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Saiko-ka-ryukotsu-borei-to, a herbal medicine, ameliorates chronic stress-induced depressive state in rotarod performance

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Abstract

Exposure to chronic stress is thought to play an important role in the etiology of depression. This disorder has been shown to involve disruption of the hypothalamo–pituitary–adrenal (HPA) system and dysfunction of the prefrontal cortex (PFC). We have demonstrated that chronic stress in rats induces similar HPA disruption or a depressive state caused by a reduction of dopaminergic and serotonergic transmission in the PFC. We have also shown that *saiko-ka-ryukotsu-borei-to*, a herbal medicine, prevents such chronic stress-induced HPA disruption. However, the behavioral and neurochemical bases of this drug remain unclear. Here we examined the effects of *saiko-ka-ryukotsu-borei-to* on the depressive behavioral state and the reduction of transmission resulting from chronic stress. The chronic stress was induced by water immersion and restraint (2 h/day) for 4 weeks followed by recovery for 10 days. The treatment with *saiko-ka-ryukotsu-borei-to* (100, 300, or 1000 mg/kg po) ameliorated the stress-induced depressive state in a dose-dependent manner, evaluated by a rotarod test. A microdialysis study indicated that the drug treatment significantly prevented the chronic stress-induced decreases in extracellular concentrations of dopamine and serotonin in the PFC. These results suggest that *saiko-ka-ryukotsu-borei-to* ameliorates the chronic stress-induced depressive state based on the prevention of PFC dysfunction. These findings provide important information for treatment of depression.

Keywords: *Saiko-ka-ryukotsu-borei-to*; Herbal medicine; Chronic stress; Rotarod performance; Dopamine; Serotonin; Prefrontal cortex; Depression; Rat

1. Introduction

Exposure to chronic stress is thought to precipitate or exacerbate several neuropsychiatric disorders including depression (Anisman and Farabollini, 1982). Several reports (Carroll et al., 1981; Kalin et al., 1982; Holsboer, 1983; Arana et al., 1985) have demonstrated that disruption of the hypothalamo–pituitary–adrenal (HPA) system is observed in approximately one half of human depressives. In general, this disruption is presented as a dexamethasone-mediated negative feedback resistance to cortisol secretion. The approach of repeatedly exposing an animal to stress has often been used in experimental models of depression. Several studies have demonstrated that chronic stress disrupts the glucocorticoid negative feedback system. For example, in chronically foot-shocked rats, elevated plasma corticosterone levels in response to acute foot-shock were not suppressed by dexamethasone (Haracz et al., 1988), and β-endorphin release upon subsequent presentation of swim stress was not inhibited by exogenous corticosterone (Young et al., 1990). Recently, we found that rats exposed to chronic stress, induced by water immersion and restraint, showed a similar attenuated feedback to dexamethasone, particularly of corticosterone secretion under resting conditions (Mizoguchi et al., 2001).

In addition, dysfunction of the prefrontal cortex (PFC) has been implicated, based on observations from clinical, neuropsychological, and neuroimaging studies, as having an important role in the pathophysiology of depression (Cummings, 1992; Deutch, 1993; Dolan et al., 1994; Fibiger, 1995; Drevets et al., 1997, 2000). Although the neurotransmitters involved remain to be elucidated, several reports have shown that the dysfunction of dopaminergic

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(Calabrese and Markovitz, 1991; Cummings, 1992; Fibiger, 1995) and serotonergic (Owens and Nemeroff, 1994; Lane et al., 1995; Drevets et al., 2000) systems in the PFC is thought to contribute to some depressive symptoms. In rats, we recently indicated that chronic stress reduced dopaminergic (Mizoguchi et al., 2000) and serotonergic transmission in the PFC (Mizoguchi et al., 2002a), and these chronically stressed animals showed a depressive behavioral state as evaluated by a rotarod test (Mizoguchi et al., 2002a). These observations appear to mimic the characteristics found in human depressives.

Several herbal medicines (called *kampo* drugs in Japan, herbal remedies composed of specified mixtures of dried plant materials) are effective in the field of neuropsychiatry (Yamada and Kanba, 1997). In particular, the *kampo* drug *saiko-ka-ryukotsu-borei-to* has been widely used in a variety of clinical cases in Japan for the treatment of stress-related neuropsychiatric disorders including depression (Yamada and Kanba, 1997). Although scientific evidence for its clinical effects is limited, our recent research indicated that *saiko-ka-ryukotsu-borei-to* ameliorated the chronic stress-induced attenuation of the glucocorticoid negative feedback (Mizoguchi et al., 2002c). This finding provided the first scientific evidence for the potential medicinal properties of this drug, because the disruption of the HPA system can contribute to some of the symptoms of depression (Steckler et al., 1999). However, whether this drug has practical, beneficial effects on the chronic stress-induced depressive behavioral state and neurochemical dysfunction remains unclear. Considering that *saiko-ka-ryukotsu-borei-to* increased activity levels in a behavioral test conducted to evaluate despair (Koshikawa et al., 1998), it is possible that *saiko-ka-ryukotsu-borei-to* has an antistressful or antidepressive action through effects on the neurotransmitter systems in the PFC.

The present study was designed to clarify the effects of *saiko-ka-ryukotsu-borei-to* on the depressive state of chronically stressed rats. For this purpose, we examined the effects of this *kampo* drug on the chronic stress-induced depressive behavioral state using the rotarod test, and decreases in the extracellular concentrations of dopamine (DA) and serotonin (5-hydroxytryptamine, 5-HT) in the PFC using a microdialysis technique.

2. Materials and methods

2.1. Drug

Saiko-ka-ryukotsu-borei-to was supplied in the form of a water-extracted dried powder that was manufactured from a mixture of the crude drugs listed in Table 1 (Tsumura & Co., Tokyo, Japan). The concentration of several effective chemicals is defined for each crude drug as an internal standard in our company's guide to Good Manufacturing Practices.

Table 1
Crude drug composition of *saiko-ka-ryukotsu-borei-to*

Plant name	Composition (g)	Major components
Bupleuri radix	5.0	saikosaponin a, c, d, e
Pinelliae tuber	4.0	homogenistic acid
Hoelen	3.0	eburicoic acid
Cinnamomi cortex	3.0	cinnamic aldehyde
Scutellariae radix	2.5	baicalin, wogonin
Zizyphi fructus	2.5	zizyphus saponin, betulinic acid
Ginseng radix	2.5	ginsenoside
Fossilia ossis mastodi	2.5	calcium base
Ostreae testa	2.5	calcium base
Zingiberis rhizoma	1.0	gingerol, shogaol

2.2. Animals and stress exposure

All animal experiments were performed in accordance with our institutional guidelines after obtaining the permission of the Laboratory Animal Committee. Naive adult male Wistar rats (Japan Clea, Tokyo, Japan) weighing 300–350 g were used. They were housed four per cage in a temperature (22 ± 2 °C)-, humidity (55 ± 10%)-, and light (12-h light/dark schedule; lights on at 7:00 a.m. and off at 7:00 p.m.)-controlled environment and were given laboratory food and water ad libitum.

Prior to any behavioral experiments, the riding ability of the animals on a rotating rod (described below) was verified. The rats were placed on the rotating rod, and the rats that immediately fell off (within 10 s) were excluded from the behavioral experiment. The rats that exceeded this criterion were used for the behavioral experiment to examine the effects of chronic stress exposure and drug treatment.

The procedure of stress exposure was described previously (Mizoguchi et al., 2000, 2001, 2002a,b,c). Briefly, the animals were placed in a stress cage made of wire net, and immersed to the level of the xiphoid process in a water bath (21 °C) for 2 h. The animals were subjected to this form of stress once a day for 4 weeks (chronic stress). To avoid the acute influence of the stress and to ensure that long-term consequences of the chronic stress were evaluated, the animals were allowed a 10-day recovery period.

2.3. Drug administration

Saiko-ka-ryukotsu-borei-to was suspended in distilled water, and was administered daily during both the 4-week stress session and the 10-day recovery period. For behavioral analysis, this drug was administered at a dose of 100, 300, or 1000 mg/10 ml/kg po. For microdialysis study, this drug was administered at a dose of 1000 mg/10 ml/kg po alone. The naive nonstressed rats were given distilled water (10 ml/kg po) daily as controls. Twenty-four hours after the last administration, either the behavioral analysis or the microdialysis study was performed.

2.4. Rotarod test

The experimental procedure has been described elsewhere (Dunhan and Miya, 1957; Commissaris and Rech, 1983; Ahmad and Nicholls, 1990; Morimoto and Kito, 1994; Mizoguchi et al., 2002a,b). Briefly, the time (seconds) that the rats stayed on a rotating rod (10 cm diameter, 7 rpm; Muromachi Kikai, Tokyo, Japan) was recorded automatically in each case for up to 180 s. When the duration of riding exceeded 180 s, the rat was removed from the rod, and the riding time was recorded as 180 s. The trial was conducted five times for each rat, and the mean value of these trials equaled the mean riding time of each rat. The number of animals in each group in all behavioral analyses was 20.

2.5. Traction test

The traction test followed the completion of the rotarod test. The experimental procedure has been described elsewhere (Kuribara et al., 1977; Mizoguchi et al., 2002a,b). Briefly, a wire (2 mm diameter, 40 cm long) was set horizontally 50 cm above the base. The rat was first forced to grasp the wire with the two forepaws, and the time (seconds) that it clung to the wire was measured for up to 60 s. When the duration of clinging exceeded 60 s, the rat was released from the wire, and the clinging time was recorded as 60 s. The trial was conducted three times for each rat, and the mean value of these trials equaled the mean clinging time for each rat.

2.6. Locomotor activity

Following the traction test, the spontaneous locomotor activity of the rat was measured over a period of 5 min using an Animex apparatus (ANIMEX AUTO, MK-110; Muromachi Kikai), as described previously (Mizoguchi et al., 2002a,b).

2.7. Brain microdialysis

Microdialysis was performed in freely moving animals according to the methods described previously (Mizoguchi et al., 2000, 2002a). Briefly, after a 2-day recovery period following the 4-week stress session, the animals were stereotaxically implanted with a guide cannula (9 mm long, 0.8 mm outer diameter; Bioanalytical Systems, West Lafayette, IN, USA) under pentobarbital anesthesia (45 mg/kg ip). The brain atlas of Paxinos and Watson (1986) was used to determine the coordinates. The following coordinates relative to the bregma were used for the cannula implantation in the PFC: anteroposterior, +3.2; lateral, +1.2; ventral, −2.0. The animals were allowed a postoperative recovery period of at least 8 days. On the day of the experiment (i.e. after the 10-day recovery period following the 4-week stress session), a microdialysis probe (PC-12; 4 mm tip length, 0.5 mm tip diameter; Bioanalytical Systems) was inserted into the guide cannula. The probe location was as follows: anteroposterior, +3.2; lateral, +1.2; ventral, −2.0 to −6.0. Then, Ringer's solution (in mM: Na^+, 147; K^+, 4.0; Ca^{2+}, 3.0) was perfused at a rate of 0.6 μl/min using a microinfusion pump. After an equilibration period of 3 h, the perfusate was collected every 70 min. To examine the response to stimuli, the KCl concentration was raised to 100 mM. Each perfusate (35 μl) was injected immediately into an HPLC system in conjunction with a coulometric electrochemical detector (ECD-200; Eicom, Kyoto, Japan) to determine the concentrations of DA and 5-HT in the perfusate. A reverse-phase ODS column (CA-5; Eicom) was used with a mobile phase consisting of 82 mM sodium phosphate (pH 6.0), 800 mg/l sodium 1-octanesulfonate, 50 mg/l EDTA, and 180 ml/l methanol.

Histological verification of the cannula position, by dye infusion performed at the end of the experiments, confirmed the correct positioning of the cannula in all animals. The number of animals in each group in this study was as follows: naive nonstressed and vehicle-treated, 10; stressed and vehicle-treated, 7 or 8; stressed and *saiko-ka-ryukotsu-borei-to*-treated, 10.

2.8. Statistics

All data were initially analyzed using one-way analysis of variance (ANOVA). Individual between-group comparisons were performed as follows: Fisher's Protected Least Significant Difference Test for the behavioral analysis; the Mann–Whitney *U* test for the microdialysis study.

3. Results

3.1. Behavioral performance

The effects of *saiko-ka-ryukotsu-borei-to* on the chronic stress-induced changes in behavioral performance are shown in Fig. 1. In the rotarod test (Fig. 1A), the riding time was significantly decreased in the chronically stressed rats [$F(4,94)=5.591$; $P<.001$]. This impaired performance was significantly ameliorated by treatment with 100–1000 mg/kg of *saiko-ka-ryukotsu-borei-to* [$F(4,94)=5.591$; 100 mg/kg, $P<.05$; 300 mg/kg, $P<.05$; 1000 mg/kg, $P<.001$]. The clinging time in the traction test (Fig. 1B) and locomotor activity (Fig. 1C) were not affected by the chronic stress or any dose of the drug tested.

3.2. Extracellular concentrations of DA and 5-HT

The effects of *saiko-ka-ryukotsu-borei-to* on the extracellular concentrations of DA and 5-HT in the PFC under the basal or KCl-stimulated conditions are shown in Fig. 2. The basal concentration of DA in the chronically stressed rats was significantly lower than that in the naive nonstressed

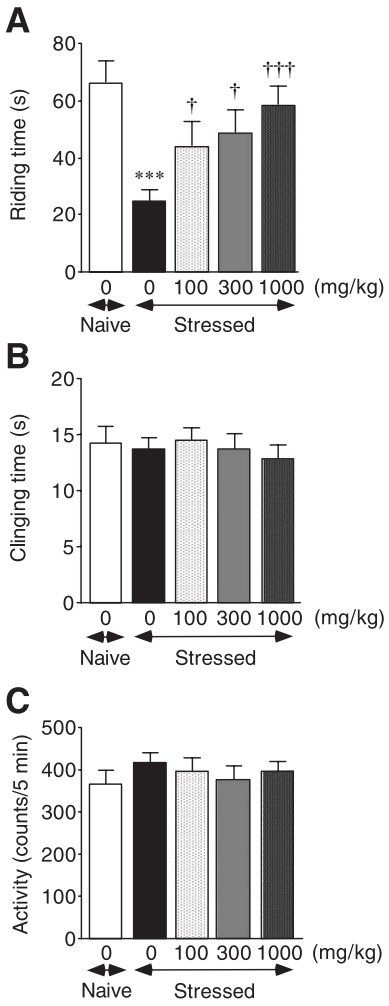

Fig. 1. Effect of *saiko-ka-ryukotsu-borei-to* on chronic stress-induced changes in behavioral performance. (A) rotarod performance; (B) traction performance; (C) locomotor activity. Each column is the mean ± S.E.M. of 20 rats per group. Asterisks indicate a significant difference from naive nonstressed and distilled water (0 mg/kg)-treated rats, $***P<.001$; daggers indicate a significant difference from stressed and distilled water-treated rats, $^{\dagger}P<.05$, $^{\dagger\dagger\dagger}P<.001$.

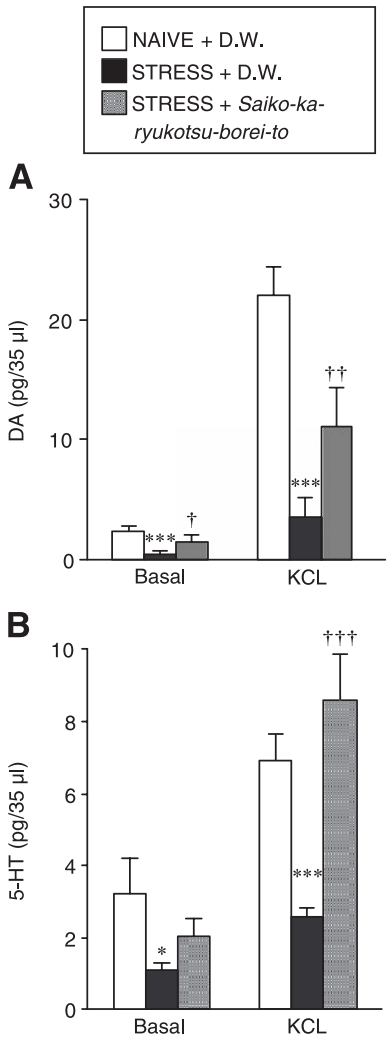

Fig. 2. Effect of *saiko-ka-ryukotsu-borei-to* on chronic stress-induced decrease in the extracellular concentration of DA (A) or 5-HT (B) in the PFC. Each column is the mean ± S.E.M. of 7, 8, or 10 rats per group. Asterisks indicate a significant difference from naive nonstressed and distilled water (D.W.)-treated rats, $*P<.05$, $***P<.001$; daggers indicate a significant difference from stressed and D.W.-treated rats, $^{\dagger}P<.05$; $^{\dagger\dagger}P<.01$; $^{\dagger\dagger\dagger}P<.001$.

rats ($P<.001$) (Fig. 2A). Under the stimulated conditions, the increasing response of the DA concentration to KCl stimulation in the naive nonstressed rats was also significantly attenuated in the chronically stressed rats ($P<.001$). These decreases in the DA concentrations were significantly prevented by the drug treatment (1000 mg/kg), respectively (basal, $P<.05$; KCl, $P<.01$).

The basal concentration of 5-HT in the chronically stressed rats was significantly lower than that in the naive nonstressed rats ($P<.05$) (Fig. 2B). Under the stimulated conditions, the increasing response of the 5-HT concentration to KCl stimulation in the naive nonstressed rats was also significantly attenuated in the chronically stressed rats ($P<.001$). The decrease in the basal concentration of 5-HT was not prevented by the drug treatment. However, the decrease under the stimulated conditions was significantly prevented by the treatment ($P<.001$).

4. Discussion

Our results showed that *saiko-ka-ryukotsu-borei-to* could ameliorate the chronic stress-induced impairment of the rotarod performance, possibly by preventing a reduction of dopaminergic and serotonergic transmission in the PFC.

As shown in Fig. 1, the chronic stress decreased the riding time in the rotarod test. This decrease is not likely to be related to muscle relaxation or motor dysfunction because the chronic stress did not affect the clinging time or locomotor activity, which is in agreement with our recent

reports (Mizoguchi et al., 2002a,b). Antidepressants such as desipramine and trazodone, which increase activity levels in a behavioral test conducted to evaluate despair, increased the riding time in the rotarod test (Morimoto and Kito, 1994). Moreover, the chronic stress-induced decrease in the riding time was reversed by trazodone (Mizoguchi et al., 2002a). Therefore, the chronic stress-induced decrease in the riding time observed in the present study is thought to represent a depressive behavioral state. In the drug treatment study for the behavioral performance, *saiko-ka-ryukotsu-borei-to* reversed the chronic stress-induced impairment of the rotarod performance in a dose-dependent manner concomitant with unchanged traction performance and locomotor activity. These results confirm that the beneficial action of this drug is not because of the changes in muscle power or locomotor activity, and suggest that this drug ameliorates the chronic stress-induced depressive behavioral state.

We have recently suggested that the chronic stress-induced impairment of the rotarod performance is caused by a reduction of dopaminergic (Mizoguchi et al., 2002b) and serotonergic (Mizoguchi et al., 2002a) transmission in the PFC. Therefore, to clarify the mechanism of the beneficial effects of *saiko-ka-ryukotsu-borei-to* on the chronic stress-induced depressive state, we hypothesized that this drug acts on these neurotransmitter systems. Hence, we examined the effects of this drug on the chronic stress-induced reduction of dopaminergic and serotonergic transmission in the PFC using the most effective dose of this drug in the rotarod performance, i.e., 1000 mg/kg. As shown in Fig. 2, the chronic stress-induced decreases in both extracellular concentrations of DA and 5-HT in the PFC were prevented by the drug treatment. These results strongly suggest that the beneficial action of *saiko-ka-ryukotsu-borei-to* on the chronic stress-induced depressive state is based on the prevention of dopaminergic and serotonergic dysfunction in the PFC.

However, the actual mechanism of the preventive action of *saiko-ka-ryukotsu-borei-to* on these neurotransmitter systems remains unclear. Three principal mechanisms may be hypothesized. Firstly, since *saiko-ka-ryukotsu-borei-to* increased activity levels in a test of behavioral despair (Koshikawa et al., 1998) that is sensitive to dopaminergic or serotonergic system (Porsolt et al., 1977), this drug may directly stimulate these neurotransmitter systems. Secondly, *saiko-ka-ryukotsu-borei-to* may suppress excitation of the brain induced by stress because this drug suppressed both elevation of the serum corticosterone level in mice (Sasaki et al., 1995) and an increase in the tissue concentrations of DA and its metabolite dihydroxyphenylacetic acid in the brain (Sasaki et al., 1998) in response to acute stress. Moreover, this drug improved both spontaneous locomotor hyperactivity and a decrease in time of sodium pentobarbital-induced sleep in El mice, suggesting the involvement of gamma-aminobutyric acid (Iizuka et al., 1998). Finally, the preventive action of this drug may be effected indirectly through glucocorticoid regulation. Several reports have demonstrated that altered glucocorticoid regulation can affect the dopaminergic (Imperato et al., 1989) and serotonergic (Mendelson and McEwen, 1992; Kuroda et al., 1992, 1993; Slotkin et al., 1996) systems in the PFC. Both animal and human studies challenging the HPA system have shown some neuroendocrine and behavioral changes similar to those observed in depression, suggesting that some of the depressive symptoms can be contributed to disruption of the HPA system (Steckler et al., 1999). Indeed, the same chronically stressed rats used in the present study exhibited not only the depressive state caused by the reduction of dopaminergic (Mizoguchi et al., 2000) and serotonergic (Mizoguchi et al., 2002a) transmission in the PFC but also disruption of the glucocorticoid feedback system (Mizoguchi et al., 2001). For that reason, the PFC dysfunction may be related to the disruption of the feedback system. Thus, the preventive action of *saiko-ka-ryukotsu-borei-to* for the chronic stress-induced disruption of the feedback system (Mizoguchi et al., 2002c) may underlie the prevention of the dopaminergic and serotonergic dysfunction in the PFC.

In regard to glucocorticoid regulation, some ingredients of *saiko-ka-ryukotsu-borei-to* may be implicated in its beneficial actions. The major components of the crude drug have been determined (Table 1). Among them, several active ingredients that influence the glucocorticoid secretion system in particular have been isolated. For example, oral administration of saikosaponins increased the plasma levels of adrenocorticotropic hormone and corticosterone in normal rats (Hiai et al., 1981; Yokoyama et al., 1981), and this corticosterone elevation could be blocked by dexamethasone, suggesting activation of the HPA axis (Hiai et al., 1981). Ginseng saponins similarly increased adrenal cAMP, a second messenger of adrenocorticotropic hormone, and thereby stimulated synthesis and secretion of corticosteroids in normal but not hypophysectomized rats (Hiai et al., 1979). Ginseng saponins also acted as a functional ligand for glucocorticoid receptors (Lee et al., 1997). Since glucocorticoids are considered to be essential hormones for overcoming acute physical stress, these ingredients may facilitate the resistance, coping, and adaptation responses of organisms to stress through modulation of the glucocorticoid secretion system. These actions may be involved in an antistress effect of *saiko-ka-ryukotsu-borei-to*.

Depression is generally precipitated by stressful life events. In this disorder, disruption of the HPA system (Carroll et al., 1981; Kalin et al., 1982; Holsboer, 1983; Arana et al., 1985) and PFC dysfunction (Cummings, 1992; Deutch, 1993; Dolan et al., 1994; Fibiger, 1995; Drevets et al., 1997, 2000) have been demonstrated. In the present and recent studies, we showed that *saiko-ka-ryukotsu-borei-to* could ameliorate two factors that mimic depression observed in the chronically stressed rats, namely PFC dysfunction (present study) and disruption of the HPA system (recent study, Mizoguchi et al., 2002c). Thus, these

findings implicate this drug as a candidate for the prevention and treatment of depression.

In conclusion, the present results suggest that *saiko-ka-ryukotsu-borei-to* ameliorates the chronic stress-induced depressive state based on the prevention of reduction of dopaminergic and serotonergic transmission in the PFC. This action may be a mechanism of the clinical relief provided by *saiko-ka-ryukotsu-borei-to* in the field of neuropsychiatry. Further research is required to clarify the effective ingredients in this *kampo* drug and to discover the mechanism underlying its actions.

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