Improvement of Fatigue and Acceleration of Recovery from Stress-Induced Deficient Sexual Behavior in Mice Following Oral Administration of Soft-Shelled Turtle Powder

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Effects of oral administration of cryomilled powder of the soft-shelled turtle (SST powder) on forced-exercise performance and stress-induced reduction in sexual and learning behavior were studied in mice. In the fatigue-preventive experiment, the SST powder was administered orally to mice which were forced to climb a descending rope for 60 min a day. The treatment significantly improved the daily performance of the mice. In the stress recovery experiment, male mice were hanged from the tail and forced to swim using their fore paws for 30 to 60 min everyday. Stress significantly reduced sexual behavior and memory retrieval (step down test). Daily oral administration of the SST powder significantly attenuated this decline in sexual behavior. However, the effects on memory retrieval were not statistically significant. These results suggest that administration of SST powder attenuates fatigue and accelerates recovery from stress in mice.

Key words fatigue; stress; sex behavior; soft-shelled turtle; mouse

Soft-shelled turtle was used as a food and robust tonic for medical purposes in ancient China and is still used today.¹⁾ The nutritional and tonic properties supplement a deficit of essential factors in the human body. Traditionally in China, the soft-shelled turtle has been used to attenuate physiological tiredness and improve weakened functions during stressful conditions. We have shown previously that chronic oral treatment of the powder from the soft-shelled turtle (SST powder) is beneficial in hypertension,²⁾ prevents liver injury³⁾ and has anticancer effects.⁴⁾ However, the effects of the soft-shelled turtle on fatigue and stress have not been investigated in detail.

MATERIALS AND METHODS

SST Powder Cryomilled SST powder was prepared by rapid freezing of the whole body of the soft-shelled turtle (*Trionychidae amyda*) in liquid nitrogen and then grinding and drying. SST powder was suspended in saline containing 0.5% carboxymethyl cellulose sodium (CMC). The suspension for oral administration was freshly prepared every day.

Fatigue-Preventive Experiment Eight-week-old adult male mice (ddy) were used. The mice were put into a rope climbing apparatus for 30 min a day and trained to climb the rope descending at a speed of 4 m/min for 4 d. An electric current was continuously passed through the grid floor of the apparatus. The mice received an electric shock when they stopped climbing the rope and touched the grid floor. Therefore, the mice were forced to keep climbing the rope. The experiment was started after a 4-d training period. The mice were forced to climb the rope for 60 min a day for 10 d and the total distance climbed was recorded. The SST powder (250 or 500 mg/kg) was given orally after the forced exercise. Since, at these doses, SST powder exhibits anti-hypertension and anti-cancer effects and improves liver injury,²⁻⁴⁾ the same doses were used in the present study. The control group received CMC-saline. Tocopherol (30 mg/kg) was used as a positive reference

drug.

In a separate series of experiments, the acute effect of the SST powder was also studied using trained mice. On the first day, mice were forced to climb the rope for 60 min. On the second day, either the SST powder or CMC-saline was administered orally and, after 10 min, the forced exercise was started. The distance climbed in 60 min was measured. An interval of 10 min was employed because fatigue is usually observed in the late stages of the experiment.

Stress Recovery Experiment At first, as a pilot experiment, 9-week-old adult male mice (IV-CS strain) were used. A male mouse was put into a cage together with three estrogenic adult female mice of the same strain which had been treated with estradiol benzoate. The male mouse was kept in the cage for 30 min a day for 4 d. Male mice intromitting every day were selected. The selected male mice were divided into four groups, namely, normal control, stress control, SST 250 and SST 500 mg/kg administration group. Each group consisted of 10 mice. The experiment was continued for 11 d (including an "opening" day) and the daily performance was monitored in the following order: recording of sexual behavior, learning trial, forced swimming stress, and then administration of the SST (250, 500 mg/kg) powder or CMC-saline (normal control, stress control). For the investigation of sexual behavior, the number of male mice which showed sexual behavior (licking, mounting and intromission), the frequency of sexual behavior per mouse during the 10 min experimental period, and the latency (the latent time before the mouse showed the first sexual behavior) were recorded. Then acquisition of passive avoidance learning was tested using a step-down type apparatus. Each mouse was placed on a rubber stand situated on the grid floor which was connected to an electrical shock generator. The experiment was continued for 10 min on the first day and for 3 min on the following 10 d. The number of mice stepping down onto the floor and the number of stepping times for each mouse were

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recorded. At the end of experiment, the number of days the mouse stepped down on the floor were recorded as the number of errors.

After the step-down test, swimming stress was given for 30 min on the first day. On the following days, the duration of the stress was gradually increased by 10 min a day until it reached a maximum of 60 min. The mouse tail was stuck with adhesive tape to a bar placed above the water surface, and the mouse had to keep swimming using forepaws in order to avoid drowning. The normal control group mice did not receive this stress.

RESULTS

Figure 1 shows the effect of chronic administration of SST powder on forced exercise in mice. During the 10 d of the experiment, the total achievement of the control group decreased gradually. However, the total motor activity of the SST powder-treated groups was unchanged. Statistically significant differences between the control group and SST powder-treated groups were observed after 5 d of forced exercise. On day one, the distance climbed for all four groups decreased, presumably because the duration of the exercise increased from 30 to 60 min. However, it recovered gradually over the following three days. Several days may be necessary for the mice to adjust to the daily exercise.

The effect of a single administration of SST powder is shown in Table 1. The treatment did not significantly affect the total distance climbed, suggesting that the SST powder does not affect directly physical performance.

The effects of the SST powder administration on sexual behavior are shown in Fig. 2. Licking (Fig. 2A), mounting (Fig. 2B) and intromission (Fig. 2C) were used as parameters. The number of mice, frequency (number of times per 10 min) and latency were measured for each parameter. The performance of the stressed group was always below that of the normal control group, *i.e.* small number of animals, low frequency and long latency. Chronic treatment with SST powder improved the stress-induced decrease. In particular the effects on licking were the most striking, and the frequency was sometimes greater than that of the normal controls.

Figure 3 shows the effect of SST powder on the stress-induced deficiency of acquisition and retrieval of memory. Stress increased the number of mice which stepped down during the test. Stress-induced deterioration was improved by administration of SST powder. However, the difference was not statistically significant.

DISCUSSION

Human fatigue is usually classified into two types, acute and chronic. Rest is necessary for recovery from the two fatigue types to supply the necessary substances lost during physical movement and to remove any accumulated superfluous substances. In the rope climbing apparatus employed in the present experiment, the rope descends continuously and, if the mouse stops climbing, it will touch the grid floor and get an electric shock. To keep climbing is the only way for the mouse to avoid the electrical shock.

Fig. 1. Effects of Chronic Administration of the SST Powder or Tocopherol on the Forced Climbing of Mice

The mice were forced to climb the rope every day for 60 min from day 1 to day 10. The data for day 0 indicate the last day of the training session in which the duration of climbing was 30 min. The ordinate indicates the total distance climbed on each day expressed as a percentage of the length of the rope descended (240 m). For clarity, only mean values are shown. Asterisks indicate significant differences from the control group (* $p < 0.05$; ** $p < 0.01$, Duncan's new multiple-range test). The number of animals was 10 for each group.

Table 1. Effects of a Single Administration of SST Powder on Forced Exercise

	Total distance climbed (%)	
	1st day	2nd day
Control	61 ± 8.0	58 ± 8.8
SST 250 mg/kg	71 ± 8.6	63 ± 7.6
SST 500 mg/kg	76 ± 7.7	66 ± 10.2

Either SST powder or CMC-saline (control) was administered 10 min before exercise on the 2nd day. Values represent mean ± S.E.M. from 10 rats.

If the physical exercise is sufficiently strong and one day is not long enough for recovery, the fatigue will be chronic. A deficient ability of main motor organs (predominantly the skeletal muscles) and the whole body (the central and autonomic nervous system and the cardiovascular system) is probably the cause of the fatigue. The former is important for acute fatigue and the latter for chronic fatigue.

We have demonstrated the beneficial effect of SST powder on fatigue. The total distance climbed per day for the control mice decreased day by day, probably indicating chronic fatigue. However, the mice in the SST powder-treated groups kept climbing almost restlessly. They seldom stopped climbing and their motor activities remained almost unchanged. Tocopherol is suggested to have anti-oxidative effects and improves micro-circulation,⁵⁾ necessary for the improvement of muscle fatigue. This effect was also confirmed in the present experiment. The single administration of SST powder just before forced exercise did not affect the performance, suggesting that SST powder does not directly stimulate the motor organ but improves recovery of whole body from fatigue.

The forced swimming stress-induced decrease in learning and sexual behavior has been shown previously.^{6,7)} We employed the same protocol to stress the mice and

Fig. 2. Effects of Chronic Treatment with SST Powder on Sexual Behavior in Chronically Stressed Mice

A male mouse was placed in a cage with estrogenic female mice for 10 min every day for 10 d, and the following three parameters were measured: licking (A), mounting (B) and intromission (C). The number of mice that conducted these acts expressed as a percentage (upper), the mean frequency of the acts expressed as the mean number per animal (middle) and the mean latency (lower) are shown in each figure. Normal unstressed control (open circles); stressed control (filled circles); 250 mg/kg SST (filled triangles); 500 mg/kg SST (filled squares). Each group consisted of 10 mice. For clarity, only mean values are shown. Asterisks indicate significant differences from the stress control group (* $p < 0.05$; ** $p < 0.01$, Duncan's new multiple-range test).

to evaluate learning and sexual behavior. The treatment of SST powder improved the sexual behavior of the stressed mouse. The effect was most significant when licking was used as an index. In a previous study, effects of *Eleutherococcus seticosus* on mounting were significant. Because beneficial effects of SST powder on mounting and intromission became significant after 8 d, longer treatment may be necessary. Since the adrenal gland is enlarged and tyrosine hydroxylase activity in adrenal gland and hypothalamic regions is decreased, the decline in sexual

behavior produced by the swimming stress is probably caused by a change in the activity of the central nervous system and the internal secretory system.^{6,7)} Therefore, it is possible that chronic administration of SST powder increases the activity of these systems. Further experiments are necessary to clarify the detailed mechanism.

The process of learning and memory can be divided into three components, acquisition, fixation and retrieval. It has been shown that the forced swimming stress employed in the present experiment does not affect acquisition or

Fig. 3. Effects of Chronic Treatment with SST Powder on Passive Avoidance Learning Responses in Mice

Normal unstressed control (open circles); stressed control (filled circles); 250 mg/kg SST (filled triangles); 500 mg/kg SST (filled squares). Cumulative ratio of mice stepping down from the rubber stand more than once (A), twice (B) and three times (C) over 10 d is shown. D: average number of errors per mouse over 10 d.

fixation, but impairs retrieval.⁷⁾ Therefore, in the present experiment we administered SST powder just after stress to investigate the effect on retrieval. The decline in memory retrieval due to stress was improved by chronic administration of SST powder. However, the effects did not reach statistical significance.

In conclusion, chronic administration of SST powder improved the recovery from the physical stress-induced deficiency in locomotion and behavior.

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