

The physiological and behavioral effects of subchronic intracisternal administration of TGF- β in rats: comparison with the effects of CRF

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(Received 10 October 2006; and accepted 29 October 2006)

ABSTRACT

We studied the physiological and behavioral effects of subchronic intracisternal administration of transforming growth factor- β (TGF- β) for 7 days. Subchronic intracisternal administration of TGF- β significantly inhibited the increase in body weight of rats but did not affect food intake. In the measurement of locomotor activity after the final intracisternal administration on day 7, the total count for 1.5 h increased significantly in the TGF- β group compared with the vehicle group. However, that for 10 h was not different between both groups. Furthermore, significant elevations in oxygen consumption were observed in the TGF- β group during both light and dark phase. Subchronic TGF- β treatment induced a significant decrease in the number of total leukocytes and lymphocytes and the relative weight of the thymus, and a significant increase in brown adipose tissue weight. Corticotropin-releasing factor (CRF) is the primary neuroendocrine factor released in response to stress. Subchronic treatment with CRF, as a positive control, significantly affected body weight, food intake, oxygen consumption, total leukocyte and lymphocyte counts, and thymus and adrenal weight. Subchronic TGF- β administration partially mimicked the stress responses, implicating a role for TGF- β in the brain in stress.

The transforming growth factor- β s (TGF- β s) are widely recognized as multifunctional cytokines, that regulate differentiation and death of many types of cells, and that consequently modulate immune function (24, 25). TGF- β s are expressed in numerous tissues including the central nervous system (CNS), where neurons, astrocytes, and microglia are the cellular sources (22).

TGF- β plays an important role in neuronal survival and the recovery of normal neuronal function

following CNS diseases (11, 33). The expression of TGF- β in the brain and the concentration of TGF- β in cerebrospinal fluid (CSF) are increased in neurodegenerative diseases, such as transient ischemia (27), Alzheimer's disease (12) and multiple sclerosis (18). In addition, we previously demonstrated that the concentration of TGF- β was increased in the CSF of exercise-fatigued rats and that intracisternal administration of TGF- β in sedentary mice decreased spontaneous motor activity (14, 15). From these results we postulated that TGF- β in the brain might be involved in the manifestation of the feeling of fatigue. Some researchers have reported increased levels of circulating TGF- β in chronic fatigue syndrome patients (4, 20). These observations imply that TGF- β plays an important role in the mechanism of the manifestation of the feeling of fatigue.

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Stress is considered to be one of the principal causes of fatigue. The stress response is comprised of two major components, *i.e.*, activation of the hypothalamus-pituitary-adrenal (HPA) axis and the sympathetic nervous system (32). The HPA axis is the primary neuroendocrine pathway by which the CNS responds to stress (8). In recent years, there has been increasing evidence that chronic fatigue syndrome patients might be related to dysfunction of the HPA axis (7, 9). One of the neuroendocrine factors that appears to be crucial in the regulation and coordination of the endocrine, autonomic and behavioral responses to stress is corticotropin-releasing factor (CRF) (3). CRF functions both as a neuroendocrine factor in the HPA axis to release ACTH, and as a neurotransmitter in various brain regions to enhance the sympathetic nervous system (32).

TGF- β and CRF have been implicated in the sensation of fatigue and stress, respectively. The increase in the concentration of TGF- β in CSF or expression of CRF mRNA in the brain is elicited by a common cause, *i.e.*, exercise, which is one of the physical stressors (15, 19, 30). A large number of studies have reported the physiological and behavioral effects of CRF. For instance, there is significant evidence supporting the effects of acute and chronic administration of CRF into the cerebral ventricles on body weight, food intake, body temperature, anxiety, locomotor activity, and endocrine, immune and sympathetic nervous systems (2, 6, 10, 23, 28). In contrast to CRF, few studies have examined the physiological and behavioral effects of TGF- β in the brain. We previously demonstrated that acute administration of TGF- β into the cisterna magna decreased spontaneous motor activity and increased fat metabolism in rats (35). Therefore, in this study we examined the physiological and be-

havioral effects of subchronic intracisternal administration of TGF- β for 7 days and compared the effects with those of CRF as a positive control.

MATERIALS AND METHODS

Animals. Male SD rats (8 weeks old, Charles river) were maintained on an inverse light-dark cycle (light on 18:00, and light off 6:00) for 1 week acclimatization period. They were housed individually in standard plastic cages (25 × 38 × 17.5 cm) at a room temperature and humidity of 22 ± 0.5°C and 55 ± 5%, respectively. Rats were fed a 30% fat diet containing 21% protein and 49% carbohydrate as kcal-base, during the dark period (6:00–18:00) to synchronize the feeding phase, and given free access to water. The animals were weighed daily at 5:00. 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).

Implantation of the cannula into the cisterna magna and TGF- β administration. Rats were anesthetized with 1 mg/kg pentobarbital sodium, fixed onto a stereotaxic apparatus, and implanted with a permanent 23-gauge guide cannula for sample administration into the cisterna magna, as described previously (35).

Experiment 1: subchronic effect of TGF- β administration for 7 days

After 5 days recovery following the surgery, the rats were treated with either 40 μ L of the vehicle solution (artificial-CSF: 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%

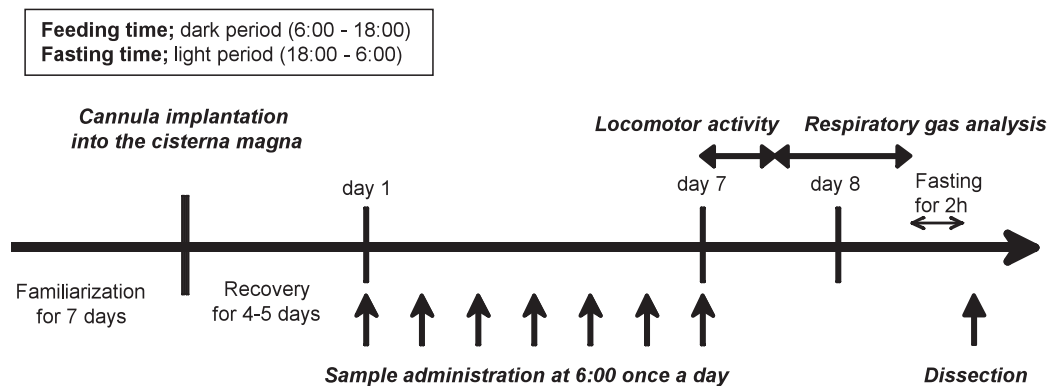


Fig. 1 Experimental protocol for the study of the physiological and behavioral effects of repeated intracisternal administration of TGF- β for 7 days (Experiment 1)

BSA, 0.5 mM HCl) or with 40 μ L of TGF- β 2 solution (R&D Systems, Minneapolis, MN; 1 ng/ μ L vehicle solution), intracisternally by syringe pump (KDS100; KD Scientific, MA, USA) once daily at 6:00. To determine whether the effects of TGF- β on the physiological and behavioral parameters were elicited by TGF- β alone, 4.97 μ g of corticotropin releasing factor (CRF; Peptide Institute, Osaka, Japan) dissolved in 40 μ L of vehicle solution was also administered into the cisterna magna as a positive control. After the last administration on the 7th day, locomotor activity of the rats was measured during the 10-hour dark period. After the completion of locomotor activity measurement, oxygen consumption was measured for 24 h, *i.e.*, following the light and dark periods. Rats were fed during the dark period, but did not receive any intracisternal treatment. After the measurement of oxygen consumption, rats were fasted for 2 h. Blood samples were collected by decapitation, and organs were removed and weighed. Plasma was isolated by centrifugation and stored at -70°C until analysis.

Experiment 2: acute effect of TGF- β administration

After 5 days recovery following surgery, rats were fasted from 18:00 to 6:00, fed for 2 h and then fasted again for 2 h. At 10:00, rats were treated with 40 μ L of the vehicle solution or with 40 μ L of TGF- β 3 (R&D Systems, 1 ng/ μ L vehicle solution), similar to Experiment 1. Blood samples were collected by decapitation, 30 min after administration, and plasma was isolated by centrifugation and stored at -70°C until analysis.

Measurement of locomotor activity and respiratory gas analysis. Locomotor activity of each rat was examined using a Supermex (Muromachi Kikai, Tokyo, Japan) for 10 h after the last intracisternal administration of TGF- β on day 7. Details of the locomotor activity measurement have been described previously (35). Briefly, locomotor activity was determined by detecting the movement of infrared radiation emitted from each animal. Locomotor activities were measured as a single count when an animal moved from one region of the measurement area, which was divided optically by multiple lenses, to a neighboring region. Total counts were calculated by summing all counts over a 10-min period.

Respiratory gas was determined using a CO₂/O₂ analyzer (RL-600; Alco System, Tokyo, Japan) to assess the metabolic rate via a metabolic chamber at a flow rate of 1.5 L/min. The details of the gas analysis have been described previously (17). Briefly,

room air was pumped through each chamber, and expired air was dried in a thin cotton column, before being directed to a CO₂/O₂ analyzer. The respiratory exchange ratio (RER), amount of oxidation of carbohydrate per body weight (CHO/w), and amount of fatty acid per body weight (FAT/w) were calculated from O₂ consumption (VO₂) and CO₂ production. Details of these calculations have been described elsewhere (17).

Hematological and biochemical analysis in blood and plasma. Hematological examination for erythrocytes, leukocytes, hemoglobin and hematocrit was performed in whole blood using FALCO biosystems (Kyoto, Japan). Glucose, free fatty acids, ketone bodies, ACTH and corticosterone in plasma were measured using the following commercially available kits: glucose (glucose CR-II; Wako Pure Chemical Industries, Osaka, Japan), free fatty acids (NEFA C; Wako Pure Chemical Industries), ketone bodies (ketone test; Sanwa Chemical Institute, Nagoya, Japan), ACTH (ACTH-RIA kit; Bachem Peninsula Laboratories, CA, USA), and corticosterone (RAT corticosterone RIA system; Amersham bioscience, Tokyo, Japan).

Statistics. Data are expressed as the mean \pm SEM. With respect to body weight and food intake per body weight, comparisons of different groups were conducted using two-way repeated-measures ANOVA, followed by a post hoc Student's t-test at each time point. Statistical analyses of differences were compared to the vehicle group using Student's t-test. Analyses were performed using the Prism software package (Graph pad software, SA) and P-values < 0.05 were considered significant.

RESULTS

Experiment 1: subchronic effect of TGF- β administration for 7 days

Body weight

On the first day of the treatment, the mean body weight of the TGF- β group was 298 ± 2.2 g, which was similar to that of the vehicle and CRF groups (303 ± 4.9 g and 300 ± 7.2 g, respectively). The vehicle-treated controls gradually gained weight, whereas the increase in body weight was significantly inhibited in the TGF- β - and CRF-treated rats (two-way repeated measures ANOVA; vehicle vs. TGF- β and vehicle vs. CRF, $P < 0.0001$, respectively) (Fig. 2). On days 5, 6, and 7 the body weight of

the TGF- β group was significantly lower than that of the vehicle group. With respect to the CRF group, the body weight was significantly lower than that of the vehicle group on days 4, 5, 6, and 7.

Food intake

Daily food intake per body weight of the TGF- β -treated group was not significantly different from that of the vehicle-treated group for 3 and 12 h after administration (Fig. 3A, B). However, food intake of the CRF-treated group was significantly lower than that of the vehicle group for 3 h after administration (two-way repeated measures ANOVA; vehicle vs. CRF, $P < 0.0001$) (Fig. 3A); this difference was significant throughout the entire test period. CRF treatment tended to decrease food intake for up to 12 h, compared to the vehicle group (two-way repeated measures ANOVA; vehicle vs. CRF, $P = 0.08$), and this difference was significant on days 1–5 (Fig. 3B).

Locomotor activity

Fig. 4 shows the total locomotor activity for 1.5 h (A) and 10 h (B) after the last treatment on day 7. The locomotor activity of TGF- β group was suppressed significantly for 1.5 h compared to that of the vehicle control group, but there was no significant difference in the locomotor activity for 10 h between two groups. CRF treatment did not change the locomotor activity at 1.5 and 10 h compared to the vehicle group.

Respiratory gas analysis

Total oxygen consumption was significantly high-

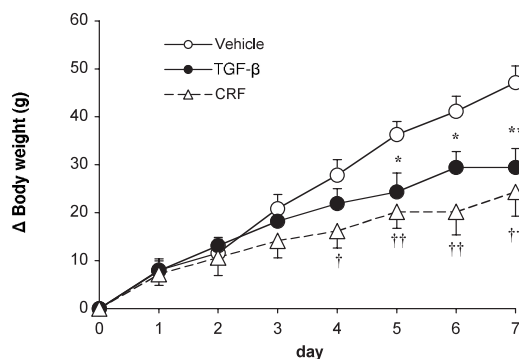


Fig. 2 Changes in body weight during the 7 days of intracisternal administration of TGF- β (40 ng/day), vehicle (artificial CSF) or CRF (4.97 μ g/day) in rats. Values are presented as the mean \pm SE from 6–10 rats. TGF- β -treated group; * and **, Significantly different from vehicle group ($P < 0.05$ and $P < 0.01$, respectively). CRF-treated group; † and ††, Significantly different from vehicle group ($P < 0.05$ and $P < 0.01$, respectively).

er in rats treated subchronically with TGF- β compared with the vehicle-treated rats throughout the light and dark period (Fig. 5). With respect to CRF-treated rats, significant increases in oxygen consumption were also observed in both the light and dark period. RER, CHO/w and FAT/w were not significantly different between both the TGF- β and CRF groups and the vehicle group.

Organ weight

Subchronic intracisternal administration of TGF- β for 7 days significantly decreased the relative thymus weight and increased that of brown adipose tissue (Table 1). The relative weights of the heart, liver, spleen, kidney, adrenal gland, visceral fat and skeletal muscle (*M. gastrocnemius*) of TGF- β -treated rats were not different from those of vehicle-treated rats. In the CRF group, the relative weights of the

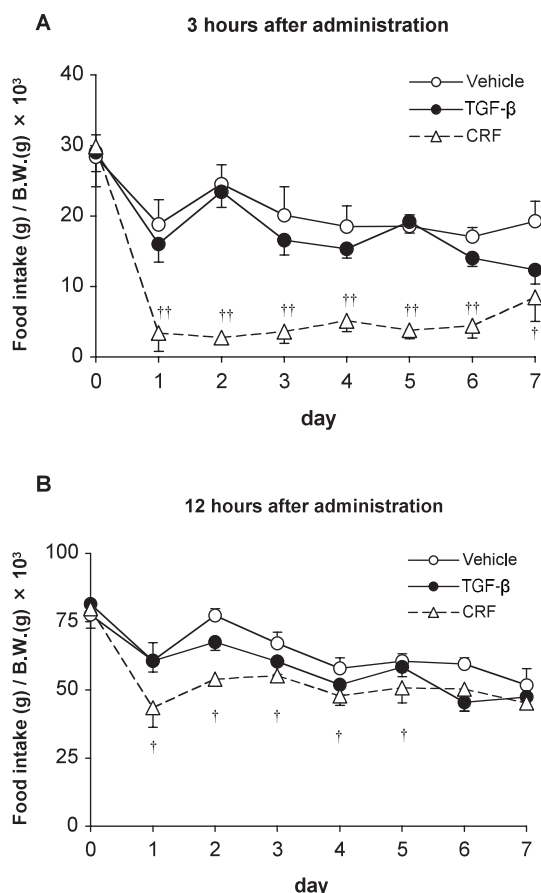


Fig. 3 Food intake for 3 h (A) or 12 h (B) normalized for body weights during the 7 days of intracisternal administration of TGF- β (40 ng/day), vehicle or CRF (4.97 μ g/day) in rats. Values are presented as the mean \pm SE from 6–10 rats. CRF-treated group; † and ††, Significantly different from vehicle group ($P < 0.05$ and $P < 0.01$, respectively).

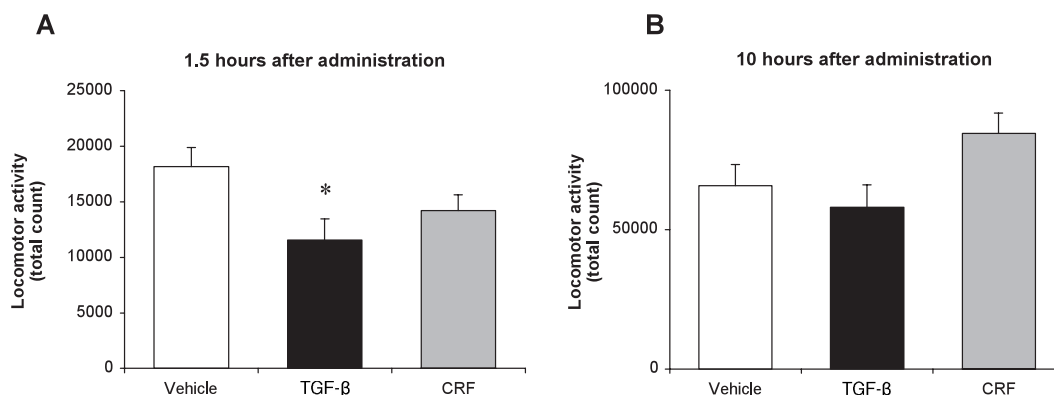


Fig. 4 Effects of subchronic intracisternal administration of TGF- β on locomotor activity of rats. Total counts of locomotor activity of rats for 1.5 h (A) or 10 h (B) after intracisternal administration at day 7. Values are presented as the mean \pm SE from 6–10 rats. TGF- β -treated group; *, Significantly different from vehicle group ($P < 0.05$).

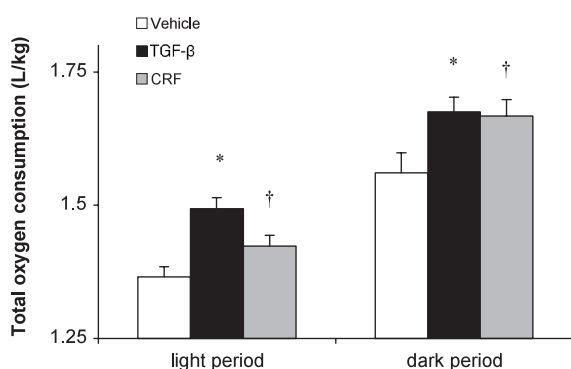


Fig. 5 Effects of subchronic intracisternal administration of TGF- β on total oxygen consumption of rats. Each rat was housed in a metabolic chamber for 12 h after intracisternal administration of TGF- β , vehicle or CRF, and oxygen consumption was measured during the subsequent light and dark period. Values are presented as the mean \pm SE from 6–10 rats. TGF- β -treated group; *, Significantly different from vehicle group ($P < 0.05$). CRF-treated group; †, Significantly different from vehicle group ($P < 0.05$).

thymus were significantly lower and those of the adrenal gland were significantly higher compared with the vehicle group.

Hematological and biochemical analysis

The hematological values are shown in Fig. 6. The number of total leukocytes, comprised of granular leukocytes, lymphocytes and monocytes, was significantly lower in the TGF- β - and CRF-treated rats compared with the vehicle-treated rats, respectively. Subchronic intracisternal administration of TGF- β for 7 days significantly decreased the number of lymphocytes compared to that of vehicle-treated rats, whereas the number of neutrophilic leukocytes, which comprise the major proportion of

granular leukocytes, was not changed by treatment with TGF- β or CRF. Other hematological values (erythrocytes, eosinophilic leukocytes, monocytes, hemoglobin, hematocrit) of both the TGF- β - and CRF-treated groups were not significantly different from the vehicle-treated group (data not shown). Glucose, free fatty acids, ketone bodies and ACTH levels in plasma of both the TGF- β - and CRF-treated groups were also not significantly different from the vehicle-treated group (Table 2).

Experiment 2: acute effect of TGF- β administration

Plasma ACTH and corticosterone concentrations were not different between the TGF- β - and vehicle-treated groups (Fig. 7). Intracisternal administration of CRF significantly increased ACTH ($P = 0.040$) and corticosterone ($P = 0.057$) concentrations in plasma. Plasma glucose levels were not changed (Table 3). Plasma levels of free fatty acids and ketone bodies were significantly higher in the TGF- β - and CRF-treated groups compared with the vehicle-treated group (Table 3).

DISCUSSION

Body weight, food intake and oxygen consumption

The significant inhibition in body weight gain of TGF- β -treated rats (Fig. 2) was primarily due to the increased basal metabolism in these rats, since there was no statistically significant difference in food intake (Fig. 3) and total oxygen consumption was elevated significantly (Fig. 5). The significant increase in total oxygen consumption during both the light and dark phase of the TGF- β -treated group is likely involved in the elevation of thermogenesis in brown

Table 1 Organ weights in the TGF- β -, vehicle- or CRF-treated groups

Variable (g/kg)	Vehicle	TGF- β	CRF
Heart	3.02 \pm 0.04	3.11 \pm 0.05	3.02 \pm 0.04
Liver	37.02 \pm 1.22	35.83 \pm 0.47	37.61 \pm 1.01
Spleen	2.01 \pm 0.14	1.86 \pm 0.04	1.94 \pm 0.14
Kidney	7.26 \pm 0.23	7.88 \pm 0.22	7.52 \pm 0.34
Adrenal gland	0.157 \pm 0.005	0.184 \pm 0.016	0.178 \pm 0.005 \dagger
Thymus	1.56 \pm 0.12	1.20 \pm 0.08*	1.06 \pm 0.14 \dagger
Epididymal fat	12.73 \pm 0.77	13.39 \pm 0.42	12.96 \pm 1.60
Perirenal fat	16.26 \pm 1.50	17.81 \pm 1.05	14.09 \pm 1.96
Mesentery fat	11.54 \pm 1.09	10.69 \pm 0.80	10.36 \pm 0.72
Brown adipose tissue	1.135 \pm 0.095	1.387 \pm 0.07*	1.113 \pm 0.171
Gastrocnemius	12.18 \pm 0.24	11.96 \pm 1.16	12.54 \pm 0.16

Organs were dissected and weighed after subchronic intracisternal administration of TGF- β , vehicle or CRF for 7 days. Values are presented as the mean \pm SE from 6–10 rats. TGF- β -treated group; *, Significantly different from vehicle group ($P < 0.05$). CRF-treated group; \dagger , Significantly different from vehicle group ($P < 0.05$).

adipose tissue (BAT) as indicated by the BAT hypertrophy (Table 1). BAT is considered the major site for nonshivering thermogenesis. In particular, it is well understood that uncoupling protein-1 (UCP1) is essential for thermogenesis in BAT (21). BAT thermogenesis and UCP1 expression are known to be up-regulated following activation of the sympathetic nervous system, *e.g.*, to produce heat following chronic cold exposure and to regulate body weight following consumption of high energy density diets (29, 31). It has been demonstrated that chronic cold exposure led to BAT hypertrophy through the synthesis of UCP1 and up-regulation of mitochondrial proteins (36). Although the cause of BAT hypertrophy in TGF- β -treated rats remains obscure, the possibility remains that this is due to an increase in the expression of UCP1. Subchronic treatment with CRF, as a positive control, resulted in marked suppression of the increase in body weight of rats (Fig. 1). Several studies have reported that subchronic and chronic intracranial administration of CRF led to decreases in food intake and inhibition of body weight gain (2, 6, 28). In this experiment, food consumption was significantly altered for 3 h in the CRF-treated group and resulted in a drastic reduction to about 25 percent of vehicle-treated group (Fig. 2). Arase *et al.* demonstrated that intracerebroventricular injection of 5 μ g of CRF significantly reduced food intake for 1 h which tended to last up to 3 h (2). CRF increases oxygen consumption through activation of the sympathetic nervous system (5). The prominent suppression of body weight gain of the CRF-treated group was likely caused by the reduction of food consumption and enhancement of oxygen consumption. Although both TGF- β and CRF resulted in a similar suppression of

body weight gain, they exhibited distinct effects on food intake.

ACTH, corticosterone and energy substrates

No significant changes were observed in plasma concentrations of glucose, free fatty acids, and ketone bodies collected 2 h after the respiratory gas analysis (Table 2). Plasma ACTH levels of both the TGF- β - and CRF-treated groups were also not significantly different from that of the vehicle-treated group. Several reports have shown that chronic intracerebroventricular CRF treatment using an osmotic pump elevated the plasma concentrations of ACTH and corticosterone in rats (10, 23). Our result demonstrating no significant changes in the plasma ACTH concentration of the CRF-treated group might be attributed to our subchronic administration protocol, *i.e.*, the dissection was performed one and half a days after the last intracisternal administration. Consequently, it remains uncertain whether or not TGF- β treatment affects the levels of these HPA-axis-related hormones. We examined the acute effects of intracisternal administration of TGF- β (Experiment 2). As a result, ACTH and corticosterone concentrations in plasma 30 min after TGF- β administration were not different from vehicle controls (Fig. 7). Acute treatment with CRF significantly increased these hormones levels, as previously reported. Several reports have demonstrated that adrenal hypertrophy is caused by activation of the HPA axis (6, 10, 23). The fact that subchronic intracisternal administration of TGF- β for 7 days did not give rise to adrenal hypertrophy (Table 2) and the result of acute TGF- β administration (Fig. 7) suggest that TGF- β in the brain does not activate the HPA axis. TGF- β , which is distinct from CRF, might only

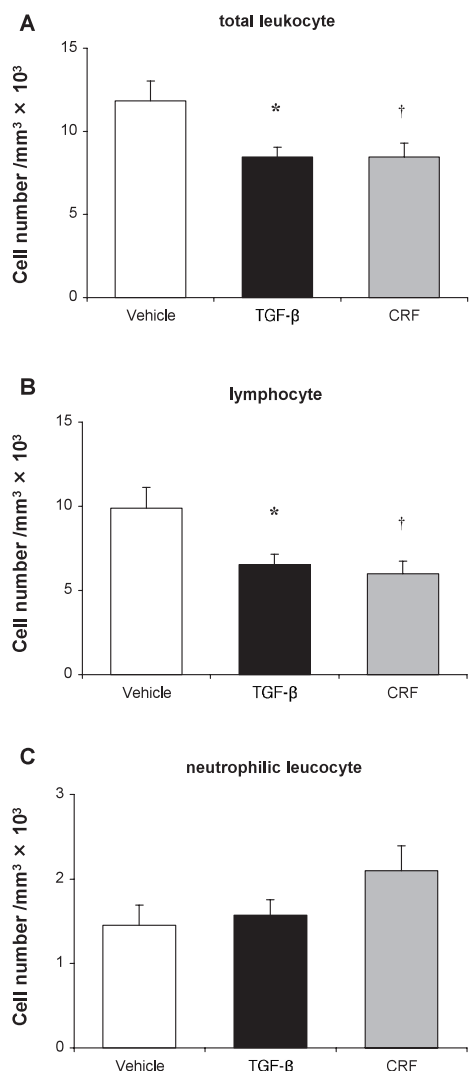


Fig. 6 Effects of subchronic intracisternal administration of TGF- β on hematological parameters. The cell numbers of total leukocytes (A), lymphocytes (B) and neutrophils (C) in whole blood from the TGF- β , vehicle or CRF groups are shown. Blood was collected by decapitation. Values are presented as the mean \pm SE from 6–10 rats. TGF- β -treated group; *, Significantly different from vehicle group ($P < 0.05$). CRF-treated group; †, Significantly different from vehicle group ($P < 0.05$).

induce activation of the sympathetic nervous system, but the details remain unclear. In the future, the effect of TGF- β on activation of the sympathetic nervous system should be investigated further. The increases in plasma free fatty acids, and ketone bodies 30 min after intracisternal administration of TGF- β (Table 3) were consistent with the result of our previous report (35).

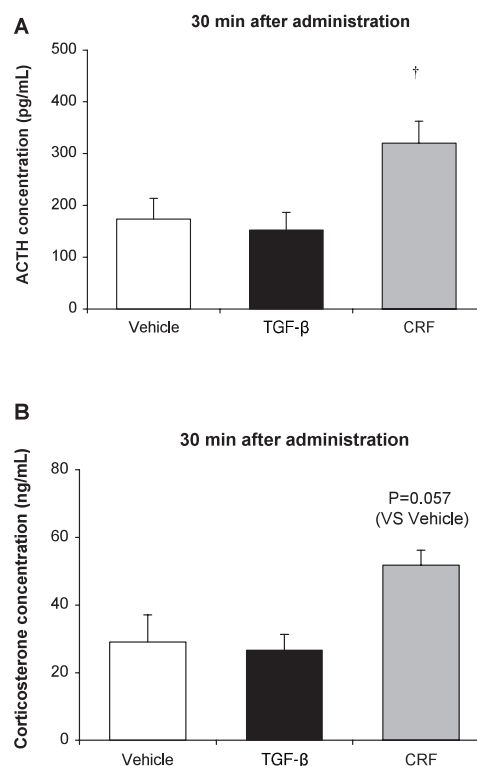


Fig. 7 Acute effects of intracisternal administration of TGF- β on the concentration of ACTH (A) and corticosterone (B). Plasma ACTH and corticosterone concentrations in rats 30 min after intracisternal administration of TGF- β , vehicle or CRF. CRF group; †, Significantly different from vehicle group ($P < 0.05$).

Hematological parameters

Subchronic treatment with TGF- β or CRF for 7 days significantly reduced the number of leukocytes (Fig. 6). While no changes were observed in the number of neutrophils and monocytes, a significant decrease in the number of lymphocytes was demonstrated. These results suggest that the decrease in the number of leukocytes is attributable to the reduced number of lymphocytes. Diminished function of the thymus, as indicated by thymus atrophy (Table 1), can be regarded as the cause of the decreased number of lymphocytes in TGF- β - and CRF-treated groups. The thymus is the primary immune organ and is the major site for the development and maturation of functional T-lymphocytes (1). Thymic involution, induced by chronic stress and chronic CRF treatment, is considered to be due to chronically elevated concentrations of corticosterone, as a consequence of long-term stimulation of the HPA axis (6, 10, 26). On the other hand, several studies have revealed that the thymus receives rich sympathetic innervation, and that thymocytes express

Table 2 Plasma concentrations of energy substrates and ACTH in the TGF- β -, vehicle- or CRF-treated groups

Variable	Vehicle	TGF- β	CRF
Glucose (mg/dL)	164.4 \pm 4.3	163.5 \pm 4.4	161.5 \pm 3.5
Free fatty acid (mEq/L)	0.317 \pm 0.032	0.323 \pm 0.049	0.293 \pm 0.018
Ketone body (μ M)	113.0 \pm 13.2	98.2 \pm 5.5	110.8 \pm 10.2
ACTH (pg/mL)	220.5 \pm 40.9	197.2 \pm 31.7	198.8 \pm 59.1

Blood samples were collected by decapitation after subchronic intracisternal administration of TGF- β , vehicle or CRF for 7 days. Values are presented as the mean \pm SE from 6–10 rats.

Table 3 Plasma concentrations of energy substrates 30 min after intracisternal administration

Variable	Vehicle	TGF- β	CRF
Glucose (mg/dL)	170.6 \pm 12.1	158.6 \pm 4.9	177.2 \pm 10.5
Free fatty acid (mEq/L)	0.289 \pm 0.026	0.382 \pm 0.027*	0.429 \pm 0.04†
Ketone body (μ M)	217.9 \pm 69.2	489.9 \pm 59.7*	531.6 \pm 76.2†

Blood samples were collected by decapitation 30 min after administration of TGF- β , vehicle or CRF. Values are presented as the mean \pm SE from 6–10 rats. TGF- β -treated group; *, Significantly different from vehicle group ($P < 0.05$). CRF-treated group; †, Significantly different from vehicle group ($P < 0.05$).

β -adrenoreceptors (34). Gu *et al.* reported that incubation of thymocytes with adrenergic receptor agonists *in vitro* induced an apoptotic response (13). These results imply that stimulation of the sympathetic nervous system, not merely elevated corticosterone, may also contribute to thymus atrophy. Thus, the decrease in thymus weight of the TGF- β -treated group likely occurred due to activation of the sympathetic nervous system.

Locomotor activity

We have previously reported that single intracisternal administration of TGF- β suppressed total spontaneous motor activity for 1 h (35), which was confirmed by the current measurement of locomotor activity for 1.5 h after the last administration on day 7 (Fig. 3). However, no significant changes were observed in the total counts of locomotor activity for 10 h. Spontaneous motor activity has been used as an indication of the sensation of fatigue (14, 15). This result suggests that subchronic intracisternal administration of TGF- β for 7 days might not cause the chronic sensation of fatigue.

Natural killer cytotoxicity

Sympathetic nervous system hyperactivity is considered to lead to immune suppression. For example, activation of the sympathetic nervous system plays a role in CRF-induced suppression of natural killer (NK) cell cytotoxicity (16). It would be intriguing to investigate whether subchronic TGF- β treatment could suppress NK cytotoxicity via the sympathetic nervous system.

Stress response

It is well known that exercise, which is a stressor, elicits increased expression of CRF in the brain (15, 19, 30). Many researchers have examined CRF in relation to stress in detail and have demonstrated that CRF initiates biological response to stress via the HPA axis and sympathetic nervous system (3). On the other hand, increased TGF- β levels in the brain are also induced by exercise. Our results regarding the central effects of TGF- β led us to postulate that subchronic TGF- β treatment activated the sympathetic nervous system, but exhibited no effect on the HPA axis. The effects of TGF- β in the brain partially overlapped the stress responses that evoke activation of the sympathetic nervous system, suggesting that TGF- β in the brain was involved in the stress. In the stress status, in which overactivity of the sympathetic nervous system, immunosuppression, body weight loss, *etc.*, are evident, TGF- β in the brain may at least partially contribute to these responses in addition to CRF.

Acknowledgement

We thank the Radioisotope Research Center of Kyoto University for providing us with the facilities to use radioisotopes.

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