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Effect of Zena F-III®, a Liquid Nutritive and Tonic Drug, on the Neurochemical Changes Elicited by Physical Fatigue in Mice

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HANAWA, M., T. ASANO, K. AKIYAMA, K. YABE, K. TSUNODA, T. TADANO AND D. SUTOO. *Effect of Zena F-III", a liquid nutritive and tonic drug, on the neurochemical changes elicited by physical fatigue in mice.* PHARMACOL BIOCHEM BEHAV **66**(4), 771–778, 2000.—The effects of a liquid nutritive and tonic drug (NTD) on the neurochemical changes elicited by physical fatigue in mice were investigated in terms of the calcium-dependent dopamine synthesizing function of the brain. In this study, Zena F-III® (Taisho Pharmaceutical Co., Ltd., Japan), one of the most popular NTDs in Japan, containing 15 crude drug extracts together with taurine, caffeine, and vitamins, and formulated based on the precepts of traditional Chinese medicine, was used. Male mice were forced to walk for 0–6 h at a speed of 3 m/min using a programmed motor-driven wheel cage. The serum and brain calcium levels in the mice were significantly increased following forced walking. The increase in brain calcium level began later and was more gradual than that in the serum calcium level, and reached its maximum value following forced walking for 3 h. The neostriatal dopamine level was also significantly increased, and locomotor activity significantly decreased following forced walking for 3 h. Prior oral administration of F-III (10 ml/kg) attenuated the increases in the serum and brain calcium levels, the increase in the brain dopamine levels, and the decrease in locomotor activity induced by forced walking. Taking into consideration these findings with our previous reports, it is suggested that physical fatigue leads to an increase in dopamine synthesis in the brain through a calcium/calmodulin-dependent system, thereby inducing behavioral changes, and that F-III inhibits this pathway and may alleviate overwork-induced physical fatigue. © 2000 Elsevier Science Inc.

Calcium/calmodulin Crude drugs Dopamine synthesis Liquid nutritive and tonic drug Locomotor activity Mice brain Overwork-induced physical fatigue Traditional Chinese medicine

CRUDE drugs obtained from natural resources, for example, plants and animals, have been used in forms of ethno-medicine such as traditional Chinese medicine. Many liquid nutritive and tonic drugs (NTDs) that contain these crude drugs together with synthetic drugs such as vitamins, caffeine, and amino acids, generally referred to as "Drink-Zai" (literally, "drinking drugs"), are widely used in Japan. The NTDs have become extraordinarily popular in Japan since the end of World War II, and most of them are classified as over-the-counter (OTC) drugs. Many users claim that the NTDs are useful for recovery from physical and psychological fatigue, for improvement of a weak constitution, and as nutritive supplements. The pharmacological mechanisms underlying the antifatigue and other effects of NTDs, however, remain to be elucidated. Although the ingredients of these drugs were chosen based on many years of experience, until now, no satisfactory logical or experimental investigations on the effects of those have been performed. Furthermore, a physiological definition of fatigue is difficult, and the developmental mechanisms responsible for fatigue have not been elucidated.

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The drugs of the Zena F-series (Zena F-I®, F-II® and F-III®, Taisho Pharmaceutical Co., Ltd., Japan) are among the most popular NTDs in Japan. These drugs were formulated based on prescriptions of Chinese herbal medicine. In particular, F-III was modified from the "Kai-xin-shu-yu-shen-qi-wan" prescription, and contains 15 crude drug extracts along with taurine, caffeine, and vitamins (see Table 1). Kai-xin-shu-yu-shen-qi-wan originated from the "Fan-wang" prescription. The Fan-wang prescription has been used for thousands of years as a folk remedy for decline in physical activity, lax muscles, lassitude, oversensitivity to cold, blurred vision, loss of sexual drive, loss of appetite, dyspepsia, palpitations, insomnia, mild depression, and forgetfulness. It has been thought from olden times that these physiological disorders occur when the internal balance between fatigue and recovery is lost. The original Fan-wang prescription was not preserved in China, but the prescription was recorded in "Ishinpo", which is the oldest Japanese medical text in existence. The Ishinpo was edited based on about 200 Chinese medical texts by Yasuyori Tamba, and was dedicated to the Japanese Emperor in 984 A.D. It was used as a textbook for medical students in the olden days, and was designated as a national treasure in 1984 A.D.

Tadano et al. (31) investigated the drugs of the Zena F-series by behavioral experiments in mice, and indicated that F-I, -II, and -III alleviate physical fatigue induced by forced walking or swimming, and that F-III is the most effective among the three. Sutoo et al. (22,23,26,28) have examined the role of calcium ions in brain functions, and have demonstrated that calcium activates tyrosine hydroxylase and increases dopamine synthesis in the brain through a calmodulin-dependent system, and that activated dopaminergic neurons regulate various brain functions. This concept was developed in an attempt to elucidate the mechanisms by which exercise and stress modify and affect brain functions. Studies have shown that exercise or exposure to cold leads to an increase in the serum and brain calcium levels, thereby inducing an increase in dopamine synthesis in the brain, and that the increased levels of dopamine induce physiological and behavioral changes (2,24,27). In the present study, based on these findings, the effects of Zena F-III on the neurochemical changes induced by forced walking were investigated in mice.

METHODS

Test Drugs

As shown in Table 1, a NTD used in the present study, i.e., Zena F-III, contains 15 crude drug extracts and a few synthetic drugs. The crude drugs were extracted by ethanol mixed with water or by water. The extracts were concentrated, measured, and mixed together with synthetic drugs, and packed into a shield bottle of 50 ml. These procedures were carried out in a clean and computerized factory.

Animals

Male ddY mice, 5 weeks of age, provided by Japan SLC, Inc. (Shizuoka, Japan), were housed in groups of 8–10 in stainless steel cages at room temperature (22 ± 2°C) for 1 week before the commencement of the experiments, under a 12L:12D cycle. Food and water were provided ad lib. However, 6 h before initiation of the experiments, the mice were deprived of food, but were given water. Animals received humane care in compliance with the "Guiding principles for the care and use of laboratory animals" formulated by the Japanese Pharmacological Society.

For exercise, they were forced to walk for 0, 1, 2, 3, or 6 h at a speed of 3 m/min using a programmed motor-driven wheel cage. Following the exercise, the changes in the serum and brain calcium levels, brain dopamine level, and locomotor activity were measured. Also, the effects of F-III on these changes elicited by exercise were analyzed. The test sample of F-III (10 ml/kg) was orally administered once 1 h before the start of the exercise, and taurine (100 mg/kg), vitamin B_2

TABLE 1
ACTIVE INGREDIENTS OF ZENA F-III

Crude Drug Extracts	Content: As Extract (mg/50 ml)	Content: As Crude Drug* (mg/50 ml)	Synthetic Drugs	Content (mg/50 ml)
Muirapuama extract	15.0	300	Taurine	500
Ginseng radix extract	90.0	600	Vitamin B_2 phosphate	5
Epimedii herba extract	100.0	1000	Vitamin B_6	5
Rehmanniae radix extract	150.0	300	Caffeine anhydrous	50
Cistanchis herba extract	151.6	500		
Cnidii monnieri fructus extract	600.0	300		
Cuscutae semen extract	33.0	300		
Poria extract	9.6	300		
Phocae testis et penis tincture	500.0	100		
	(ml/50ml)			
Corni fructus fluid extract	0.5	500		
Dioscoreae rhizoma fluid extract	0.3	300		
Eucommiae cortex liquid extract	0.3	300		
Schizandrae fructus fluid extract	0.3	300		
Cervi parvum cornu tincture	1.08	300		
Agkistrodon japonicae tincture	1.25	250		

*Each value represents the original weight of crude drug in a bottle (50 ml).

phosphate (1 mg/kg), vitamin B_6 (1 mg/kg), caffeine anhydrous (10 mg/kg), and tap water were used as placebo or control. Experiments were carried out between 1000 and 1600 h.

Calcium Levels

Calcium levels in the serum and brain were measured according to the method employing an *o*-cresolphthalein complexone (20). The calcium levels in the mice exercised for 0–6 h, which were administered F-III or water 1 h before the start of the exercise, and in unexercised mice 1–7 h after the administration of F-III were compared. Also, the serum calcium levels in the unexercised mice and mice exercised for 1 h, which were prior administered F-III, taurine, vitamin B_2, vitamin B_6, caffeine or water, were compared. Blood were obtained from each mice, and the serum was separated quickly and assayed. Whole brains were homogenized with saline at 4°C and centrifuged for 1 h at 25,000 × *g* (4°C), after which the supernatant was assayed. Data were analyzed by Student's *t*-test or an analysis of variance (ANOVA) and Dunnett's *t*-test.

Dopamine Levels

Brain dopamine levels were determined immunohistochemically and analyzed quantitatively by a brain mapping analyzer (30). Four animal groups (10 mice/group), i.e., unexercised control mice administered water, unexercised mice administered F-III, water-preadministered mice exercised for 3 h, and F-III-preadministered mice exercised for 3 h, were prepared. The mice were anesthetized with pentobarbital sodium and perfused intracardially with 50 ml of a solution of 0.1 M cacodylate and 1% sodium metabisulfite (SMB) containing 2.5% glutaraldehyde (pH 7.5). The whole brains were removed and postfixed in the same solution for 30 min, then frozen on dry ice. The frozen brains were sectioned coronally at 20-μm thickness in a cryostat. For the analysis of dopamine, brain slices approximately 5 mm rostral from the interaural line (Fig. 3), which largely contain the neostriatal region, were chosen in the light of a previous report (23) that the dopamine levels in this region were increased following intracerebroventricular administration of $CaCl_2$.

The immunohistochemical staining procedure was performed according to a previous report (23). The brain slices were washed with Tris-SMB buffer (Tris 0.05 M and SMB 0.85%, pH 7.5). The dopamine antibody was diluted 1:800 with Tris-SMB buffer and 50 μl of the antibody solution was applied to each brain slice, followed by incubation of the slices at 4°C for 12 h. After the reaction, the slices were washed with phosphate-buffered saline (PBS). Then, the second antibody, antirabbit IgG goat serum labeled with fluorescein isothiocyanate, was diluted 1:100 with PBS and applied to the sections; the sections were incubated in the dark at 20°C for 3 h, and then washed with PBS. Nonspecific fluorescence with glutaraldehyde was reduced by 0.1 M $NaBH_4$ in PBS.

The distribution of dopamine was quantitatively analyzed using a brain mapping analyzer (30). This analyzer is a measuring microscope for the distribution of immunohistochemical fluorescence intensity in the tissue slice. The average fluorescence intensity in a small brain region can be measured by a photomultiplier tube through a pinhole and objective of a microscope. The brain slice is continuously moved in the *x*- or *y*-direction by means of a scanning stage under the objective lens and the data in each brain region are collected. The operating conditions of the analyzer were as follows: excitation range, 420–490 nm; interference filter, 530 nm; photomultiplier voltage, 850 V; and objective, 20×/0.75 (magnification/numerical aperture). In this study, the immunohistochemical fluorescence intensities obtained in the slices were indicated relatively compared with that of standard uranium glass. The slices stained with the dopamine antibody were measured reticulately at 20-μm intervals. The background value was subtracted from the total fluorescence intensity value at each measuring point. The background value was obtained from the slices that were treated with the same procedure, but without the dopamine antibody. The average fluorescence intensity in each region was analyzed by ANOVA and the Newman–Keuls *t*-test for comparisons among the four groups (20 slices/group).

Locomotor Activity

The mice were placed in locomotor activity cages (30 × 40 × 40 cm, Animex type S, LKB) for 30 min to allow them to adapt to the environment. They were then removed from the cages and forced to walk for 3 h. After walking, the mice were returned to the locomotor activity cages and locomotor activity counts/10 min were measured for 2 h. All observations were made in an isolated environmental room, maintained at a constant temperature of 22 ± 2°C. The locomotor activity was measured in unexercised mice pretreated with F-III or

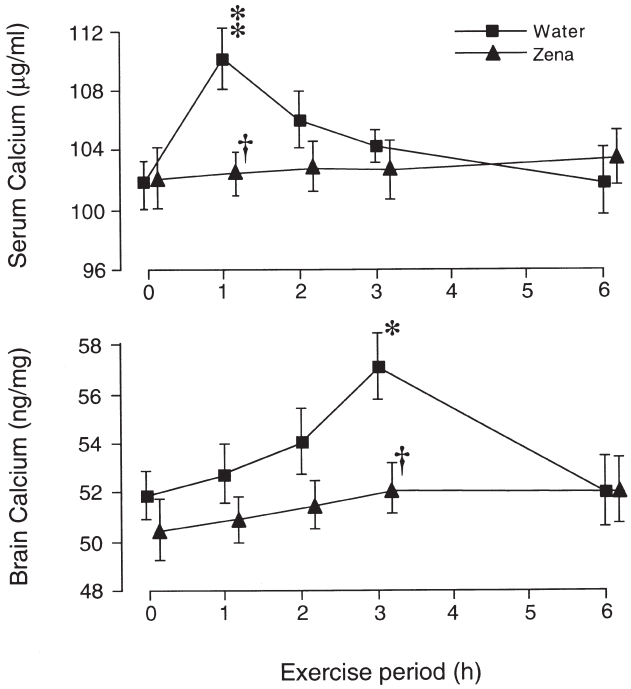

FIG. 1. Effect of Zena F-III on the changes in calcium levels in the serum (upper) and brain (bottom) elicited by exercise. Mice were administered tap water or F-III (10 ml/kg) orally 1 h before the start of forced walking. (3 m/min). Results are expressed as the mean ± SEM of 9–16 mice. The serum calcium levels in the mice exercised for 1 h and brain calcium levels in the mice exercised for 3 h were significantly increased compared to those in the unexercised mice. In the F-III preadministered group, the increases in serum and brain calcium levels induced by exercise were inhibited. $^*p < 0.05$, $^{**}p < 0.01$ compared with the levels in the unexercised mice of the same group, by Dunnett's *t*-test. †$p < 0.01$ compared with the water-treated group during the same exercise period by Student's *t*-test.

water. Also, the locomotor activity was compared among three groups, i.e., exercised mice pretreated with F-III or water, and unexercised mice pretreated with water. The total locomotor counts per 2 h were analyzed by Student's *t*-test or by ANOVA and Newman–Keuls *t*-test.

RESULTS

As shown in Fig. 1, increases in the serum and brain calcium levels were noted during exercise. The serum calcium levels increased temporarily, and then returned to their original levels during exercise. The serum calcium levels in the mice exercised for 1 h were 8% higher ($p < 0.01$) than those in the unexercised mice. The brain calcium levels increased more slowly, and returned to their original levels during exercise. The brain calcium levels in mice exercised for 3 h were 10% higher ($p < 0.05$) than those in the unexercised mice. On the other hand, when F-III was administered orally once 1 h before the exercise, the increases in the serum and brain calcium levels by exercise were inhibited. In the F-III-treated group, the serum and brain calcium levels in the exercised mice were not significantly different when compared to the levels in unexercised mice, and the serum calcium levels in the mice exercised for 1 h and brain calcium levels in the mice exercised for 3 h were 7% ($p < 0.01$) and 9% ($p < 0.01$) lower, respectively, than those in the water-treated group.

The effects of F-III and its synthetic ingredients on the serum calcium levels in the unexercised mice and mice exercised for 1 h are shown in Fig. 2. In the unexercised group, the serum calcium levels in the mice administered F-III, taurine, vitamin B_2, or vitamin B_6 were not significantly different as compared with those in the water-administered mice, while the serum calcium levels in mice administered caffeine were significantly increased by 7% ($p < 0.05$) compared to those in the water-administered mice. F-III administration also did not significantly change the serum calcium levels in the unexercised mice until 7 h after the administration (data not shown). The increase in serum calcium levels induced by exercise was not significantly inhibited by prior administration of taurine, vitamin B_2, vitamin B_6, or caffeine.

Figure 3 shows the profile of the brain dopamine levels. The neostriatum showed the highest immunohistochemical fluorescence intensity of dopamine in unexercised control mice pretreated with water. The mean ± SEM of the fluorescence intensity in this region was 0.45 ± 0.02. The neostriatal dopamine levels in the unexercised mice treated with F-III were not significantly different from those in the control mice. In the mice exercised for 3 h, on the other hand, the neostriatal dopamine levels were approximately 30% higher ($p < 0.01$) compared with those in the unexercised control mice. Pretreatment with F-III inhibited the exercise-induced increase in the dopamine levels, and the levels in exercised mice pretreated with F-III were not significantly different from those in the unexercised control mice.

Figure 4 shows the locomotor activities in unexercised mice pretreated with F-III and water. The total locomotor activity in a 2-h period in unexercised mice administered F-III was approximately 75% higher ($p < 0.001$) than that in water-administered mice. In Fig. 5, the locomotor activities in unexercised control mice, water-preadministered mice exercised for 3 h, and F-III-preadministered mice exercised for 3 h, were compared. The total locomotor activity in a 2-h period in the exercised mice was approximately 60% lower ($p < 0.01$) than that in the unexercised mice. In the exercised mice, F-III preadministration increased the total locomotor activity

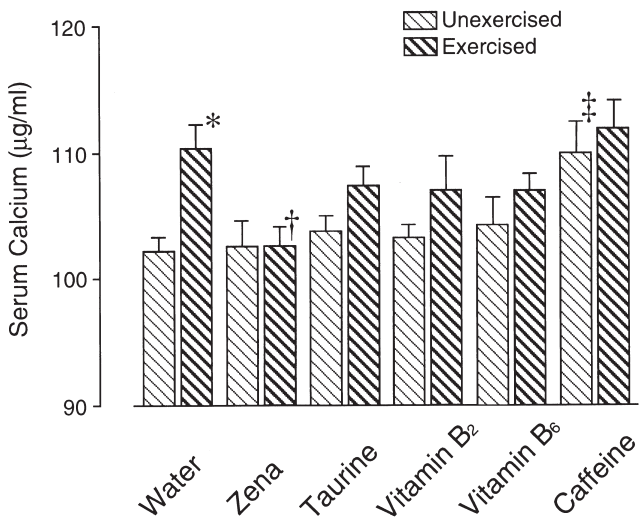

FIG. 2. Effect of Zena F-III, taurine, vitamin B_2, vitamin B_6, or caffeine on the serum calcium levels in unexercised and exercised mice. Mice were administered tap water, F-III (10 ml/kg), taurine (100 mg/kg), vitamin B_2 phosphate (1 mg/kg), vitamin B_6 (1 mg/kg), or caffeine anhydrous (10 mg/kg) orally, 2 h before being sacrificed in the unexercised group, or 1 h before the start of the exercise in the exercised group. The mice were forced to walk for 1 h at a speed of 3 m/min. Results are expressed as the mean ± SEM of 8–10 mice. The serum calcium levels in water-preadministered mice exercised for 1 h were 8% higher compared with those in the unexercised mice. The increase in serum calcium levels induced by exercise was significantly inhibited by prior administration of F-III. In the unexercised mice, administration of caffeine increased the serum calcium levels by 7% compared to those in the water-administered group. *$p < 0.01$ compared with the unexercised group administered the same drug by Student's *t*-test. †$p < 0.05$ compared with the exercised group preadministered water by Dunnett's *t*-test. ‡$p < 0.05$ compared with the unexercised group administered water by Dunnett's *t*-test.

by approximately 160% ($p < 0.01$) compared with that in the water-preadministered mice. Pretreatment with F-III, thus, improved the lower locomotor activity induced by exercise, while also increased the locomotor activity in unexercised mice.

DISCUSSION

Sutoo et al. (22,23,26,28) have previously reported that calcium ions activate tyrosine hydroxylase and increase dopamine levels in the neostriatum through an intracerebral calmodulin-dependent system. This concept was developed, by animal experiments using mice, in an attempt to elucidate the mechanism by which exercise modifies brain functions. Studies demonstrated a significant increase in the serum and brain calcium levels in mice following running (20 m/min) for 15–60 min and 60–120 min, respectively. Furthermore, the levels of dopamine in the neostriatum, as determined immunohistochemically, and ethanol-induced sleeping time, were increased by exercise. The increase in ethanol-induced sleeping time was inhibited by prior administration of α-methyltyrosine (a tyrosine hydroxylase inhibitor) or EDTA. Therefore, it has been suggested that exercise induces an increase in serum calcium levels, that the serum calcium is transported to the brain where it stimulates dopamine synthesis through the calmodulin-dependent system, as does calcium injected di-

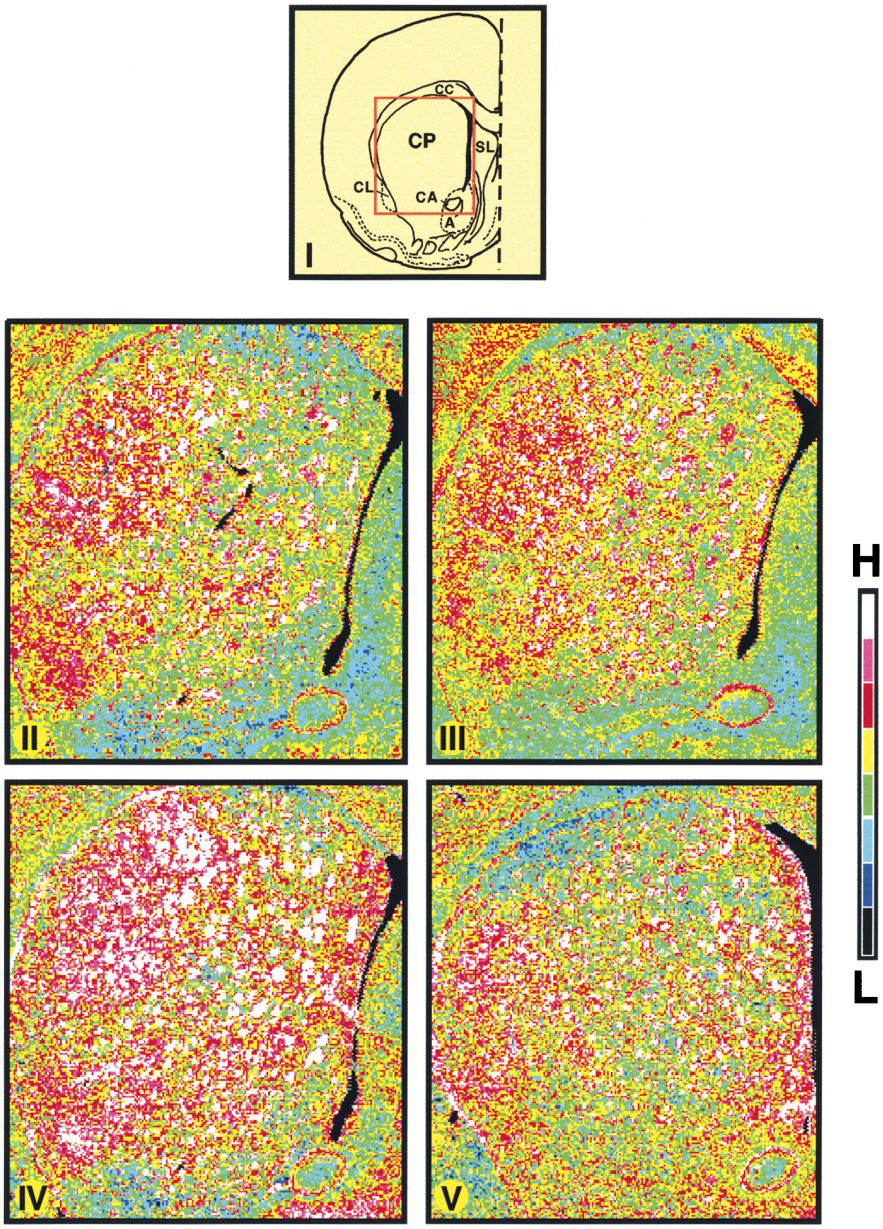

FIG. 3. Effect of Zena F-III on the changes in the striatal dopamine distribution elicited by exercise. (I) Left side of coronal section approximately 5 mm rostral from the interaural line in the mice brain. The data of the immunohistochemical dopamine distribution in the framed area are shown in II–V, which were analyzed at 20-μm intervals through a pinhole (6 μm in diameter) using a brain mapping analyzer. A, nucleus accumbens septi; CA, commissura anterior; CC, corpus callosum; CL, claustrum; CP, neostriatum; SL, nucleus septi lateralis. (II–V) Examples of the quantitative immunohistochemical distribution of dopamine in unexercised control mice administered water (II), unexercised mice administered 10 ml/kg of F-III (III), water-preadministered mice exercised for 3 h (IV), and F-III-preadministered mice exercised for 3 h (V). The immunohistochemical fluorescence intensities were classified into eight ranks and indicated by color coding. The dopamine levels in the neostriatum in the mice exercised for 3 h were approximately 30% higher ($p < 0.01$) than those in the unexercised control mice. Pretreatment with F-III inhibited the exercise-induced increase in the neostriatal dopamine level.

rectly into the brain, and that the subsequent increase in brain dopamine levels induces behavioral changes (27). Moreover, the obtained results on calcium and dopamine levels in the brains of mice following exercise were similar to those obtained in mice exposed to cold in a previous study: that is, exposure to cold also increases brain calcium and dopamine levels, and the increased brain dopamine levels induce behavioral changes (24). These studies, combined with previous related

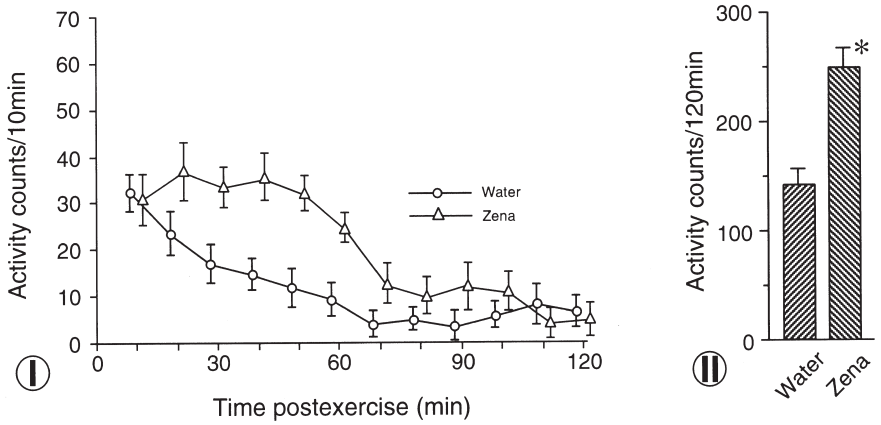

FIG. 4. Effect of Zena F-III on locomotor activity in unexercised mice. The locomotor activities in unexercised mice 1 h after oral administration of tap water or F-III (10 ml/kg) were compared. The activity counts at 10-min intervals for the 2-h recording period are provided in (I). The total counts over the 2-h period are provided in (II). Each value represents the mean ± SEM of 10 mice. The total locomotor activity in the F-III–preadministered mice was 75% higher compared to that in the water-preadministered mice. $*p < 0.001$ compared with the water-treated group by Student's *t*-test.

reports, have suggested that exercise and cold stress have similar effects on brain functions and induce physiological and behavioral changes through the actions on calcium and dopamine in the brain (24,27), and that daily exercise produces a refreshing feeling, reduces blood pressure and activates other physiological functions through calcium-dependent dopamine synthesis (2,27); excessive exercise, physical fatigue, or stress, however, abnormally enhances this synthesis and induces physiological disorders such as depression and gastric ulcer formation (24,29).

Tadano et al. (31) indicated from studies on mice that oral administration of the Zena F-series (F-I, -II, and -III) attenuates exercise-induced behavioral changes such as the decrease in locomotor activity and increase in immobility during swimming, and that F-III is the most effective among the three. In addition to this report, some pharmacological functions of each drug contained in the Zena F-series drugs have been reported as follows, although the functions of the drug mixture have not been reported. *Ginseng radix* was reported to improve or invigorate physiological conditions that is weakened by stress or disease (14). *Ginseng radix* extract produced rapid, reversible reduction of voltage-gated Ca^{2+} currents in trigeminal ganglion neurons of the adult rat, and the resultant decrease in Ca^{2+} influx could be considered to influence a

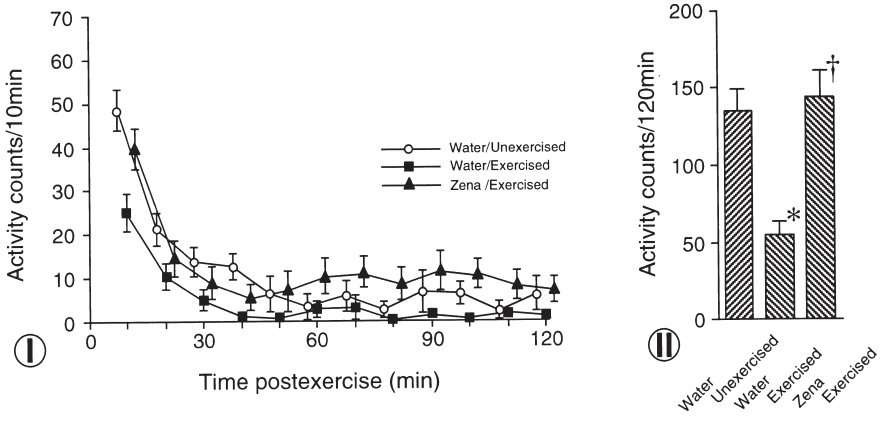

FIG. 5. Effect of Zena F-III on the changes in the locomotor activity in mice elicited by exercise. The locomotor activities in unexercised control mice administered tap water, tap water-preadministered mice exercised for 3 h, and F-III (10 ml/kg)-preadministered mice exercised for 3 h were compared. The activity counts at 10-min intervals for the 2-h recording period are shown in (I). The total counts over the 2-h period are shown in (II). Each value represents the mean ± SEM of 10 mice. The total locomotor activity in mice following exercise for 3 h was 60% lower compared to that in the unexercised mice. Preadministration of F-III prior to walking for 3 h, increased the total locomotor activity by 160% compared with that in the water-preadministered exercised mice. $*p < 0.01$ compared with the unexercised control group, and $\dagger p < 0.01$ compared with the water-preadministered exercised group by Newman–Keuls *t*-test.

wide variety of intraneuronal processes, such as Ca^{2+}-dependent enzyme activity (16). Ginseng saponins decreased the uptake of biogenic monoamines in synaptosomes of the rat brain (34). The ginsenosides Rbl and Rgl, the major components of ginseng saponins, inhibited methamphetamine-induced dopaminergic activity, such as hyperactivity and conditioned place preference in mice (12). *Cnidii monnieri* exhibited Ca^{2+}-blocking activity, and its active constituent was determined to be osthole (36). Gomisin A and schizandrin, which are constituents of *Schizandrae fructus*, showed inhibitory effects on gastric contraction and stress-induced gastric ulceration, and schizandrin showed, in addition, an inhibitory effect on gastric secretion (15). Taurine plays a role in the regulation of calcium homeostasis (19,37). Intraperitoneal injection of a high dose of taurine decreased the locomotor activity and increased the striatal dopamine concentrations in mice; however, that of low dose, but higher than that contained in Zena F-III, did not alter these (1).

In previous studies, the serum and brain calcium levels and neostriatal dopamine levels in mice were found to be significantly increased following running at a speed of 20 m/min (27). The increases in calcium levels were reproduced by the forced walking at a speed of 3 m/min in the present study, although a long walking period was necessary. Namely, the serum calcium levels in mice were significantly increased following walking for 1 h, and the brain calcium levels were increased following walking for 3 h. Also, the increase in the immunohistochemically determined dopamine levels in the neostriatum were reproduced by the forced walking for 3 h. To examine the effects of exercise on the calcium and dopamine levels, the mice were forced to run at a speed of 20 m/min in Sutoo's laboratory (27), and to examine the effects of Zena F-series drugs on physical fatigue, the mice were forced to walk at a speed of 3 m/min in Tadano's laboratory (31). In the present study, a walking speed of 3 m/min was used in all the experiments for combining the results obtained from the tests under the two different conditions. In addition to our observations, other laboratories reported that the blood calcium levels (3,6,7,21), the levels of dopamine and its metabolites in the brain (5,8,11), and brain tyrosine hydroxylase activity (5) were increased following exercise such as running, swimming, and cycling. Moreover, the serum and brain calcium levels (13,18), the brain dopamine levels and dopamine turnover (9,10,17,33), and the brain tyrosine hydroxylase activity (32,38) were increased following cold- or electric foot shock-stress. In our study, these exercise-dependent changes in calcium and dopamine levels were inhibited by prior administration of F-III.

In this study, the locomotor counts were observed to be lower in the exercised mice, in association with an increase in the striatal dopamine levels, than those in the unexercised controls. A number of studies have shown that the administration of dopamine (35) and L-tyrosine (catecholamine precursor) (4) increases locomotor activity in normal animals. However, the locomotor response to the administration of catecholamine was often reversed under abnormal environments such as exposure to stress (24,25). For example, although locomotor activity in unstressed mice was increased by supplementing L-tyrosine in the diet, that in cold-stressed mice was decreased (33). Here, the exercise-dependent decrease in locomotor activity was inhibited together with the changes in the calcium and dopamine levels, by prior administration of F-III. These findings suggest that F-III inhibits the increases in serum and brain calcium levels in exercised mice and thereby inhibits calmodulin-dependent dopamine synthesis and the dopamine-dependent behavioral changes. These actions may result in alleviation of overwork-induced physical fatigue or stress-induced physiological disorders. We think that this is one of the mechanisms underlying the antifatigue effects of F-III.

The effect of F-III in attenuating the neurochemical changes following exercise in mice was not seen with caffeine alone, which is one component of F-III; i.e., the oral administration of caffeine (10 mg/kg) showed a converse action, and increased the serum and brain calcium levels, independent of exercise. Also, locomotor activity was increased in unexercised mice without significant change of neostriatal dopamine levels by pretreatment with F-III. We consider that the actions of F-III are the result of combined actions of the various component drugs, and that F-III may thus extensively affect other pathways and functions.

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