Blood Lactate Functions as a Signal for Enhancing Fatty Acid Metabolism during Exercise via TGF-β in the Brain

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Summary Moderate-intensity running (treadmill velocity of 21 m/min) increased blood lactate and actived transforming growth factor-β (TGF-β) concentration in rat cerebrospinal fluid (CSF). On the other hand, low-intensity running (15 m/min) did not increase blood lactate and caused no change in CSF TGF-β. Intraperitoneal (i.p.) administration of lactate to anesthetized rats caused an increase in blood lactate similar to that observed after a 21 m/min running exercise and increased the level of active TGF-β in CSF. Intraperitoneal administration of lactate at the same dose to awake and unrestricted rats caused a decrease in the respiratory exchange ratio, that is, enhancement of fatty acid oxidation and depression of spontaneous motor activity (SMA). Given that intracisternal administration of TGF-β to rats has been reported to enhance fatty acid metabolism and to depress SMA, we surmise that the observed changes caused by i.p. lactate administration in this study were mediated, at least in part, by TGF-β in the brain.
Key Words lactate, TGF-β, exercise, respiratory gas analysis, spontaneous motor activity

Recently, lactate has been recognized as a good energy source for the central nervous system. Wyss et al. (*1*) demonstrated that lactate instead of glucose could maintain neuronal activities, and that when both substrates are available, the brain preferentially utilizes lactate. The study, however, implies that lactate is not a direct cause of feelings of fatigue by acting on the brain, and negated the classical view of lactate as a fatigue substance. Blood lactate increases during physical exercise, with skeletal muscles being the major sites of lactate production [see Bergersen (*2*) for review]. Increased lactate concentration serves as a signal indicating an increase in the relative ratio of glycolytic energy production, and this signal may function to induce changes in whole-body energy metabolism. Lactate directly acts on neurons and affects their activities. The activities of some neurons in the ventromedial hypothalamus are affected by glucose, whereas some neurons show increased firing rate upon lactate administration (*3*). Patil and Briski (*4*, *5*) reported the activation of catecholaminergic neurons in the hindbrain by blocking the supply of lactate into the brain with α-cyano-4-hydroxycinnamic acid. These findings indicate that lactate functions as an index for monitoring the energy metabolism status of peripheral organs.

We developed a model (Sim-Ex) that simulates physical exercise by inducing skeletal muscle contraction through electrical stimulation in the hindlimbs of anesthetized rats (*6*). Sim-Ex with a frequency of 2 Hz could reproduce an exercise load that corresponded to low-intensity exercise. We reported significant increases in the concentrations of blood lactate and active transforming growth factor-β (TGF-β) in cerebrospinal fluid (CSF) 5 and 10 min after the onset of Sim-Ex, respectively (*7*). A fraction of active TGF-β increased in rat CSF after exhaustion by exercise (*8*). Intracisternal administration of TGF-β to sedentary animals caused depression of spontaneous motor activity (SMA) in mice (*8*) and enhancement of fatty acid oxidation in rats (*9*). The former was considered as a fatigue-like behavior and the latter as a regulatory action on whole-body metabolism for endurance during exercise. These findings indicate that TGF-β is a factor in the manifestation of the feeling of fatigue and modulation of energy metabolism during physical exercise. In this study, we investigated whether increase in blood lactate caused augmentation of active TGF-β in CSF, and showed that lactate affected the energy metabolism and SMA of rats via TGF-β in the brain.

MATERIALS AND METHODS

Animals. This study was conducted in accordance with the ethical guidelines of the Kyoto University Animal Experimentation Committee and was in complete compliance with the National Institutes of Health Guide for the Care and Use of Laboratory Animals. All procedures were approved by the Kyoto University Animal Care and Use Committee. Male Sprague-Dawley rats (225–280 g; CLEA Japan, Inc., Tokyo, Japan) were used. All animals were maintained under controlled environmental conditions (22±0.5°C, 12-h light-dark cycle, and food and water ad libitum). All experiments were carried out during the dark cycle.

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Surgery.

Cannula implantation into the cisterna magna. Surgery was performed on the animals on the day of purchase. Cannulas for implantation in the cisterna magna were prepared according to Bouman and van Wimersma Greidanus (*10*), and consisted of an outer guide (length 22.7 mm, 23G) and an inner dummy cannula (23.2 mm, 29G) made of stainless steel. A CSF outflow component was omitted for simplification. The surgical approach for cannula implantation into the cisterna magna has been described previously (*9*). Briefly, the rats were anesthetized with isoflurane (2.0–2.5%, by inhalation) and fixed to a stereotaxic apparatus. A guide cannula was introduced 3.0 mm posterior to the lambda suture, inclining anteriorly at an angle of 60°, and inserted to a depth of 8.7 mm. Cannulas were fixed with Loctite 454 (Loctite Japan, Yokohama, Japan). Rats were allowed to recover for 1 wk after surgery, and only animals with body weights greater than those before surgery were used. After the completion of experiments, brains were removed, sectioned, and stained with thionine to confirm cannula placement accuracy. Animals that were improperly cannulated were excluded from the analysis.

Cannulation of jugular vein. For collecting blood for determination of time-course changes in the concentration of lactate after intraperitoneal (i.p.) lactate administration, a sampling cannula was acutely implanted according to Shibakusa et al. (*7*). Rats were anesthetized as described above, and a Hydrocoat catheter (Access Technologies, Skokie, IL) filled with 0.1% heparinized saline was inserted in the right atrium via the jugular vein.

Intraperitoneal administration and determination of blood concentration of lactate. Sodium L-lactate (Sigma-Aldrich Japan, Tokyo, Japan) and sodium chloride (Nacalai Tesque, Inc., Kyoto, Japan) were intraperitoneally administered. The concentrations of the administration solutions were adjusted by dilution with saline (Shiono Chemical Co., Ltd., Tokyo, Japan), and the pH was adjusted to 7.0. The administration amount of lactate was determined to be 900 mg/kg BW (450 mg/mL solution) by preliminary examination. The concentration of sodium chloride solution (234 mg/kg BW, 234 mg/mL solution) was determined to be of the same molar concentration (4.0 M). For collecting CSF from implanted cannulas or time-course sampling of blood (0, 5, 10, and 20 min after administration, and 40 min as additional data in a separate experiment) from jugular cannulas, rats were administered lactate solution under anesthesia. Blood was collected and mixed with an equal volume of 0.8 M perchloric acid, and the mixture was centrifuged (5,000 ×*g* for 5 min); the supernatant was stored at −70°C until determination. Lactate concentrations were determined with Determiner LA (Kyowa Medex Co., Ltd., Tokyo, Japan).

Cerebrospinal fluid collection. Cerebrospinal fluid was collected from rats immediately after the completion of treadmill running or 10 min after i.p. administration of lactate. CSF collections were made through the cannula

implanted in the cisterna magna. The rats were anesthetized with isoflurane, which has little effect on plasma energy substrate levels in short-term experiments (*7*), and fixed in the supine position; the dummy cannula was removed from the guide cannula, and CSF that leaked through the cannula was collected; 100 μL of CSF was collected in approximately 30 s. After collection, CSF was centrifuged at 2,000 ×*g* at 4°C, and the supernatant was stored at −70°C until TGF-β concentration determination. CSF visibly contaminated with red blood cells was discarded.

Determination of active form of TGF-β in rat CSF. Active TGF-β concentrations in CSF were determined using a bioassay with TGF-β-responsive mink lung epithelial cells (TMLCs) (*11*). This cell line was constructed from mink lung epithelial cells by stable transfection with a TGF-β-responsive human plasminogen activator inhibitor-1 promoter fused to a luciferase reporter gene (kindly provided by Dr. M. Abe, Department of Nanomedicine, Tokyo Medical and Dental University, and Dr. D. Rifkin, Department of Cell Biology, New York University Medical Center). The luciferase activity is completely selective for and dependent on TGF-β in the medium. All samples were diluted with DMEM containing 0.1% BSA. Purified TGF-β (R&D Systems, Minneapolis MN, USA) was used to establish a standard curve. TMLCs suspended in DMEM with 10% FBS were seeded on 96-well plates (1×10^4 cells/well) and allowed to attach for 6 h; the medium was then replaced with 100 μL of sample solution. After 16 h, luciferase activity was measured using a Veritas microplate luminometer (Promega, Tokyo, Japan) according to the manufacturer's instructions. All experiments were performed in triplicate.

Treadmill running of rats. After the recovery period, all rats were trained to run for 30 min/d on a treadmill (inclination was set at 3°) for 6 d, so that they were well accustomed to experimental handling and running at 21 m/min as described before (*12*). The animals that could not complete this schedule were excluded from the experiment. On the day of experiment, food and water were removed 2 h before running. For moderate-intensity running, during the first 5 min, the treadmill speed at the start was 16 m/min and was then increased in a stepwise manner (1 m/min increment) up to 21 m/min. For low-intensity running, during the first 5 min, the treadmill speed at the start was 10 m/min and then was similarly increased to 15 m/min. Total running time was 30 min for each condition. After running, some of the rats were decapitated and blood samples were collected; the other rats were anesthetized with sodium pentobarbital and CSF was collected as described above.

Measurement of respiratory exchange ratio in rats. The respiratory exchange ratio (RER) was measured by indirect calorimetry as previously described (*9*). In brief, a respiratory gas analysis system (ARCO-2000, Arcosystem Inc., Chiba, Japan) consisting of 12 acrylic chambers (21×10×13 cm), a mass spectrometer for CO_2 and O_2, and a switching system for collecting samples from
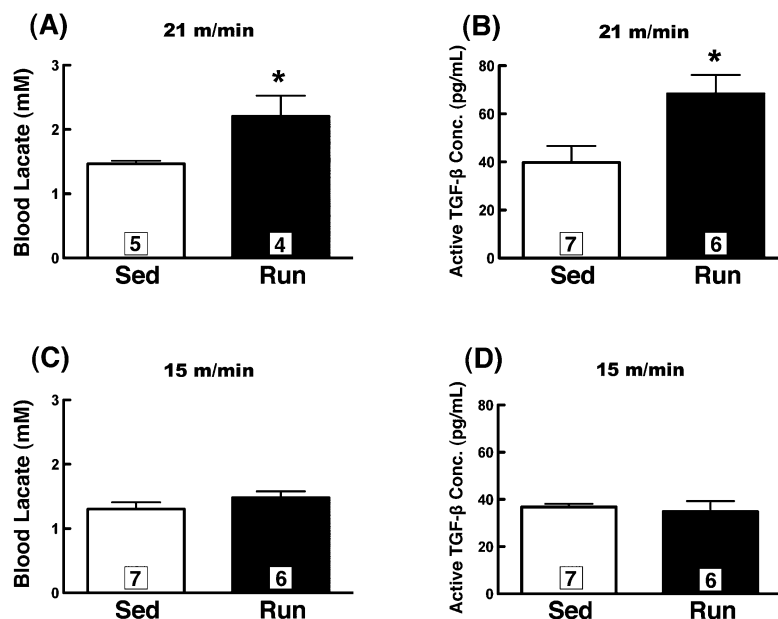
Fig. 1.   Concentration of blood lactate and increase in active TGF-β in CSF in rats during treadmill running. Rats were made to run at 15 m/min or 21 m/min for 30 min on a treadmill (Run). The inclination of belts was set at 3°. After completion of the activity, blood lactate and active TGF-β in CSF were determined. The rats that were sedentary (Sed) were used as a control. The number of animals used for data analysis is indicated in each column. *$p<0.05$.
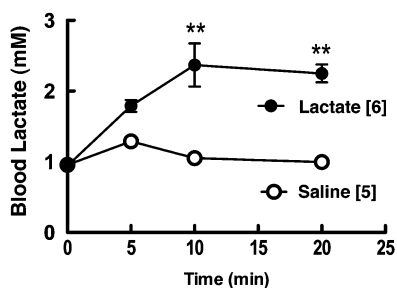

Fig. 2.   Time-course changes in blood lactate concentration after i.p. administration of lactate. The time-course changes in blood lactate were determined after i.p. administration of lactate (900 mg/kg BW) in anesthetized rats. Saline was used as vehicle and administered as a control. $n=6$ for lactate and $n=5$ for saline administration. **$p<0.01$.

Fig. 3.   Increase in active TGF-β in CSF by i.p. administration of lactate. Lactate (900 mg/kg BW) was intraperitoneally administered to anesthetized rats; CSF samples were collected 10 min after the i.p. administration, and the concentration of active TGF-β was determined. Control CSF was collected from rats intraperitoneally administered saline. $n=6$ for each group. *$p<0.05$.

and serially changing the airflow in each metabolic chamber was used. The rats were placed individually in a chamber for 2 h for stabilizing the RER, and the data for the last 30 min were used as the baseline value. Then, lactate, NaCl, or saline was intraperitoneally administered, and the determination was immediately resumed. Food and water were given ad libitum during the measurement.

Determination of SMA of rats.   Determination of SMA of rats was done according to the method of Yamazaki et al. (*9*). Briefly, the SMA of the rats was examined for 2 h after injection of samples with a Supermex (Muromachi Kikai, Tokyo, Japan). This apparatus surveys the measurement area with multiple lenses that optically divide the area and detect the animals' infrared radiation. The motor activity was assessed as one count when the animal moved from one part of the measurement area to a neighboring part. The cage for measure-
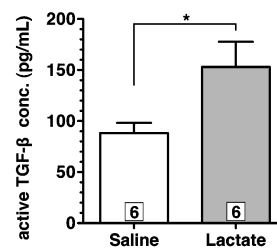
ment ($25\times38\times17.5$ cm) was a completely novel environment for each rat.

Statistics.   Comparisons between two groups were done using Student's unpaired *t*-test. For comparison of time-course changes between two groups, data were tested with a two-way repeated analysis of variance, followed by the Bonferroni multiple comparisons test. All values were shown as mean±SE. A *p* value of <0.05 was considered statistically significant. All statistical analyses were carried out using the GraphPad Prism 5 software package (GraphPad Software, Inc., La Jolla, CA, USA).

RESULTS

Exercise intensity, blood lactate, and activation of TGF-β in CSF of rats

We investigated the effects of low-intensity running (15 m/min, 3° inclination) and moderate-intensity running (21 m/min, 3° inclination) on blood lactate and
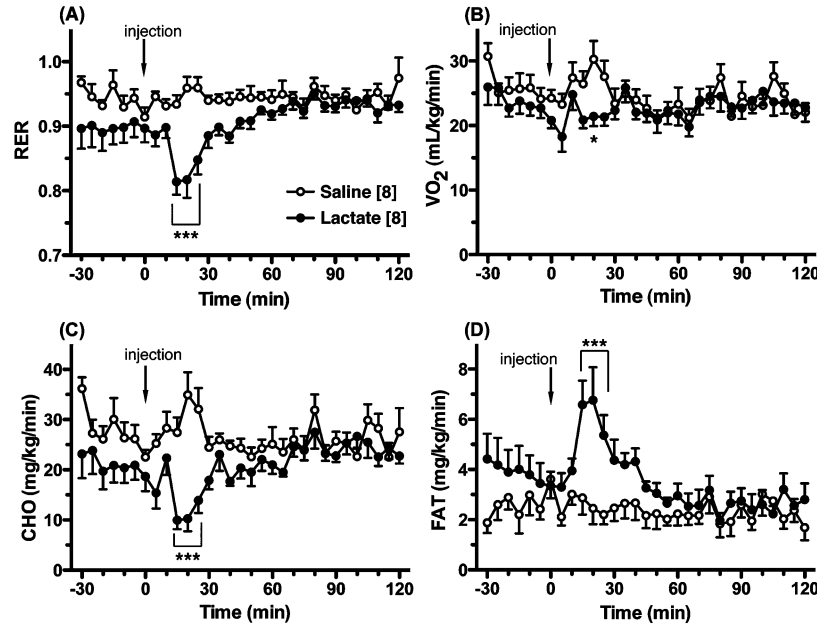
Fig. 4. Respiratory gas analysis of rats intraperitoneally administered lactate. Lactate (900 mg/kg BW) was intraperitoneally administered to awake rats, and (A) respiratory exchange ratio (RER), (B) oxygen consumption, (C) carbohydrate oxidation, and (D) fatty acid oxidation were determined. $n=8$ for each group. ***$p<0.001$.
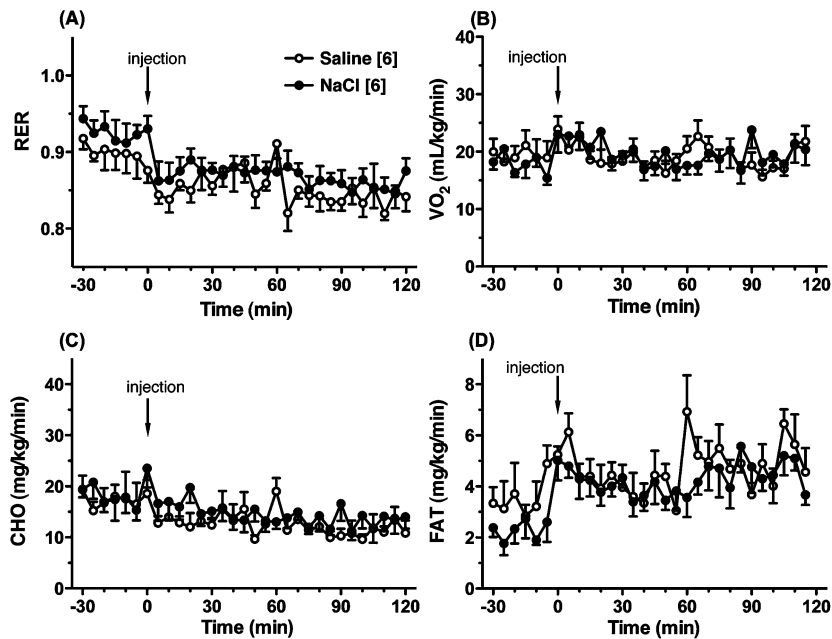

Fig. 5. Respiratory gas analysis of rats intraperitoneally administered NaCl. NaCl solution (234 mg/kg BW) was intraperitoneally administered to awake rats, and (A) respiratory exchange ratio (RER), (B) oxygen consumption, (C) carbohydrate oxidation, and (D) fatty acid oxidation were determined. $n=6$ for each group.

the concentration of active TGF-β in CSF of rats. As shown in Fig. 1, moderate-intensity running caused significant increase in the levels of blood lactate and active TGF-β in CSF as compared with sedentary controls. On the other hand, low-intensity running resulted in no change in blood lactate or CSF TGF-β when compared with sedentary controls.

Time-course change in the concentration of lactate in blood after i.p. lactate administration

The association of increase in blood lactate induced by moderate-intensity running with the activation of

TGF-β in CSF was investigated in rats. As actual exercise changes the concentrations of other energy substrates and hormones in blood, we examined whether a similar increase in blood lactate as that induced by exercise could be achieved by i.p. administration of lactate under anesthesia. The appropriate administration amount of lactate was determined to be 900 mg/kg BW from a preliminary experiment. As shown in Fig. 2, i.p. administration of lactate at 900 mg/kg BW in rats caused an increase in blood lactate, which, 10 min after administration, reached a concentration comparable
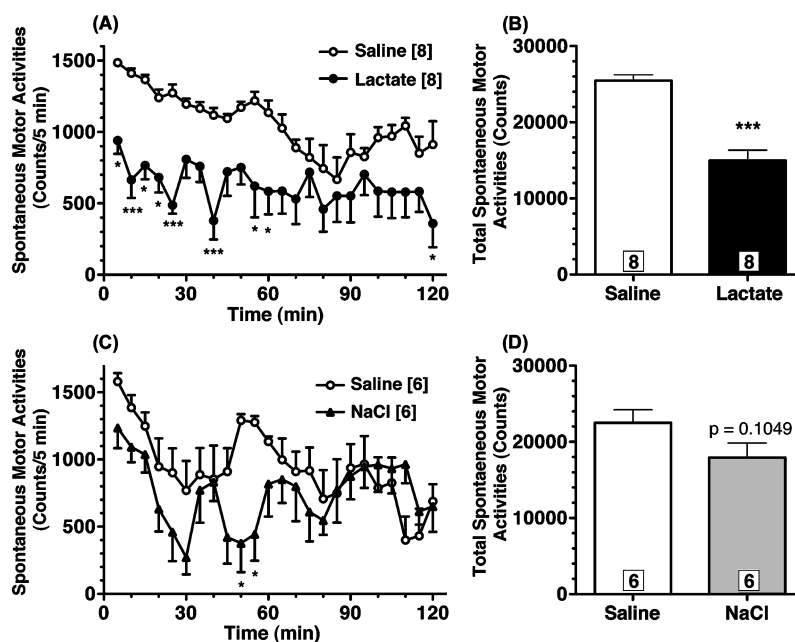
Fig. 6. Spontaneous motor activities of rats administered with lactate and NaCl. Lactate (900 mg/kg BW) and NaCl solution (234 mg/kg BW) were intraperitoneally administered to awake rats, and spontaneous motor activities (SMA) were determined for 120 min. (A) Time-course changes in SMA with lactate administration; (B) total counts of SMA of the lactate-administered group; (C) time-course changes in SMA with NaCl administration; and (D) total counts of SMA of NaCl-administered group. Intraperitoneal administration of saline was used as a control. The number of animals used for data analysis was indicated in each column. $*p<0.05$; $***p<0.001$.

with that caused by moderate-intensity running. Additional data from 40 min after the injection showed that the level of lactate would be maintained at a similar level to that recorded at the from 10-min mark (1.22 mM at 0 min vs. 2.88 mM at 40 min, $n=3$, respectively).

Increase in active TGF-β in CSF by i.p. administration of lactate

Lactate was intraperitoneally administered under the same conditions as described above; 10 min after the administration, CSF from rats was collected, and the concentration of active TGF-β was assayed. As shown in Fig. 3, compared with the control administered saline, the rats administered lactate showed a significant increase in the concentration of active TGF-β in CSF.

Changes in RER by i.p. administration of lactate

Next, the same amount of lactate was intraperitoneally administered to awake and unrestricted rats, and analyzed the RER. As shown in Fig. 4, although the changes were transient, lactate significantly decreased the RER and enhanced fatty acid oxidation during the period from 15 to 25 min after administration. In order to confirm that this RER decrease was not caused by osmotic shock with hypertonic solution, NaCl solution, which was prepared to the same molar concentration as the lactate solution, was similarly administered to rats. As shown in Fig. 5, no significant change was observed in the RER of rats administered hypertonic NaCl solution.

Changes in SMA by i.p. administration of lactate

We previously reported that increase in active TGF-β in CSF caused depression of SMA in mice (8) and rats (9). Thus, we examined whether i.p. administration of

lactate causes depression of SMA by inducing an increase in active TGF-β in CSF. Figure 6 shows the time-course changes in SMA and total amount of SMA of rats after i.p. administration of lactate and NaCl at the same molar concentration. Intraperitoneal administration of lactate caused a significant decrease in SMA at several time points (Fig. 6A) and in total SMA as compared with the saline-administered control (Fig. 6B). Intraperitoneal administration of NaCl caused a similar depression in SMA, which showed significant difference at two time points (Fig. 6C), and a decrease in total SMA, which was not statistically significant (Fig. 6D).

DISCUSSION

Previous reports showed that an increase in blood lactate induced changes in the concentration of blood catecholamine and whole-body glucose metabolism (13–15). These findings indicate the role of lactate as an index for monitoring energy metabolism, and that increase in lactate affects energy metabolism. Changes in blood lactate concentration are mainly detected in important sites in the brain such as the hypothalamus and nucleus solitary tract. Administration of lactate has been shown to have a modulatory effect on neuronal activities in these sites (3, 16, 17). On the other hand, in peripheral tissues, an increase in lactate was detected in the hepatic portal system, which affected the catecholamine response to hypoglycemia (18). These findings imply the presence of a mechanism which regulates whole-body energy metabolism in response to the changes in the concentration of blood lactate.

We previously showed that physical exercise increased the level of active TGF-β in CSF of fatigued rats (*8*), and that intracisternal administration of TGF-β enhanced fatty acid oxidation (*9*). In addition, noradrenergic projections to the hypothalamus, which plays a pivotal role in the regulation of energy metabolism, were activated by intracisternal administration of TGF-β (*19*), and inhibition of fatty acid oxidation by i.p. administration of mercaptoacetate led to an increase in active TGF-β in rat CSF (*20*). From these data, TGF-β in the brain seemed to be closely linked to the energy metabolism state in the body and to play an important role in the regulation of energy metabolism. In addition, a positive correlation was observed between the increase in blood lactate after the onset of physical exercise (in this case, Sim-Ex) and the augmentation of active TGF-β in the CSF of the same rats (*7*). These data led us to postulate that increase in blood lactate during physical exercise has a signaling function in the regulation of energy metabolism via TGF-β in the brain.

Exercise intensity, blood lactate, and activation of TGF-β in CSF of rats

First, we investigated the relationship between blood lactate concentration and active TGF-β level in CSF in the rats subjected to treadmill running. An increase in active TGF-β concentration in CSF was observed only under conditions that consequently increased blood lactate (Fig. 1). Previously, Kitaoka et al. (*21*) showed the difference in energy substrate utilization by respiratory gas analysis during exercise at velocities of 15 m/min and 20 m/min (3° inclination) in rat treadmill running. At 15 m/min, rats efficiently oxidized fatty acid, and the dominance of fatty acid utilization could be concluded from the lack of increase in blood lactate after the completion of a 30-min run at this velocity. In the present study, 21 m/min running significantly increased blood lactate concentration, and the concentration of active TGF-β in CSF similarly increased. These data indicate that the increase in blood lactate enhances the activation of TGF-β in CSF. This notion is supported by the observation that the increase in blood lactate was followed by an increase in active TGF-β in CSF in Sim-Ex (*7*).

Increase in active TGF-β in CSF by i.p. administration of lactate

Next, we investigated the effect of blood lactate increase on the activation of TGF-β in CSF by i.p. administration of lactate to anesthetized rats in order to minimize the unnecessary changes in energy substrates, metabolites, and hormones that usually occur during actual exercise. Ten minutes after i.p. administration of lactate, CSF samples from rats were collected, and the concentration of active TGF-β was determined. Intraperitoneal administration of lactate at 900 mg/kg BW in rats produced an increase in blood lactate similar to that observed during actual exercise (Fig. 2), and the treatment induced a significant increase in active TGF-β in CSF (Fig. 3). In the anesthetized rats without Sim-Ex, no significant changes in the levels of hormones or energy substrates (e.g., glucose) in blood or in the con-

centration of active TGF-β in CSF were found (*7*). Therefore, it seems reasonable to infer that the increase in active TGF-β in CSF in this study was caused by i.p. administration of lactate. The concentration of active TGF-β in control CSF in Fig. 1 and that in Fig. 3 were different. A little variation in baseline values was observed among the determinations in our bioassay for TGF-β using TMLCs. However, the quantitative relationship between the values of the control and treatment groups was accurately reflected in each determination because cells were prepared in the same lot and treated in the same culture plate.

Changes in RER by i.p. administration of lactate

Further, the effect of i.p. administration of lactate on the whole-body energy metabolism was examined. As shown in Fig. 4A, when the same amount of lactate that caused an increase in active TGF-β in CSF in anesthetized rats was intraperitoneally administered to awake rats, a significant decrease in the RER was induced, although it was only about 20 min in duration. Although lactate administration suppressed SMA of rats (discussed later), there was only one time point at which a significant decrease in oxygen consumption was observed (Fig. 4B). From these data, calculated carbohydrate and fat oxidation showed transient but significant decrease (Fig. 4C) and increase (Fig. 4D), respectively. Blood lactate increase is hypothesized to be caused by (i) insufficient enhancement of heart beat/blood flow and energy substrate mobilization at the onset of exercise or (ii) relatively higher intensity of exercise. Under these conditions, energy production via the glycolytic pathway using cellular glycogen store would be dominant. In the well-trained human, fatty acid oxidation is maximized at 65% VO₂max exercise, and its metabolism relatively decreases with further increase in exercise intensity (*22, 23*). Because at high intensity exercise like 80% VO₂max lactate will accumulate but fatty acid oxidation will decrease, the function of lactate to enhance fatty acid oxidation (via TGF-β) seems to be limited to the intensity of aerobic exercise. At the onset of exercise, enhancing fatty acid utilization would be advantageous for animals to maintain endurance during exercise. In this study, the waking rats intraperitoneally administered with lactate were unrestricted but resting, and considered to have only a little physical load. Therefore, the increase in blood lactate in this condition would resemble that in the early phase of exercise. Thus, blood lactate might function as a signal for mobilization of energy substrates, especially for fatty acid recruitment, and drive the activation of TGF-β, which acts as an important modulator in the brain. As a result, enhancement of fatty acid oxidation was thought to be induced, as indicated by a decrease in the RER. Intracisternal administration of TGF-β caused increases in the concentration of non-esterified fatty acid in blood and lipoprotein lipase activity (*9*), but showed no significant effect on blood flow (*7*).

Changes in RER by i.p. administration of NaCl

Because the concentration of lactate administered was relatively high, it might cause osmotic stress or

nociceptive stimulation in awake rats. Therefore, rats were intraperitoneally administered with the same molar concentration of NaCl, and the RER was analyzed. As shown in Fig. 5, NaCl administration did not affect the RER or oxygen consumption. This confirmed that osmotic stress or pain was not a major cause of the decrease in the RER in rats i.p. administered lactate. The intracisternal administration of 40 ng of TGF-β was reported to enhance fatty acid oxidation for at least 60 min in rats (9). Intraperitoneal lactate administration induced only a transient increase in fatty acid oxidation because (i) few organs could take up fatty acids in spite of an increase in its supply because the rats did not execute actual exercise, and (ii) since TGF-β was activated by lactate administration, its amount might be lower than that induced by intracisternal administration. Other regulatory factors might also be induced, some of which might have a counter-regulatory response to fatty acid mobilization.

Changes in SMA by i.p. administration of lactate

Recently, neurons have been demonstrated to utilize lactate preferentially as an energy source (1), and this implies that lactate will not cause a feeling of fatigue by directly acting on the central nervous system. We reported that the increase in active TGF-β in CSF caused not only enhancement of fatty acid oxidation but also depression of SMA in animals, which is a kind of tiredness behavior (8, 9). If the decrease in the RER with i.p. administration of lactate was caused by an increase in active TGF-β in the brain, it would be predicted to depress SMA of animals as well. Therefore, lactate and NaCl were intraperitoneally administered to rats under the same conditions as in the respiratory gas analysis, and their SMAs were determined (Fig. 6). The administration of lactate depressed the SMA of rats over a 120-min determination period. The lactate-treated group showed significantly lower counts at several time points (Fig. 6A), with their total counts of activity also being significantly lower than those of the control group administered saline (Fig. 6B). On the other hand, the time-course changes in SMA of rats administered NaCl at the same molar concentration as lactate generally showed a tendency of depressing SMA, with a significant decrease observed at two time points (Fig. 6C), and lower total counts of activity, which were not statistically significant (Fig. 6D). These data imply that TGF-β is involved, at least in part, in the depression of SMA caused by i.p. administration of lactate. However, the duration of lactate action was different for the RER and SMA. The decrease in the RER was transient, whereas the depression of SMA lasted a relatively long time. This discrepancy indicated that not all the physiological changes induced by i.p. administration of lactate were related to the increase of active TGF-β in the brain. The finding that NaCl administration induced a tendency of SMA depression in rats suggested that i.p. administration of high concentration of salt solution might be a contributing factor to the depression of SMA.

Concerning the validity of the experimental conditions, additional data from 40 min after injection showed the continuation of a similar lactate level as at 10 min (1.22 mM at 0 min vs. 2.88 mM at 40 min). Because these data were obtained from anesthetized rats, there still might be a possibility that the metabolism of lactate would differ from those in active rats. The increase in the blood concentration of lactate from physical exercise would rapidly revert to the basal level just after the completion of the exercise (24, 25), and our result was different from these data. Immediately after the exercise, the metabolism of lactate will continue to be sufficiently activated and the ability of metabolizing exogenously administered lactate could be different from those under anesthesia or those of waking but sedentary animals. As for determination of TGF-β concentration in the CSF, we collected samples shortly (10 min) after the injection, and the notion that the increase in the lactate level triggered the activation of the latent form of TGF-β would be appropriate.

When lactate is oxidized, the RER shows 1. In this study, it is intriguing that the RER of waking but sedentary rats administered lactate showed a lowering of RER and an enhancing fatty acid metabolism just after the administration. The administered lactate seemed to function as a signal to facilitate the recruitment and oxidation of fatty acid to be oxidized preferentially as a substrate. On the other hand, the pattern of depression of SMA was largely different from that of the RER change after lactate administration. In order to elucidate the effect of lactate on energy metabolism and SMA, it will be necessary to determine the detailed changes in blood lactate concentration and in active TGF-β concentration in CSF after lactate administration.

This study showed that an increase in blood lactate activates TGF-β in CSF, which in turn enhances fatty acid oxidation and depresses SMA in rats. In addition to its involvement in the regulation of energy metabolism, lactate may have an indirect role in the manifestation of fatigue through its actions on TGF-β. TGF-β, which is usually inactive (latent form of TGF-β) because of the masking of its active part by latency-associated peptide (LAP), is activated upon removal of LAP through degradation or conformational change. TGF-β is activated by various factors, including proteases (e.g., plasmin and matrix metalloproteases), extracellular matrix proteins (e.g., thrombospondin-1), integrins, and reactive oxygen species (26–28). However, the activation mechanism of TGF-β observed in this study was not clarified. Taking into account our previous report that intracisternal administration of TGF-β affected the electroencephalogram of rats (29) and modulated the noradrenergic projections to the ventromedial hypothalamus (19), TGF-β most likely influences neuronal activities, although its mechanism is unknown. Further investigations are warranted to elucidate the mechanisms underlying the TGF-β effects observed in this study.

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