Effect of Saiko-ka-ryukotsu-borei-to on the Stress-Induced Increase of Serum Corticosterone in Mice

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The effect of Saiko-ka-ryukotsu-borei-to (SRBT) on the stress-induced enhancement of serum corticosterone in various stress models was investigated in mice. The serum corticosterone was elevated significantly by immobilized stress, forced-swim stress, electric-shock stress, psychological stress and conditioned-fear stress, respectively. The concentration in the last two models was decreased in a dose-dependent manner by pre-administration of SRBT (10, 60, 100, 600 and 1000 mg/kg, p.o.). Therefore, this action seems to be effective in stress involving emotional factors but ineffective in physical stress models. These results indicate that the anti-stress effect of SRBT is dependent strongly on the degree of psychological change compared with physical change in mice.

Key words Saiko-ka-ryukotsu-borei-to; diazepam; serum corticosterone; anti-stress effect; stress model; mouse

Some Chinese medicinal preparations seem to be effective in treating the symptoms induced by stress. Saiko-ka-ryukotsu-borei-to (SRBT), an important Chinese medicinal preparation, is widely used in a variety of clinical situations in Japan and occasionally to treat various neurological symptoms. In this paper, we report the effect of this drug on the increase in serum corticosterone in mice stressed by various methods.

MATERIALS AND METHODS

Materials Chopped crude drugs were purchased from Nakai-kohshindo (Kobe). Corticosterone (Guaranteed Reagent) was purchased from Nacalai Tesque (Kyoto). Diazepam (for positive control) was obtained from Sigma Chemical Co.

Animals ddY Male mice weighing 28—32 g (Nihon SLC, Hamamatsu, Japan) were housed in groups of 10 in plastic cages with free access to food and water. They were kept in a room maintained at an ambient temperature of 25 ± 5°C under a day/night regime, day 7:00—19:00 and night 19:00—7:00.

Prescription The following crude drug preparation was used as SRBT; Bupleuri Radix 5.0 g, Pinelliae Tuber 4.0 g, Hoelen 3.0 g, Cinnamomi Cortex 3.0 g, Scutellariae Radix 2.5 g, Zizyphi Fructus 2.5 g, Zingiberis Rhizoma 1.0 g, Ginseng Radix 2.5 g, Fossillia Osis Mastodi 2.5 g, Ostreae Testa 2.5 g, Rhei Rhizoma 1.0 g.

Samples for Examination A one-day dose of the preparation was decoted in a beaker (1000 ml) with 600 ml of water by boiling for 30 min using an electric heater (600 W) followed by filtration and freeze-drying.

Administration of Test Samples SRBT (1000 mg/kg; psychological stress, 10, 60, 100, 600 and 1000 mg/kg) and diazepam (5 mg/kg) dissolved in distilled water in a volume of 0.2 ml/10 g body weight were administered perorally 1 h before each stress treatment. In the case of the conditioned-fear stress, SRBT (1000 mg/kg; 1 h before being returned, 10, 60, 100, 600 and 1000 mg/kg) and diazepam (5 mg/kg) were given perorally 1 h before electric-shock exposure and 0, 1 and 3 h before being returned to the electric-shock compartment. Distilled water alone was given to the control group in the same manner.

Immobile Stress Animals were restricted in polyethylene tubes (2.8 cm i.d. × 12 cm) for 20 min, according to the method of Weiss. 2

Forced-Swim Stress Animals were forced to swim by being put in a water bath (40 cm(L) × 35 cm(W) × 20 cm(H)) with water 15 cm deep at 20 ± 1°C for 15 min, based on the method of Porsolt et al. 3

Electric-Shock Stress This method was carried out according to Takahashi et al. 4 Animals were exposed to an inescapable and unsignalled electric shock (2 mA, 0.2 Hz, 5 s) for 20 min using a foot-shock stress system (Muromachi Kagaku, model FSK-01).

Psychological Stress This stress model was carried out according to Iimori et al. 5 A communication box consisting of 2 compartments was used. Animals were placed individually into the compartments and an electric shock (2 mA, 0.2 Hz, 5 s) was delivered through floor for 20 min. Animals placed in a compartment in which the floor was covered with a plastic plate were prevented from receiving the electric shock, but they were exposed to psychological stress by watching and hearing the struggling, jumping and vocalization of the shocked animals.

Conditioned-Fear Stress According to Fanselow et al., 6 animals were placed individually into compartments, and an electric shock (2 mA, 0.2 Hz, 5 s) was delivered through the floor for 20 min. 24 h later, the animals were returned to the compartments without the electric shock for 20 min.

Measurement of Serum Corticosterone Serum corticosterone was determined by fluorescence analysis. 7 Serum samples were collected by the decapitation of the stressed animals at suitable times. The corticosterone was extracted with dichloromethane then the fluorescence was measured in an ethanol–sulfuric acid (3:7) mixture (Shimadzu RF-1500; excitation, 468; emission, 520).

Statistical Analysis The results were expressed as means ± S.E. following a two-way analysis of variance for repeated measurements with the overall data being used to assess statistical significance, differences between

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the individual mean values in various groups were analyzed by the Bonferroni method. A difference was considered significant at \( p < 0.05 \).

RESULTS

Changes of Serum Corticosterone after Immobilized Stress, Forced-Swim Stress, Electric-Shock Stress and Psychological Stress

The serum corticosterone in the control group was 22.50 ng/100 ml, in the immobilized stress group, 21.82 ng/100 ml, in the forced-swim stress group, 25.64 ng/100 ml, in the electric-shock stress group and 26.88 ng/100 ml in the psychological stressed group. The corticosterone concentrations in stressed mice significantly increased in comparison with the control value, namely by 230.0% in immobilized stress, 250.0% in forced-swim stress and 146.2% in electric-shock stress. Pre-administration of diazepam reduced the corticosterone concentrations in comparison with the stress-control value, namely by 23.0% in immobilized stress, 14.5% in forced-swim stress and 26.5% in electric-shock stress. SRBT, however, had no influence on the stress-induced increase. On the other hand, the increase in psychologically stressed mice (180.5%) was reduced to 126.4% of the control by diazepam and 122.7% of the control by SRBT (Fig. 1).

Changes in Serum Corticosterone in Conditioned-Fear Stress

The serum corticosterone in conditioned-fear stressed mice was significantly increased (176.5%). This increase was reduced to 130.0% and 126.4% of the control by pre-administration of diazepam and SRBT at 1 h before returning the mice to the compartment. However, pre-administration of SRBT at 0, 3, and 25 h before returning had no significant influence (Fig. 2).

Effect of Various Doses of SRBT on Serum Corticosterone in Psychological Stress

The increase of serum concentration in psychologically stressed mice was significantly reduced to 68.1% and 49.2%, in comparison with stress-control values, by pre-administration of SRBT (100 and 600 mg/kg, respectively). However, pre-administration of doses of 10 and 60 mg/kg SRBT had no significant effect (Fig. 3).

Effect of Various Doses of SRBT on Serum Corticosterone in Conditioned-Fear Stress

The increased serum corticosterone in conditioned-fear stressed mice was significantly reduced to 70.9%, 60.4% and 56.0%, in comparison with stress-control values, by pre-administration of SRBT in doses of 60, 100 and 600 mg/kg. However, pre-administration of 10 mg/kg SRBT had no significant effect.

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Fig. 2. Change in Serum Corticosterone in Conditioned-Fear Stressed Mice Following Administration of SRBT

Mice were pre-administered a freeze-dried decoction of SRBT, (1000 mg/kg, p.o., shaded columns) and DZP (5 mg/kg, p.o., hatched columns), then the effect on the increase in serum corticosterone induced by conditioned-fear stress exposure was compared. (−25), pre-administration 1 h before electric shock exposure; (−3), pre-administration 3 h before returning to the electric-shock compartment; (−1), pre-administration 1 h before returning to the electric-shock compartment; (0), simultaneous administration and returning to the electric-shock compartment. Each value represents the mean of 6–8 mice per group, and horizontal lines show the standard error of the mean. * Significantly different from stress-control value, \( p < 0.05 \).

Fig. 3. Effect of Various Doses of SRBT on Serum Corticosterone in Psychologically Stressed Mice

Mice were pre-administered a freeze-dried decoction of SRBT, (10, 60, 100 and 600 mg/kg, p.o., shaded columns) and DZP (5 mg/kg, p.o., hatched columns). The effect on the increase in serum corticosterone induced by psychological stress exposure was compared with pre-administration 1 h before stress exposure. Each value represents the mean of 6–8 mice per group, and horizontal lines show the standard error of the mean. * Significantly different from stress-control value, \( p < 0.05 \).
AMP from the pituitary gland.\(^{12}\) We suppose that some of these are target sites for the action of SRBT.

In psychologically stressed mice, SRBT exerted an anti-stress effect in a dose-dependent manner and, in conditioned-fear stressed mice, SRBT exhibited an anti-stress effect when given in a suitably scheduled manner. A marked reduction in serum corticosterone was observed at 1 h pre-administration before returning to the compartment and this effect was dose-dependent. On the other hand, the increase in serum corticosterone was not reduced by pre-administration of SRBT at 0, 3 or 25 h before returning to the compartment. Therefore, this action may be related to the concentration of drug in the blood.

Further investigations are necessary for clarify the contributions of the crude drugs blended in this Chinese medicinal preparation and to discover the mechanism of action.

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