body temperature were detected every 5 min by a flat-panel receiver (model RA1010; Data Science Inc.) and analyzed using the Dataquest IV program (Data

Science Inc.).

Collection of CSF and serum

CSF was collected according to Inoue et al. (14). The rats were anesthetized with pentobarbital, and CSF was collected from the cisterna magna after puncturing the atlantooccipital membrane with a 26G needle. After the collection, CSF was centrifuged at 2000 x g and 4°C; then the supernatant was collected and stored at -70°C until the measurement of TGF- β concentration. CSF visibly contaminated with red blood cells was discarded.

After the collection of CSF, blood was collected from the heart. A glass capillary was inserted into the left ventricle, then blood was collected and stored on ice for 2 h to completely remove blood coagulation components that may have disturbed the TMLC bioassay. Serum was recovered by centrifugation at 1500 x g and 4°C and stored at -70°C until the measurement of cytokine concentration.

Determination of cytokines

TGF- β is produced in an inactive form referred to as latent TGF- β . The amino terminal part of the protein masks the active part (TGF- β) and is called a latency-associated protein (LAP). The dissociation of LAP by an as-yet-undetermined mechanism leads to the activation of the latent form of TGF- β . We determined the concentration of active TGF- β in samples unless otherwise noted. The concentration of active TGF- β was determined without any pretreatment of the samples, whereas total concentration, that is, the sum of the concentrations of the latent and active forms of TGF- β , was determined after the activation of latent TGF- β under a transient acidic condition.

To determine the concentration of TGF- β in CSF and serum, we conducted a bioassay using TGF- β -responsive mink lung epithelial cells (TMLCs) (1). This cell line was comprised of mink lung epithelial cells stably transfected with the TGF- β -responsive human plasminogen activator inhibitor 1 (PAI-1) promoter fused to a luciferase reporter gene (kindly provided by Dr. M. Abe, Department of Nanomedicine, Tokyo Medical and Dental University, Japan, and Dr. D. Rifkin, Department of Cell Biology, NYU Medical Center, USA). All samples were diluted with Dulbecco's modified Eagle's medium (DMEM) containing 0.1% BSA. For establishing a standard curve, purified TGF- β (R&D Systems) was used. TMLCs suspended in DMEM with 10% fetal bovine serum (FBS) were seeded onto 96-well plates (10,000 cells/well) and allowed to attach for 6 h; the medium was then replaced with 100 μ l of a sample solution. Sixteen hours

after the medium-sample exchange, luciferase activity was measured using a luciferase assay system (Promega, Ann Arbor, MI) according to the manufacturer's instructions. The sample solution was removed and replaced with 20 μ l of cell lysis reagent, and the plate was shaken for 30 min. Ten microliters of cell lysate was mixed with luciferase substrate, and luciferase activity was determined using a Veritas™ microplate luminometer (Promega). All experiments were carried out in triplicate. The concentrations of serum interleukin-1 β (IL-1 β), IL-2, IL-6 and tumor necrosis factor- α (TNF- α) were determined using rat specific ELISA kits (BioSource International, Camarillo, CA).

Confirmation of the specificity of anti-TGF- β antibody

Using a TMLC bioassay system, the specificity of anti-TGF- β antibody was confirmed. A medium containing an aliquot of rat TGF- β was prepared by mixing DMEM with pooled CSF of rats. The concentrations of anti-TGF- β antibody in the medium were set at 0 ~ 100 μ g/ml. The residual TGF- β activity after the immunoneutralization by the antibody was assessed as described above.

Statistical analysis

All values are presented as the means $\pm$ SEM. Body temperature was expressed as the average of temperatures measured for 30 min (i.e., the average of 5 temperatures measured every 5 min). The effects of the administrations of poly I:C and anti-TGF- β antibody, or TGF- β on the increase in body temperature were examined by two-way repeated-measures ANOVA. The values at each time point were compared by unpaired Student's *t*-test as a post-hoc test. The concentrations of cytokines in CSF and blood, and SMA were examined by one-way ANOVA. Fisher's PLSD was used as a post-hoc test.

Results

Decrease in SMA following poly I:C injection

Poly I:C was intraperitoneally administered 2 h after the onset of the dark period. One hour after the administration, the SMA of the rats significantly decreased and afterwards remained low throughout the dark period (Fig. 1). During the light period, the SMA of the control group also decreased. Both groups showed similarly low SMAs. The comparison of total motor activities from 2 h after poly I:C administration up to the end of the dark period demonstrated a significantly lower SMA for the poly I:C-treated group than for the control group

(saline: 60631 ± 3084 , $n=7$; poly I:C: 27913 ± 2614 , $n=8$; $p < 0.001$, unpaired Student's *t*-test). There was no difference in SMA between the two groups 24 h after the administration, and the rats treated with poly I:C showed SMA recovery (data not shown).

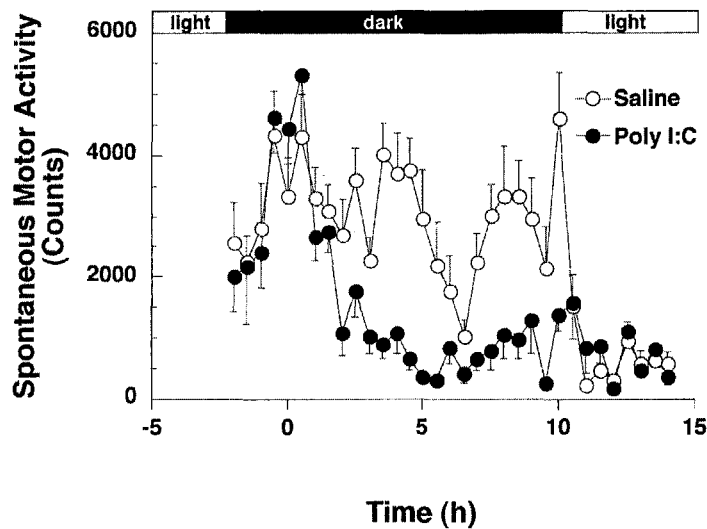


Figure 1. Effect of i.p. administration of poly I:C on spontaneous motor activity (SMA) of rats.

Two hours after the onset of the dark period (time 0), poly I:C (3 mg/kg body weight, $n=6$) or saline ($n=8$) was administered. Values are the means $\pm$ SEM. The SMA of rats was counted and added up every 30 min.

Increase in TGF- β concentration in CSF following poly I:C injection

The concentration of active TGF- β in CSF peaked 1 h after poly I:C administration, and remained high even 4 h after the treatment (Fig. 2A). The changes in total TGF- β which included the concentration of both active and latent TGF- β were not statistically significant (Fig. 2B). Sixteen hours after the administration, the concentration of active TGF- β in the CSF of the poly I:C-treated group was similar to that of the control group and considered to have reverted to the baseline concentrations (data not shown). The concentrations of TGF- β in CSF in the control group 1, 2 and 4 h after the administration with saline were 60.0 ± 10.9 , 60.2 ± 9.6 and 40.4 ± 5.9 in active form, and 1156 $\pm$ 153, 1081 $\pm$ 86 and 901 $\pm$ 67 in total, respectively (pg/ml, mean $\pm$ SEM), and showed no significant change.

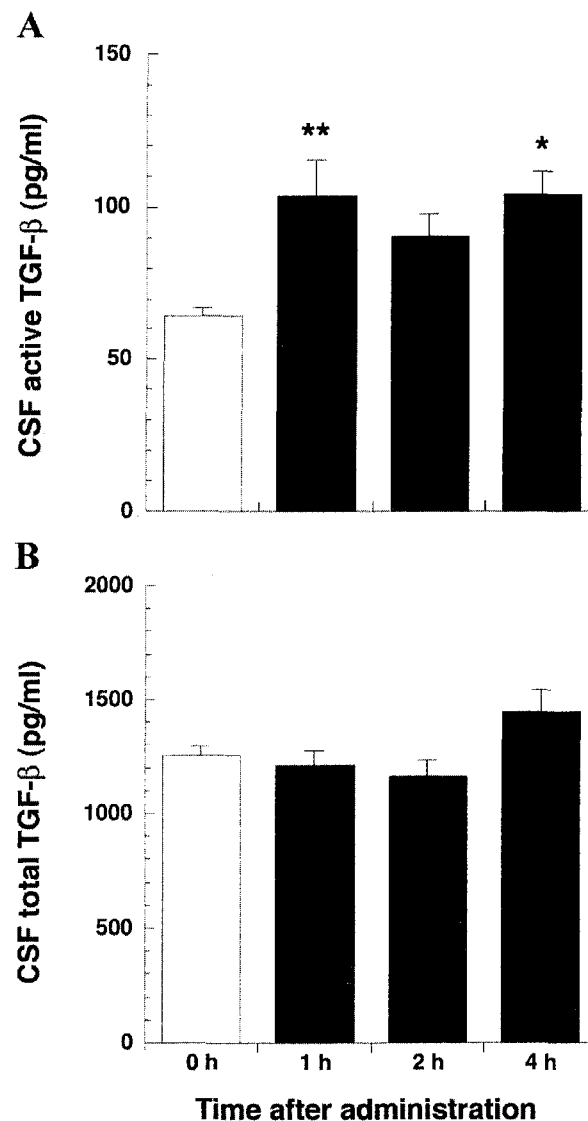


Figure 2. Effect of i.p. administration of poly I:C on the concentration of active TGF- β in CSF. Poly I:C (3 mg/kg body weight, $n=5\sim10$) was administered 2 h after the onset of the dark period (set at time 0), and CSF was collected from the cisterna magna of rats 0, 1, 2, and 4 h after the administration. The concentrations of active (A) and total (B) TGF- β were determined by luciferase assay using TGF- β -responsive mink lung epithelial cells (TMLCs). Values are the means $\pm$ SEM (* $p<0.05$, ** $p<0.01$ vs. 0 h, Fisher's PLSD test).

Changes in concentrations of cytokines in serum

The changes in the concentrations of active TGF- β (Fig. 3A) and total TGF- β (Fig. 3B) in serum were not statistically significant between saline and poly I:C-administered group. The concentrations of TNF- α and IL-6 peaked 2 h after poly I:C administration, and decreased thereafter (Fig.3). Some rats showed elevated concentrations of IL-1 β 4 h after poly I:C administration; however, no significant change in the concentration in the control group was observed at any other time points (data not shown). There was no significant difference between

control group and poly I:C-administered group in the concentration of IL-2 in serum at all time points (data not shown).

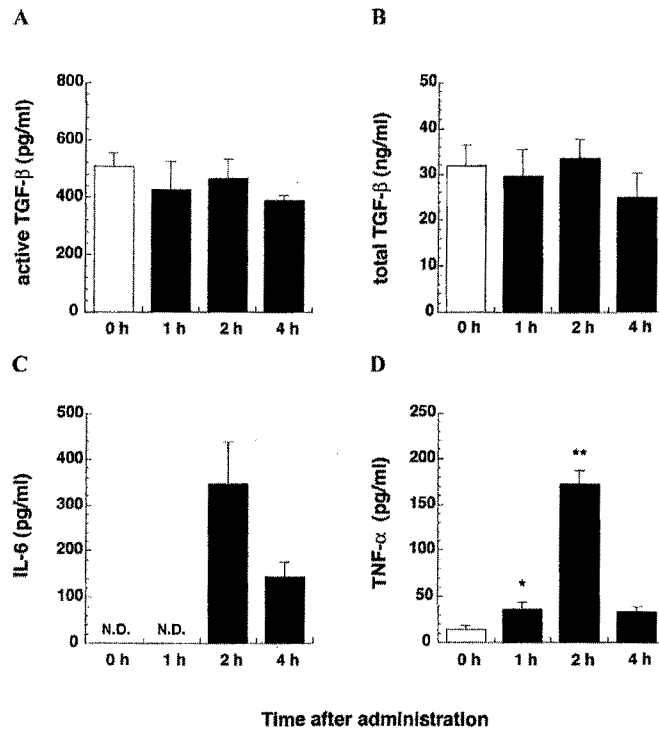


Figure 3. Effects of i.p. administration of poly I:C on the concentrations of cytokines in serum. Poly I:C (3 mg/kg body weight, n=5~10) was administered 2 h after the onset of the dark period, and blood samples were collected by heart puncture 0, 1, 2 and 4 h after the administration. The concentrations of active (A) and total (B) TGF-β were determined by luciferase assay using TMLCs. The concentrations of IL-6 (C) and TNF-α (D) were measured using a corresponding ELISA kit. Values are the means±SEM (*p<0.05, **p<0.01 vs. 0 h, Fisher's PLSD test).

Specificity of anti-TGF-β antibody

The anti-TGF-β antibody used in this study was raised against a mixture of purified recombinant human TGF-β1, porcine TGF-β1.2, porcine TGF-β2 and recombinant amphibian TGF-β5. The specificity of the anti-TGF-β antibody was examined by addition of this antibody to the medium which contained an aliquot of rat CSF. The ability of the antibody to neutralize rat TGF-β activity was determined by measuring the inhibition of luciferase production, which is controlled by a TGF-β-responsive promoter. The concentration of TGF-β in the medium was determined as 55.87 pg/ml. The luciferase activities (relative light units) were dose-dependently inhibited by the addition of the anti-TGF-β antibody, and at 100 μg/ml the activity was suppressed by 98% compared to the control (Fig.4). This indicates that the anti-TGF-β antibody used in this experiment was sufficient to neutralize the activity of rat TGF-β.

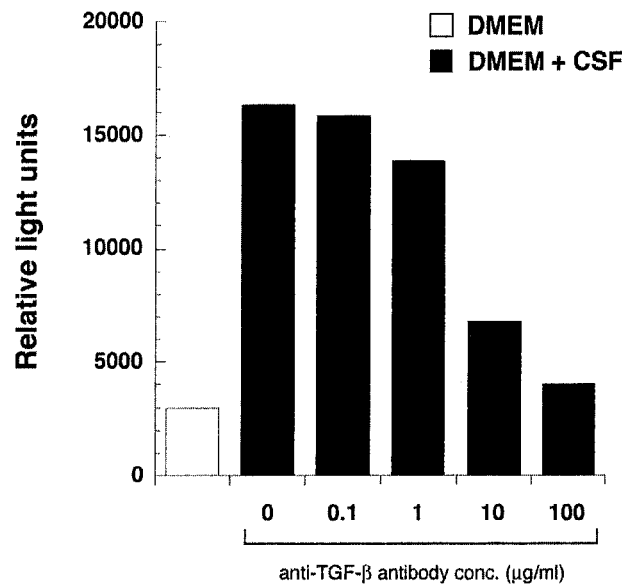


Figure 4. Immunoneutralization of rat TGF- β by pan-specific anti-TGF- β antibody. The efficacy of anti-TGF- β antibody against rat TGF- β was confirmed. A luciferase reporter assay was conducted with a medium containing an aliquot of the cerebrospinal fluid of rats, and rat TGF- β in the medium was immunoneutralized by adding anti-TGF- β antibody (#AB-100-NA; R&D Systems). The baseline induction was determined by administration of DMEM alone. The luciferase expression (relative light units) was dose-dependently inhibited by anti-TGF- β antibody.

Rise in body temperature following poly I:C administration and partial decrease in body temperature by intracisternal administration of anti-TGF- β antibody

The average baseline body temperature was calculated using data from 2 to 0 h before the administration. The baseline body temperature of the group administered the anti-TGF- β antibody was $37.59 \pm 0.11^\circ\text{C}$ ($n=7$) and that of the group administered control IgG was $37.73 \pm 0.12^\circ\text{C}$ ($n=5$). Poly I:C and IgG were administered at the same time. The peak increase in core body temperature was recorded 4~5 h after the administration. The peak body temperature of the group intracisternally treated with control IgG was 1.17°C higher than the baseline temperature. On the other hand, the peak body temperature of the group intracisternally administered the anti-TGF- β antibody was 0.84°C higher than the baseline temperature. Consequently, the rise in body temperature was significantly inhibited by the intracisternal administration of the anti-TGF- β antibody (Fig. 5), indicating that TGF- β in the brain plays some role in the mechanism underlying the body temperature rise induced by poly I:C. On the other hand, intracisternal administration of either anti-TGF- β antibody or nonimmune IgG to the control rats that were administered saline caused no significant increase in body temperature.

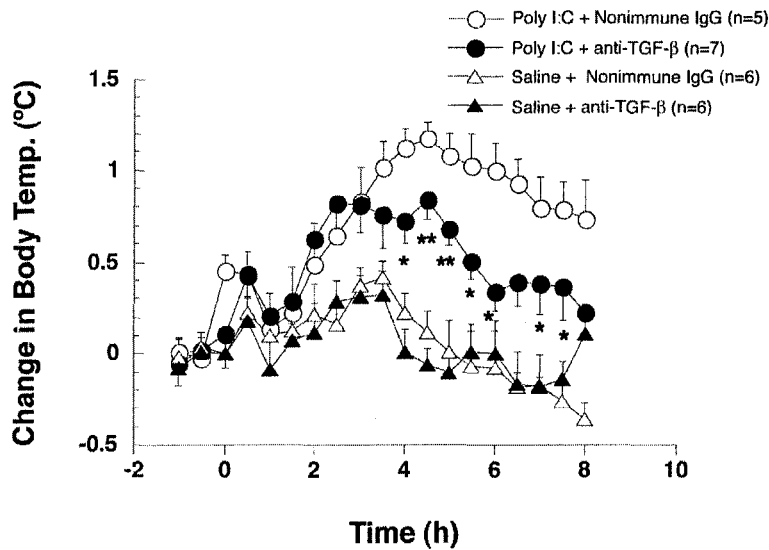


Figure 5. Effect of intracisternal administration of anti-TGF- β antibody on the rise in body temperature induced by i.p. administration of poly I:C. Two hours after the onset of the dark period (time 0), the i.p. administration of poly I:C (3 mg/kg body weight) or saline and the intracisternal administrations of an anti-TGF- β antibody or nonimmune IgG were carried out. Values are the means $\pm$ SEM (* p <0.05, ** p <0.01, anti-TGF- β antibody vs. nonimmune IgG, two way repeated ANOVA, followed by Student's t -test as a post hoc test).

Decrease in SMA following poly I:C administration and effect of intracisternal administration of anti-TGF- β antibody

Two hours after the onset of the dark period, poly I:C was intraperitoneally administered and the corresponding IgGs were intracisternally administered to the rats, whose SMA was recorded for 8 h. The SMA of the control IgG-treated group significantly decreased 1 h after poly I:C administration and remained low thereafter, similarly to that of the group administered poly I:C alone, as shown in Fig. 1. The SMA of the group administered the anti-TGF- β antibody similarly decreased and showed almost the same time-course changes as that of the control IgG-treated group. The total amount of SMA of the poly I:C-treated group was significantly lower than that of the saline-treated group, regardless of whether they were administered the anti-TGF- β antibody or control IgG (Fig. 6). Consequently, the intracisternal administration of the anti-TGF- β antibody did not affect the SMA of the rats or inhibit the decrease in SMA induced by poly I:C administration, which was different from the effect of the antibody on the body temperature rise.

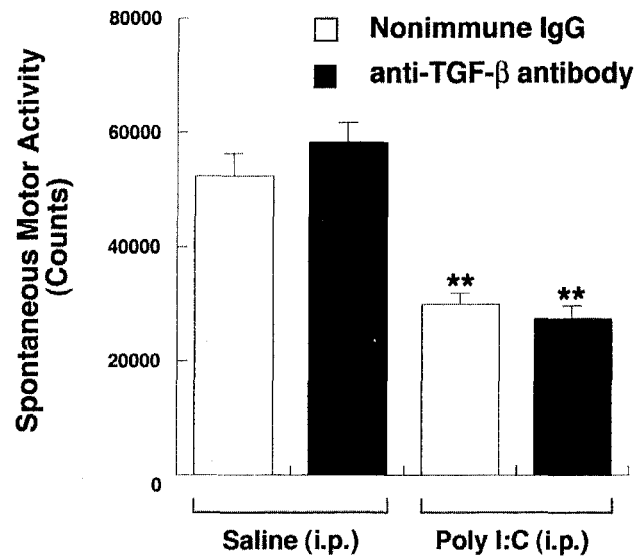


Figure 6. Effect of intracisternal administration of anti-TGF- β antibody on the decrease in SMA induced by i.p. administration of poly I:C. Two hours after the onset of the dark period, the i.p. administration of poly I:C (3 mg/kg body weight) or saline, and the intracisternal administration of an anti-TGF- β antibody or nonimmune IgG were carried out. SMA is represented as the total count for 8 h after the administrations (saline + nonimmune IgG, n=6; saline + anti-TGF- β antibody, n=6; poly I:C + nonimmune IgG, n=7; poly I:C + anti-TGF- β antibody, n=5). Values are the means $\pm$ SEM. There was no significant difference in the counts between the saline + nonimmune IgG and saline + anti-TGF- β antibody administrations, or between the poly I:C + nonimmune IgG and poly I:C + anti-TGF- β antibody administrations. **p<0.01, for saline + nonimmune IgG vs. Poly I:C + nonimmune IgG and for saline + anti-TGF- β antibody vs. poly I:C + anti-TGF- β antibody (Fisher's PLSD test).

Rise in body temperature by intracisternal administration of TGF- β

In the vehicle-administered group, the rise in temperature continued for 3 h, then gradually decreased. On the other hand, the body temperature of the TGF- β -administered group showed a significant and prolonged rise compared with that of vehicle-administered group. The significantly higher temperature was observed from 4 to 9 h after intracisternal administration of TGF- β (Fig. 7A). The SMA of the TGF- β -administered group was significantly lower than that of the vehicle-administered group (Fig. 7B).

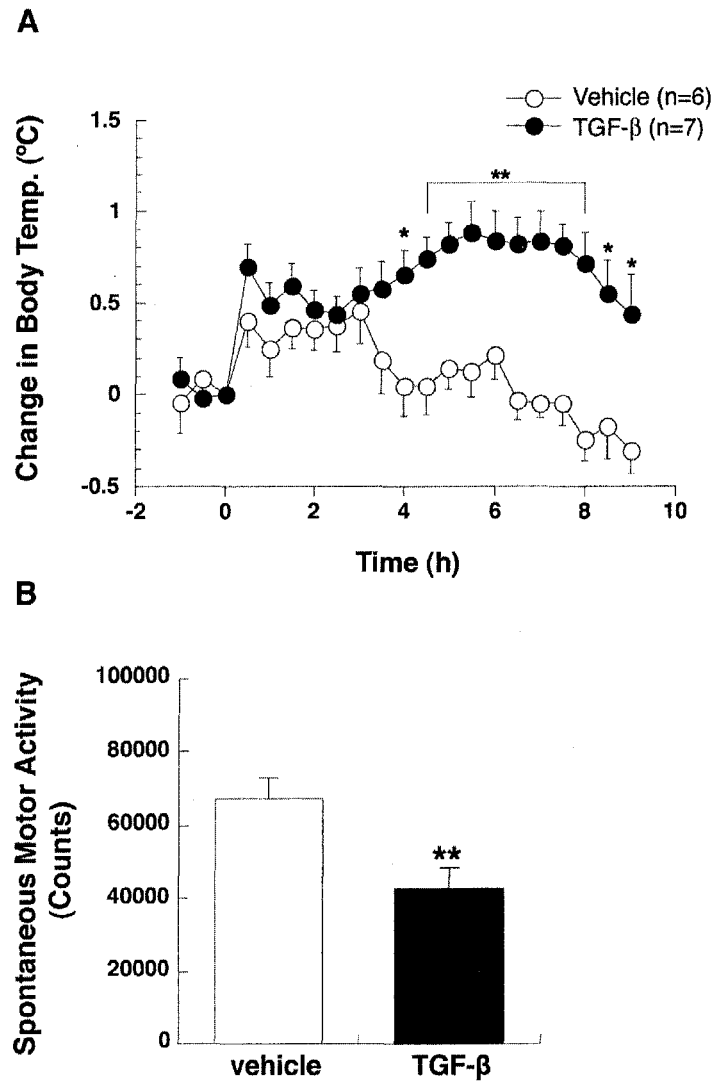


Figure 7. Changes in body temperature and spontaneous motor activity after intracisternal administration of TGF- β . (A) Two hours after the onset of the dark period (time 0), the intracisternal administrations of TGF- β (n=7) or its vehicle (n=6) were carried out and changes in body temperature were measured. Values are the means $\pm$ SEM. **P<0.01, *P<0.05 (two-way repeated ANOVA, followed by Student's *t*-test as a post-hoc test). (B) Total spontaneous motor activity over 8 h following intracisternal administration of TGF- β . Values are the means $\pm$ SEM. **P<0.01 (Student's *t*-test).

Discussion

We previously showed that the concentration of active TGF- β in CSF is increased by exercise and that this increase is associated with the manifestation of the feeling of fatigue and various physiological changes (2, 15, 34). Therefore, we considered that TGF- β in the brain is also related to the tiredness during infection. As an index of the feeling of fatigue, we

determined the SMA of the rats treated with poly I:C during the dark period, when the rats were active (Fig.1). The SMA of the saline-treated group was consistently higher than that of the poly I:C-treated group. Poly I:C treatment decreased SMA significantly 2 h after the administration and thereafter. This indicates that Poly I:C administration decreased SMA even during the active period of the rats. A similar decrease in SMA was previously observed by the administration of LPS, which is another well-known pyrogenic substance (10, 21). It seems likely that these substances cause the feeling of fatigue, which is similar to the tiredness we feel when we catch a cold or are infected with an influenza virus, and depress the motivation to move spontaneously.

The concentration of active TGF- β in CSF peaked 1 h after poly I:C administration, remained high afterwards, and was still significantly high 4 h after administration (Fig. 2A). This increase indicates the presence of mechanisms that transduce information on pathogenic invasion from peripheral tissues (in this study, the i.p. administration of the virus-like substance) to the brain. On the other hand, regarding cytokines in serum, the concentrations of TNF- α and IL-6 peaked 2 h after poly I:C administration (Fig. 3C, D). Similar increases were reported previously (13). No changes were observed in the concentrations of IL-1 β , IL-2, and active and total TGF- β in serum (Fig. 3A, B) under this experimental condition. The increase in the concentration of active TGF- β in CSF occurred earlier than those of other cytokines in serum. Therefore, the increase in TGF- β in CSF was not likely induced by cytokines in serum. In the case of LPS, Romanovsky pointed out the involvement of the vagus nerves with the early phase of sickness syndrome (29). Although it has not been elucidated how information of the invasion of the virus-like substance (poly I:C) is transmitted via the vagus nerves, this system may be a possible mechanism for the early increase in active TGF- β in the brain. Of course we could not exclude the possibility of the contribution of cytokines which have not yet been determined and whose concentration may increase more rapidly.

We next investigated the possible role of a TGF- β increase in CSF following poly I:C administration. The intracisternal administration of the anti-TGF- β antibody to rats partially but significantly inhibited the rise in body temperature induced by poly I:C (Fig. 5). This partial inhibition of body temperature rise suggests the possibilities that the administered anti-TGF- β antibody could not completely neutralize the activity of TGF- β or that multiple mechanisms that are independent of TGF- β in the brain are involved in the rise in body temperature. Furthermore, the intracisternal administration of TGF- β itself caused a significant rise in body temperature (Fig. 7A). Because this experiment was conducted during the dark period, the rise in body temperature of the vehicle-administered group was partly due to the increase in SMA. The

body-temperature increases that occurred just after drug administration in both groups were caused by stress associated with handling and intracisternal administration. In the TGF- β -administered group SMA was significantly lower (Fig. 7B), and the SMA was unlikely to contribute to an increase in body temperature. Therefore, the intracisternal administration of TGF- β itself was responsible for the significant rise in body temperature from 4 to 9 h after the administration, and TGF- β in the brain can be considered to increase the body temperature.

Infection or proinflammatory cytokines induce cyclooxygenase-2 (COX-2) and membrane-associated prostaglandin E synthase (mPGES) mainly in vascular endothelial cells. These enzymes ultimately synthesize prostaglandin E2 (PGE2) from arachidonic acid (7, 24, 33). PGE2 acts on the thermoregulatory center in the hypothalamus and causes a rise in body temperature (12, 31, 32). TGF- β induces COX-2 in neurons and astrocytes *in vitro* (23). Therefore, the inhibition of the body temperature rise observed in this study may have been caused by the inhibition of this signal transduction pathway. The concentration of TGF- β in CSF peaked 1 h and remained high 4 h after poly I:C administration. The peak time of this increase in concentration was earlier than that of the rise in body temperature (4~5 h). These dynamics also suggest the involvement of TGF- β in the induction of enzymes associated with the modulation of body temperature.

There have been studies demonstrating the attenuation of the production and activity of proinflammatory cytokines in peripheral tissues (11) and the suppression of the activation of lymphocytes (8, 28) and microglia (5, 18, 25) by TGF- β . In all of these studies, the effects of TGF- β appeared to be in direct opposition to the effects of proinflammatory cytokines. However, our results, which suggest the involvement of TGF- β in the mechanism underlying the development of fever, indicate that the effects of this cytokine are not necessarily limited to anti-inflammatory processes. Indeed, it has been reported that TGF- β both activates and inactivates macrophages (3) and activates nuclear factor- κ B (NF- κ B) (20, 22), a member of the proinflammatory signal transduction system. These data indicate that the effects of TGF- β are bidirectional, that is, pro- or anti-inflammatory, depending on the class and status of the cell that receives the action of TGF- β .

In contrast to the inhibition of the body temperature rise, intracisternal administration of anti-TGF- β antibody did not eliminate the decrease in SMA following poly I:C treatment in the present study. The administration of a large dose of poly I:C markedly decreased SMA. To date, no study has reported a treatment that could eliminate the decrease in SMA induced by a large dose of poly I:C or LPS (4, 13, 16). Thus, we conclude that TGF- β in the brain is also not

associated with this decrease in SMA. Biologically, a marked decrease in SMA during infection indicates an advantage of this inactivity, and implies a tight regulation by multiple factors. It has been reported that various cytokines are released following the administration of poly I:C and LPS (10, 13), and our study confirmed this phenomenon. Because no study on proinflammatory cytokines has yet been conducted from the point of view of the depressive effect on SMA, the effect of cytokines other than TNF- α and IL-6 should be investigated.

The proinflammatory cytokines described above are therapeutically administered to humans. Side effects such as fever, anorexia, tiredness and (psychological) depression are problems associated with medication with these cytokines. Among these side effects, fever can be treated with appropriate antipyretics, but the feeling of fatigue cannot. This dissociation indicates that the mechanisms underlying the development of fever and the manifestation of the feeling of fatigue are different. To prevent the above-mentioned side effects of therapeutic cytokines, the mechanisms by which these cytokines cause the feeling of fatigue or decrease SMA in experimental animals should be elucidated.

References

1. **Abe M, Harpel JG, Metz CN, Nunes I, Loskutoff DJ, and Rifkin DB.** An assay for transforming growth factor-beta using cells transfected with a plasminogen activator inhibitor-1 promoter-luciferase construct. *Anal Biochem* 216: 276-284, 1994.
2. **Arai M, Yamazaki H, Inoue K, and Fushiki T.** Effects of intracranial injection of transforming growth factor-beta relevant to central fatigue on the waking electroencephalogram of rats: comparison with effects of exercise. *Prog Neuropsychopharmacol Biol Psychiatry* 26: 307-312, 2002.
3. **Ashcroft GS.** Bidirectional regulation of macrophage function by TGF-beta. *Microbes Infect* 1: 1275-1282, 1999.
4. **Bluthe RM, Laye S, Michaud B, Combe C, Dantzer R, and Parnet P.** Role of interleukin-1beta and tumour necrosis factor-alpha in lipopolysaccharide-induced sickness behaviour: a study with interleukin-1 type I receptor-deficient mice. *Eur J Neurosci* 12: 4447-4456, 2000.
5. **Brionne TC, Tesseur I, Masliah E, and Wyss-Coray T.** Loss of TGF-beta 1 leads to increased neuronal cell death and microgliosis in mouse brain. *Neuron* 40: 1133-1145, 2003.
6. **Cannon JG, Fielding RA, Fiatarone MA, Orencole SF, Dinarello CA, and Evans WJ.** Increased interleukin 1 beta in human skeletal muscle after exercise. *Am J Physiol* 257:

R451-455, 1989.

7. **Cao C, Matsumura K, Yamagata K, and Watanabe Y.** Cyclooxygenase-2 is induced in brain blood vessels during fever evoked by peripheral or central administration of tumor necrosis factor. *Brain Res Mol Brain Res* 56: 45-56, 1998.
8. **Chen W and Wahl SM.** TGF-beta: the missing link in CD4+CD25+ regulatory T cell-mediated immunosuppression. *Cytokine Growth Factor Rev* 14: 85-89, 2003.
9. **Conti B, Tabarean I, Andrei C, and Bartfai T.** Cytokines and fever. *Front Biosci* 9: 1433-1449, 2004.
10. **Dantzer R.** Cytokine-induced sickness behaviour: a neuroimmune response to activation of innate immunity. *Eur J Pharmacol* 500: 399-411, 2004.
11. **Dennler S, Goumans MJ, and ten Dijke P.** Transforming growth factor beta signal transduction. *J Leukoc Biol* 71: 731-740, 2002.
12. **Feldberg W and Saxena PN.** Further studies on prostaglandin E 1 fever in cats. *J Physiol* 219: 739-745, 1971.
13. **Fortier ME, Kent S, Ashdown H, Poole S, Boksa P, and Luheshi GN.** The viral mimic, polyinosinic:polycytidylic acid, induces fever in rats via an interleukin-1-dependent mechanism. *Am J Physiol Regul Integr Comp Physiol* 287: R759-766, 2004.
14. **Inoue K, Yamazaki H, Manabe Y, Fukuda C, and Fushiki T.** Release of a substance that suppresses spontaneous motor activity in the brain by physical exercise. *Physiol Behav* 64: 185-190, 1998.
15. **Inoue K, Yamazaki H, Manabe Y, Fukuda C, Hanai K, and Fushiki T.** Transforming growth factor-beta activated during exercise in brain depresses spontaneous motor activity of animals. Relevance To central fatigue. *Brain Res* 846: 145-153, 1999.
16. **Katafuchi T, Kondo T, Take S, and Yoshimura M.** Enhanced expression of brain interferon-alpha and serotonin transporter in immunologically induced fatigue in rats. *Eur J Neurosci* 22: 2817-2826, 2005.
17. **Katafuchi T, Kondo T, Yasaka T, Kubo K, Take S, and Yoshimura M.** Prolonged effects of polyriboinosinic:polyribocytidylic acid on spontaneous running wheel activity and brain interferon-alpha mRNA in rats: a model for immunologically induced fatigue. *Neuroscience* 120: 837-845, 2003.
18. **King VR, Phillips JB, Brown RA, and Priestley JV.** The effects of treatment with antibodies to transforming growth factor beta1 and beta2 following spinal cord damage in the adult rat. *Neuroscience* 126: 173-183, 2004.

19. **Kluger MJ.** Fever: role of pyrogens and cryogens. *Physiol Rev* 71: 93-127, 1991.
20. **Konig HG, Kogel D, Rami A, and Prehn JH.** TGF- β 1 activates two distinct type I receptors in neurons: implications for neuronal NF- κ B signaling. *J Cell Biol* 168: 1077-1086, 2005.
21. **Kozak W, Conn CA, and Kluger MJ.** Lipopolysaccharide induces fever and depresses locomotor activity in unrestrained mice. *Am J Physiol* 266: R125-135, 1994.
22. **Lu T, Burdelya LG, Swiatkowski SM, Boiko AD, Howe PH, Stark GR, and Gudkov AV.** Secreted transforming growth factor β 2 activates NF- κ B, blocks apoptosis, and is essential for the survival of some tumor cells. *Proc Natl Acad Sci USA* 101: 7112-7117, 2004.
23. **Luo J, Lang JA, and Miller MW.** Transforming growth factor β 1 regulates the expression of cyclooxygenase in cultured cortical astrocytes and neurons. *J Neurochem* 71: 526-534, 1998.
24. **Matsumura K, Cao C, Ozaki M, Morii H, Nakadate K, and Watanabe Y.** Brain endothelial cells express cyclooxygenase-2 during lipopolysaccharide-induced fever: light and electron microscopic immunocytochemical studies. *J Neurosci* 18: 6279-6289, 1998.
25. **McCartney-Francis N, Jin W, and Wahl SM.** Aberrant Toll receptor expression and endotoxin hypersensitivity in mice lacking a functional TGF- β 1 signaling pathway. *J Immunol* 172: 3814-3821, 2004.
26. **Ohnuki K, Moritani T, Ishihara K, and Fushiki T.** Capsaicin increases modulation of sympathetic nerve activity in rats: measurement using power spectral analysis of heart rate fluctuations. *Biosci Biotechnol Biochem* 65: 638-643, 2001.
27. **Pedersen BK and Hoffman-Goetz L.** Exercise and the immune system: regulation, integration, and adaptation. *Physiol Rev* 80: 1055-1081, 2000.
28. **Roes J, Choi BK, and Cazac BB.** Redirection of B cell responsiveness by transforming growth factor β receptor. *Proc Natl Acad Sci USA* 100: 7241-7246, 2003.
29. **Romanovsky AA.** Signaling the brain in the early sickness syndrome: are sensory nerves involved? *Front Biosci* 9: 494-504, 2004.
30. **Smith BK and Kluger MJ.** Human IL-1 receptor antagonist partially suppresses LPS fever but not plasma levels of IL-6 in Fischer rats. *Am J Physiol* 263: R653-655, 1992.
31. **Stitt JT.** Prostaglandin E1 fever induced in rabbits. *J Physiol* 232: 163-179, 1973.
32. **Williams JW, Rudy TA, Yaksh TL, and Viswanathan CT.** An extensive exploration of the rat brain for sites mediating prostaglandin-induced hyperthermia. *Brain Res*

120: 251-262, 1977.

33. **Yamagata K, Matsumura K, Inoue W, Shiraki T, Suzuki K, Yasuda S, Sugiura H, Cao C, Watanabe Y, and Kobayashi S.** Coexpression of microsomal-type prostaglandin E synthase with cyclooxygenase-2 in brain endothelial cells of rats during endotoxin-induced fever. *J Neurosci* 21: 2669-2677, 2001.

34. **Yamazaki H, Arai M, Matsumura S, Inoue K, and Fushiki T.** Intracranial administration of transforming growth factor-beta3 increases fat oxidation in rats. *Am J Physiol Endocrinol Metab* 283: E536-544, 2002.

SUMMARY

CHAPTER 1.

The author investigated the effects of intracranial transforming growth factor (TGF)- β on spontaneous motor activity and energy metabolism. After injection of TGF- β into the cisterna magna of the rat, spontaneous motor activity decreased significantly for 1 h. The intracranial injection of TGF- β produced an immediate decrease in respiratory exchange ratio (RER). However, no significant changes were observed in energy expenditure. TGF- β induced a significant increase in total fat oxidation and a decrease in total carbohydrate oxidation. Furthermore, the serum substrates associated with fat metabolism were significantly altered in rats injected with TGF- β . Both lipoprotein lipase activity in skeletal muscle and the concentration of serum ketone bodies increased, suggesting that the increase in fat oxidation caused by TGF- β may have occurred in the liver and muscle. Intracranial injection of TGF- β appeared to evoke a switch in the energy substrates accessed in energy expenditure. The author's data suggests that the release of TGF- β in the brain by exercise is a signal for regulating energy consumption.

CHAPTER 2.

The author investigated the relationship between TGF- β in the brain and both fever and the feeling of fatigue as typical symptoms during infection. In this study, the i.p. administration of polyinosinic:polycytidylic acid (poly I:C), a synthetic double-stranded RNA, to rats was used as an infection model. Poly I:C decreased spontaneous motor activity (SMA) 2 h after i.p. administration, and this decrease was maintained thereafter. The concentration of active transforming growth factor- β (TGF- β) in cerebrospinal fluid (CSF) increased 1 h after

the administration. This increase occurred earlier than those in the concentrations of other proinflammatory cytokines, such as interleukin-6 (IL-6) and tumor necrosis factor-alpha (TNF-alpha), in serum. However, serum concentration of TGF- β did not changed. The intracisternal administration of an anti-TGF- β antibody partially inhibited fever induced by poly I:C administration; however, this treatment did not affect the decrease in SMA. Furthermore, intracisternal administration of TGF- β raised the body temperature. The author's data indicates that TGF- β in the brain, which was increased by poly I:C administration, is associated with fever but not with a decrease in SMA.

ACKNOWLEDGEMENTS

The author wishes to express his grateful acknowledgment to Dr. Tohru Fushiki, Professor of Nutrition Chemistry, Division of Food Science and biotechnology, Graduate School of Agriculture, Kyoto University, for useful advice, helpful discussions and his continuing interest and encouragement of this research.

The author would like to express his sincere gratitude to Dr. Kazuo Inoue, Associate Professor of the Laboratory of Nutrition Chemistry for his useful advices and encouragement of this research.

The author is deeply grateful to Dr. Satoshi Tsuzuki, Assistant Professor of the Laboratory of Nutrition Chemistry for his useful suggestion and advices.

The author is deeply grateful to Dr. Teruo Kawada, Professor of Laboratory of Molecular Function of Food (Ex-associated Professor of Nutrition Chemistry) for useful advice and generous encouragement.

The author would like to thank Dr. Toshihiko Iwanaga, Professor of Histology and Cytology, Graduate School of Medicine, Hokkaido University, for his useful advice and technical advices.

The author would like to thank Dr. Kiyoshi Matsumura, Professor of Faculty of Information Science and Technology, Osaka Institute of Technology, for his useful discussion and encouragement.

The author would like to thank Dr. Yoshihisa Nakano, Professor of Laboratory of Nutrition Chemistry, Osaka Prefecture University, and Dr. Junko Matsuzaki, the senior member of the laboratory of Nutrition Chemistry, Osaka Prefecture University, for their useful discussion and encouragement.

The author appreciates to Dr. Tetsuro Shibakusa, the member of the same research group, for his useful discussion and encouragement.

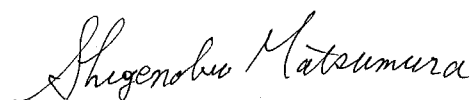
The author would acknowledges the members of same research group (Exercise-group:UnG; Fatigue-group: HiroG) for their useful discussion and encouragement.

The author appreciates to Dr. Hanae Yamazaki, Dr. Wataru Mizunoya, Dr. Yasuko Manabe, Dr. Takayuki Kawai, Dr. Ayako Suzuki, Dr. Naohiko Inoue, Dr. Hiroya Kawasaki and Dr. Nobuyuki Takahashi, the senior member of the laboratory, for respectful instruction of experimental technique and attitude.

Thanks are due to all the members of the Laboratory of Nutritional Chemistry, Kyoto University, for their helpful support and assistance.

Finally, the author thanks his parents for their encouragement and support.

Laboratory of Nutrition Chemistry
Kyoto University

A handwritten signature in cursive script, reading "Shigenobu Matsumura".

LIST OF PUBLICATIONS

1. **Intracranial administration of transforming growth factor- β 3 increases fat oxidation in rats.**

Am J Physiol Endocrinol Metab. 2002 Sep;283(3):E536-44.

2. **Increase in transforming growth factor-beta in the brain during infection is related to fever, not depression of spontaneous motor activity.**

Neuroscience. 2007 Feb 9;144(3):1133-40.