Available online at www.sciencedirect.com
ScienceDirect
Pharmacology, Biochemistry and Behavior 86 (2007) 55–61
PHARMACOLOGY BIOCHEMISTRY AND BEHAVIOR
www.elsevier.com/locate/pharmbiochembeh

Saikokaryukotsuboreito, a herbal medicine, prevents chronic stress-induced dysfunction of glucocorticoid negative feedback system in rat brain

Kazushige Mizoguchi [a,*], Nan Sun [a,b], Xue-Long Jin [a,c], Yoshio Kase [d], Shuichi Takeda [d], Wakako Maruyama [a], Takeshi Tabira [e]

[a] *Department of Geriatric Medicine, National Institute for Longevity Sciences, National Center for Geriatrics and Gerontology, 36-3 Gengo, Morioka, Obu, Aichi 474-8522, Japan*
[b] *Department of Thoracic Surgery, The First Clinical College and The First Affiliated Hospital, China Medical University, 155 Nanjingbei Road, Heping, Shenyang, Liaoning 110001, China*
[c] *Department of Physiology, Tianjin Medical University, 22 Qixiangtai, Tianjin 300070, China*
[d] *Central Research Laboratories, Tsumura and Co., 3586 Yoshiwara, Ami-machi, Inashiki-gun, Ibaraki 300-1192, Japan*
[e] *National Institute for Longevity Sciences, National Center for Geriatrics and Gerontology, 36-3 Gengo, Morioka, Obu, Aichi 474-8522, Japan*

Received 17 August 2006; received in revised form 29 November 2006; accepted 12 December 2006
Available online 20 December 2006

Abstract

Disruption of the hypothalamo–pituitary–adrenal (HPA) axis characterized by dysfunction of the glucocorticoid negative feedback system is frequently observed in human depressives and is thought to involve a reduction in glucocorticoid receptor (GR) function in the feedback sites including the brain. Recently, we found that chronic stress in rats induces similar HPA disruption that is caused by abolishment of feedback ability in the prefrontal cortex (PFC) and hippocampus, which involves decreased cytosolic GRs or increased nuclear GRs, respectively. Also, we found that *saikokaryukotsuboreito* (SRBT), a herbal medicine, prevents the chronic stress-induced HPA disruption. We therefore examined here the effects of this drug on the chronic stress-induced changes in GRs in the PFC and hippocampus. Chronic stress was induced in rats by water immersion and restraint (2 h/day) for 4 weeks. SRBT significantly prevented decreased cytosolic GRs in the PFC and increased nuclear GRs in the hippocampus in the chronically stressed rats. Moreover, SRBT significantly prevented the abolishment of feedback ability in both regions. These results suggest that the beneficial effects of SRBT on the GR level are involved in its ameliorating actions on the HPA disruption. This finding provides information important for the prevention and treatment of depression.

Keywords: *Saikokaryukotsuboreito*; Herbal medicine; *Kampo* drug; Glucocorticoid receptor; Glucocorticoid negative feedback; Corticosterone; Dexamethasone; Prefrontal cortex; Hippocampus; Depression; Rat

1. Introduction

Exposure to chronic stress is thought to precipitate or exacerbate several neuropsychiatric disorders including depression (Mazure, 1995). Several reports have demonstrated that disruption of the hypothalamo–pituitary–adrenal (HPA) axis presenting as attenuation of the dexamethasone (DEX)-mediated negative feedback on cortisol secretion is observed in approximate one half of human depressives (Carroll et al., 1981; Kalin et al., 1982; Holsboer, 1983; Arana et al., 1985). Although glucocorticoid secretion is negatively regulated by glucocorticoids at the level of the anterior pituitary gland (Miller et al., 1992), several regions of the brain, such as the hypothalamus, hippocampus, and prefrontal cortex (PFC), that have abundant glucocorticoid receptors (GRs), are also involved (Feldman and Conforti, 1985; Magarinos et al., 1987; Kovács and Makara, 1988; Diorio et al., 1993; Feldman and Weidenfeld, 1999; Mizoguchi et al., 2003a). Recently, we have indicated that chronic stress in rats induces similar HPA disruption to human depressives, which is caused by the abolishment of feedback ability in the PFC and hippocampus (Mizoguchi et al., 2001, 2003a). Moreover, we have shown that chronic stress simultaneously decreases GR levels in the cytosolic fraction of the PFC and increases the levels in the nuclear fraction of the

* Corresponding author. Tel.: +81 562 46 2311x5553; fax: +81 562 48 2373.
E-mail address: k-mizo@nils.go.jp (K. Mizoguchi).

0091-3057/$ - see front matter © 2006 Elsevier Inc. All rights reserved.
doi:10.1016/j.pbb.2006.12.007

hippocampus, suggesting the involvement of these changed GRs in the abolishment of feedback ability in both regions (Mizoguchi et al., 2003a).

Several traditional herbal medicines (called *Kampo* drugs in Japan, herbal remedies composed of specified mixtures of dried plant materials) are effective in the field of neuropsychiatry (Kanba et al., 1998). In particular, *saikokaryukotsuboreito* (SRBT, *chai-hu-jia-long-gu-mu-li-tang* in Chinese), one of the *Kampo* drugs, has been widely used in a variety of clinical situations for the treatment of stress-related neuropsychiatric disorders, such as depression (Sarai, 1992), neurosis (Kanba et al., 1998), anxiety (Sarai, 1992), and insomnia (Kikutani, 1984), with very low side effects. Although scientific evidence of the clinical effects of this drug is scarce, several basic researches have demonstrated that SRBT attenuates acute stress-induced elevation of serum corticosterone (CORT) levels in mice (Sasaki et al., 1995) and that this drug increases behavioral activity in a despair test (Koshikawa et al., 1998). This drug also suppresses an excessive increase in the glutamate release induced by high concentration of K^+ in the hippocampus of normal and zinc-deficient rats, a neurological disease model (Tamano and Takeda, 2004). Moreover, SRBT prevents both disruption of the HPA axis (Mizoguchi et al., 2002) and behaviorally depressive state (Mizoguchi et al., 2003b) in chronically stressed rats. These findings have suggested that SRBT has an anti-stress or anti-depressive action and that it may thereby act as a modulator of the glucocorticoid secretion system.

Thus, because SRBT might have a psychotropic action through the glucocorticoid secretion system, we considered a possibility that this drug modifies the GR levels in the brain regions that contribute to the feedback system and regulates feedback ability of these regions. The present study was designed to clarify this possibility. For this purpose, we examined the effects of SRBT on abnormal changes in the GR levels in the PFC and hippocampus using a Western immunoblot technique and on abolishment of feedback ability mediated by DEX locally placed in the PFC and hippocampus in chronically stressed rats.

2. Materials and methods

2.1. Drugs

SRBT 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. The powdered drug was suspended in distilled water. SRBT (100, 300, or 1000 mg/10 ml/kg, p.o.) was administered daily during both a 4-week stress session and a 10-day recovery period, described below.

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.

Table 1
Crude drug composition of *saikokaryukotsuboreito* (SRBT)

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

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 fed laboratory food and water ad libitum. The procedure of stress exposure was described previously (Mizoguchi et al., 2000, 2001, 2003a). 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 maintained at 21 °C for 2 h. The animals were subjected to this stress session once a day for 4 weeks (chronic stress). To avoid the acute influence of the last stress session and to evaluate the influence of chronic stress as a consequence of the cumulative stress effects, animals were allowed a 10-day recovery period. In our preliminary experiments, gastric ulcer was not produced by single or chronic exposure. Although relatively severe, this stress is not intense enough to produce gastric ulcer.

2.3. Immunoprecipitation and Western immunoblot analysis

The amount of GR proteins was measured according to the method established previously by us (Mizoguchi et al., 2003a). Briefly, the rats were sacrificed by decapitation after the 10-day recovery period following the 4-week stress session. After decapitation, the PFC and hippocampus were quickly dissected on an ice plate, immediately frozen on dry ice, and stored at −80 °C. On the day of the experiment, the sample for immunoprecipitation was prepared according to the method of Beck et al. (1993) with minor modifications. Each tissue was homogenized in 1 ml of hypotonic buffer [10 mM HEPES (pH 7.4) containing 1 mM EDTA–2Na, 10 mM molybdate, 0.1 mM *p*-amidinophenylmethanesulfonyl fluoride (Wako Pure Chemicals, Osaka, Japan), 0.1 mg/ml leupeptin (Sigma, St. Louis, MO, USA), and 0.1 mg/ml antipain (Sigma)] in a Teflon-glass homogenizer. The homogenates were ultracentrifuged at 100,000 ×*g* for 1 h to yield a cytosolic fraction (the supernatant) and a fraction containing nuclei (the pellet). The pellets were extracted with hypotonic buffer containing 0.5 M NaCl for 1 h on ice, and then ultracentrifuged at 100,000 ×*g* for 30 min to yield soluble nuclear extracts. The extracts were desalted, and an aliquot of each cytosolic or nuclear fraction was used for the determination of protein concentrations according to Lowry's methods (Lowry et al., 1951).

For immunoprecipitation, a monoclonal mouse anti-GR antibody (3 μg, BuGR2; Affinity BioReagents, Golden, CO, USA) was added to each sample (500 μl) and incubated for 1 h. Protein G-Sepharose (20 μl per sample, Amersham Biosciences) was then added and mixed by rotation for 1 h. The protein-bound protein G-Sepharose was collected by centrifugation and washed. The immunoadsorbed GR complexes were solubilized and denatured. The samples were clarified by centrifugation for 5 min and subjected to gel electrophoresis.

For immunoblot analysis, the proteins were separated by electrophoresis on 12.5% SDS-polyacrylamide gels (ExcelGel; Amersham Biosciences) and electroblotted onto nitrocellulose filters. The anti-GR antibody diluted to 1 μg/ml in phosphate-buffered saline (PBS) containing 1% (w/v) bovine serum albumin and 0.1% (v/v) Triton-X100 (incubation buffer) was applied overnight at 4 °C. The filters were then incubated for 2 h with peroxidase-linked sheep anti-mouse IgG (Amersham Biosciences) diluted to 1:5000 in the incubation buffer. The peroxidase was visualized on X-ray film using an Enhanced Chemiluminescence system (Amersham Biosciences).

Semiquantitative densitometric analyses of GR signals were performed on X-ray film using Macintosh-driven image-processing and analysis software (BioMax 1D; Eastman Kodak, Rochester, NY, USA). The accuracy of our immunoprecipitation–Western immunoblot analysis system for GR was confirmed by our previous study (Mizoguchi et al., 2003a). Data are expressed as relative change (%) for the GR levels in each cytosolic fraction of the control (naive non-stressed) rats.

2.4. DEX infusion into brain

The infusion experiments were performed according to the method described previously (Mizoguchi et al., 2000, 2003a). Briefly, after a 2-day recovery period following the 4-week stress session, the animals were stereotaxically and bilaterally 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, i.p.). The brain atlas of Paxinos and Watson (1982) was used to determine the coordinates. The following coordinates relative to the bregma were used for the cannula implantation (in mm): the PFC, anteroposterior,+3.2; lateral, ±1.0; vertical, −2.5; the hippocampus, anteroposterior, −4.2; lateral, ±2.4; vertical, −2.6. In our previous study (Mizoguchi et al., 2003a), we verified histologically the cannula position and confirmed the correct position. The animals were initially treated with Xylocaine (Astellas Pharma, Tokyo, Japan) to minimize pain and were monitored on a daily basis for signs of distress or infection. Thus, the animals were free of the water immersion and restraint stress session during the 10-day recovery period, but they were subjected to surgical stress during this period.

Animals were adapted to a mock infusion protocol to minimize any stress associated with the procedure before the start of infusion experiments. After an 8-day recovery period from the surgery (i.e., 10 days after the 4-week stress session), the animals were restrained gently while the stylets were removed and replaced with an infusion cannula (PC-12; Bioanalytical Systems) that extended 1 mm below the guide cannula. The animals received bilateral infusions of DEX (Sigma) at a concentration of either 0 (vehicle) or 10 ng in 1 μl sterile saline containing 0.2% (v/v) ethanol at a rate of 0.2 μl/min using a microinfusion pump. This dose of DEX was optimized by our preliminary study (data not shown). The infusion was performed between 10:00 A.M. and 11:30 A.M. The cannula remained in place for 2 min after the completion of the infusion. Stylets were inserted back into the guide cannula, and the animals were returned to the home cage. Four hours after the infusion, the animals were sacrificed by decapitation, the trunk blood was collected, and the plasma was separated and stored at −20 °C.

2.5. CORT radioimmunoassay

The [^{125}I]-labeled CORT (46.3 kBq) double antibody radioimmunoassay kit for rat (Amersham Biosciences) was used to measure the plasma CORT concentration. To displace CORT from corticosteroid-binding globulin, the plasma was heated for 30 min at 60 °C. The assay was performed in duplicate at room temperature, using rabbit anti-CORT serum as the first antibody and donkey anti-rabbit serum coated on magnetizable polymer particles as the second antibody. According to the manufacturer, the cross-reactivity is low. The highest cross-reactivity is found with 11-deoxycorticosterone (2.4% in contrast to 100% for CORT). The possible range of the assay is between 0.78 and 200 ng/ml.

2.6. Statistics

All data were initially analyzed using one-way analysis of variance (ANOVA). Individual between-group comparisons of changes in the GR levels were made using Fisher's Protected Least Significant Difference test, and comparisons of changes in the plasma CORT levels following intracranial infusions of DEX were analyzed using the unpaired *t* test.

3. Results

3.1. Detection of GR protein

As shown in Fig. 1, two immune-specific bands (molecular weight: 54 kDa and 97 kDa, respectively) were detected in the cytosolic preparations derived from the hippocampus (lane 1) and PFC (lane 2). Considering general information and our previous study (Mizoguchi et al., 2003a), we determined that the 54 kDa band is IgG and the 97 kDa band is GR.

3.2. Effect of SRBT on GR levels

The effects of chronic stress and SRBT treatment on the GR levels in the cytosolic and nuclear fractions of the PFC and hippocampus were examined by using the immunoprecipitation–Western immunoblot technique (Fig. 2). In the PFC (Fig. 2A and C), cytosolic GRs were significantly decreased by chronic stress [$F(4,35)=2.363$, $p<0.05$] compared with the

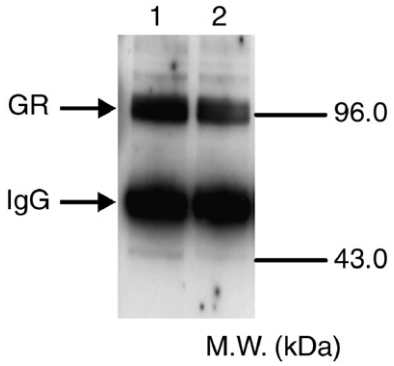

Fig. 1. Western immunoblot analysis of immunoadsorbed GR. The blot shows detection of GR proteins from the cytosolic fraction prepared from the hippocampus (lane 1) and PFC (lane 2).

naive non-stressed rats (Fig. 2A). Although this decrease was not prevented by 100 mg/kg of SRBT, it was significantly prevented by 300 and 1000 mg/kg of the drug [$F(4,35)=2.363$; 300 mg/kg, $p<0.05$; 1000 mg/kg, $p<0.01$], and there was no significant difference between naive non-stressed and SRBT-treated (300 and 1000 mg/kg) stressed rats. The nuclear GR levels were not significantly changed by chronic stress and SRBT treatment (Fig. 2C). In the hippocampus (Fig. 2B and D), cytosolic GRs tended to increase following chronic stress compared with the naive non-stressed rats [$F(4,35)=1.272$, $p=0.06$] (Fig. 2B). This increase was not affected by 100–1000 mg/kg SRBT. The nuclear GR levels were significantly increased by chronic stress compared with the naive non-stressed rats [$F(4,35)=5.532$, $p<0.01$] (Fig. 2D). Although this increase was not prevented by 100 and 300 mg/kg of SRBT, it was 23significantly prevented by 1000 mg/kg of the drug [$F(4,35)=5.532$, $p<0.001$], and there was no a significant difference between naive non-stressed and SRBT-treated (1000 mg/kg) stressed rats.

3.3. Effect of SRBT on feedback ability

The effects of chronic stress and SRBT treatment on the feedback ability mediated by bilateral infusions of DEX into the PFC or hippocampus are shown in Fig. 3. In the PFC (Fig. 3A), the plasma CORT levels of the naive non-stressed rats were significantly suppressed by the DEX infusion compared with the vehicle infusion [$F(1,14)=17.697$, $p<0.001$]. However, the CORT levels in the chronically stressed rats were not suppressed by DEX [$F(1,14)=0.381$, $p=0.547$], and the levels were significantly higher than those of the DEX-infused naive non-stressed rats [$F(2,21)=22.487$, $p<0.001$]. The CORT levels in the chronically stressed and SRBT-treated rats were significantly suppressed by the DEX infusion compared with the vehicle infusion [$F(1,14)=18.350$, $p<0.001$], and the levels were significantly lower than those of the DEX-infused and chronically stressed rats [$F(2,21)=22.487$, $p<0.001$]. Similarly, in the hippocampus (Fig. 3B), the plasma CORT levels of the naive non-stressed rats were significantly suppressed by the DEX infusion [$F(1,14)=22.091$, $p<0.001$]. However, the CORT levels in the chronically stressed rats were not suppressed by DEX [$F(1,14)=1.410$, $p=0.255$], and the levels

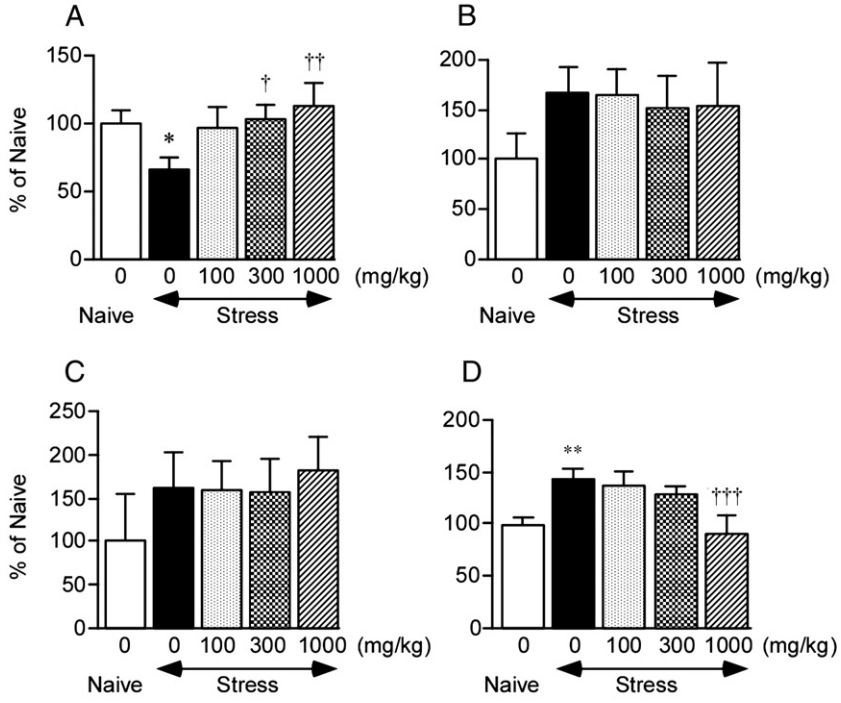

Fig. 2. Effect of *saikokaryukotsuboreito* (SRBT) on chronic stress-induced increases or decreases in GR proteins in the cytosolic (A and B) or nuclear (C and D) fraction of the PFC (A and C) or hippocampus (B and D). GR proteins were measured by using an immunoprecipitation–Western immunoblot technique (see Materials and methods section). The cytosolic GR levels of naive non-stressed rats were used as control (100%). Each column is the mean±S.E.M. of 5 rats per group. Asterisks indicate a significant difference from naive non-stressed rats: *, $p<0.05$; **, $p<0.01$; daggers, a significant difference from stressed rats: †, $p<0.05$, ††, $p<0.01$, †††, $p<0.001$.

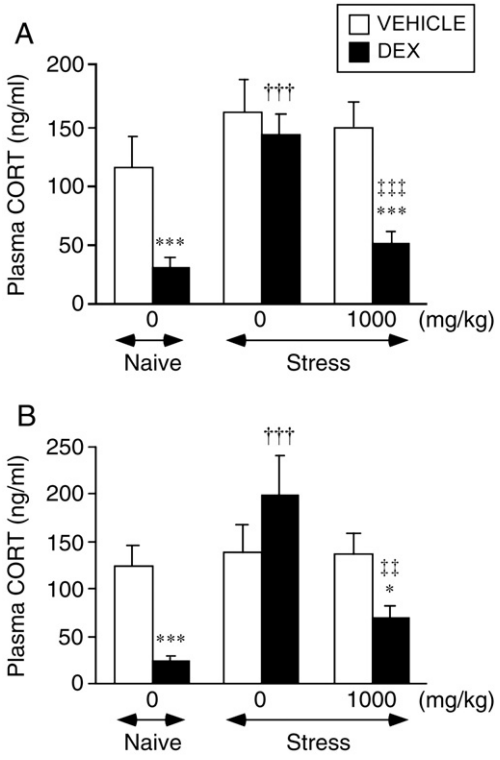

Fig. 3. Effect of *saikokaryukotsuboreito* (SRBT) on chronic stress-induced abolishment of feedback ability in the PFC (A) or hippocampus (B). DEX (10 ng) was infused after a 10-day recovery period following a 4-week stress session. Four hours after the DEX infusions, the concentrations of plasma CORT were measured (see Materials and methods section). Each column is the mean±S.E.M. of 8 rats per group. Asterisks indicate a significant difference from vehicle-infused rats in each group: *, $p<0.05$; ***, $p<0.001$; daggers, a significant difference from naive non-stressed and DEX-infused rats: ††††, $p<0.001$; double daggers, a significant difference from stressed and DEX-infused rats: ‡‡, $p<0.01$, ‡‡‡, $p<0.001$.

were significantly higher than those of the DEX-infused naive non-stressed rats [$F(2,21)=12.989$, $p<0.001$]. The CORT levels in the chronically stressed and SRBT-treated rats were significantly suppressed by the DEX infusion compared with the vehicle infusion [$F(1,14)=6.555$, $p<0.05$], and the levels were significantly lower than those of the DEX-infused and chronically stressed rats [$F(2,21)=12.989$, $p<0.01$].

4. Discussion

The present results suggest that SRBT can prevent chronic stress-induced abolishment of glucocorticoid negative feedback ability in the PFC and hippocampus through the improvement of abnormal changes in the GR levels. This mechanism is thought to underlie the improving effects of this drug on chronic stress-induced disruption of the HPA axis (Mizoguchi et al., 2003b), which is thought to be related to its anti-depressive action.

As shown in Fig. 2, chronic stress significantly decreased cytosolic GRs in the PFC and significantly increased nuclear GRs in the hippocampus, which are in agreement with our previous report (Mizoguchi et al., 2003a). Although cytosolic GRs in the hippocampus significantly increased in the stressed rats in our previous study (Mizoguchi et al., 2003a), they tend to increase in the present study ($p=0.06$).

In the drug treatment study, SRBT prevented these abnormal changes in GRs, except the increase in cytosolic GRs in the hippocampus. In particular, the preventive effect of SRBT on the decrease in GRs in the PFC was a dose-dependent manner, suggesting that this effect is based on a pharmacological property. Regarding the underlying mechanisms, regulation of glucocorticoid secretion by SRBT may have a role. Although several factors can regulate the expression levels of GRs, the ligand, glucocorticoid, negatively regulates the GR expression at the transcription level (Rosewicz et al., 1988). In particular, GRs in the hippocampus as well as in the PFC are sensitive to changes in endogenous glucocorticoid secretion (Lowy, 1991; O'Donnell et al., 1995). We previously reported that a single exposure to water immersion and restraint produced a marked elevation of CORT secretion, and this response was maintained during the 4-week stress session (Mizoguchi et al., 2001). Indeed, cytosolic GRs in the hippocampus were down-regulated in the stress duration-dependent manner and up-regulated conversely after the recovery period (Mizoguchi et al., 2003a). However, in the PFC, cytosolic GRs were initially down-regulated at the 4-week stress period, and this down-regulation was maintained until the end of the recovery period. Because sensitivity of cytosolic GRs in the PFC to excessive glucocorticoids during a chronic stress situation may be lower than that in the hippocampus, the ligand-dependent down-regulation may occur later (Mizoguchi et al., 2003a). Thus, the elevation of plasma CORT levels in response to stress may be involved in the chronic stress-induced abnormal changes in GRs. Considering that SRBT suppresses elevated plasma CORT levels in response to acute stress (Sasaki et al., 1995), the preventive effects of SRBT on GRs in the present study may be due to its suppressive action for elevated CORT. Alternatively, SRBT may attenuate excitation of the brain induced by stress. For example, this drug reduces hyperactivity of locomotor and prolongs the sleep induced by pentobarbital sodium in El mice whose brains excite easily in response to stimuli, suggesting the involvement of gamma-aminobutylic acid (Iizuka et al., 1998). Moreover, this drug decreases the elevation of the concentrations of dopamine and its metabolite dihydroxyphenylacetic acid in the brain in response to stress (Sasaki et al., 1998). Thus, SRBT might prevent the abnormal GR changes as a result of attenuation of the brain excitation induced by stress. The mechanisms underlying the effects of this drug on GRs should be clarified by the detailed studies.

The accumulation of nuclear GRs in the hippocampus may be due to the results of the changes in cytosolic GRs. Steroid receptors are shuttling proteins that traffic continuously between cytoplasm and nucleus when liganded (Guiochon-Mantel et al., 1991; Dauvois et al., 1993; Madan and DeFranco, 1993; Galigniana et al., 1999). Hormone binding is transient, and the loss of hormone from the receptor leads to a recycling of the receptor (Scherrer et al., 1990). Although there is no evidence indicating that SRBT modifies the GR traffic system, it may be possible that SRBT influences the chronic stress-induced abnormality of nucleocytoplasmic shuttling of GRs.

GRs are a candidate molecule that contributes to the feedback system. Therefore, to explore a functional significance for the preventive effects of SRBT on the abnormal changes in GRs (Fig. 2), we next examined the effects of this drug on the chronic stress-induced dysfunction of the feedback system in the PFC and hippocampus. As shown in Fig. 3, when DEX was infused into the PFC or hippocampus of the chronically stressed rats, no suppressive response to DEX was observed, which is in agreement with our previous report (Mizoguchi et al., 2003a). In the drug treatment study, SRBT certainly prevented the abolishment in both regions, suggesting that the preventive effects of SRBT on the chronic stress-induced abnormal changes in GRs in the PFC and hippocampus (Fig. 2) contribute to the beneficial effects of this drug on the feedback system in these regions.

The fact that the maximum dose, i.e. 1000 mg/kg, of SRBT was effective for the chronic stress-induced GR changes and dysfunction of the feedback system in the PFC and hippocampus in the present study is consistent with our previous findings that the same dose of SRBT prevented the chronic stress-induced disruption of the HPA system (Mizoguchi et al., 2002) as well as decreased the extracellular concentrations of dopamine and serotonin in the PFC and behaviorally depressive state (Mizoguchi et al., 2003b). This consistency suggests that the GR regulating actions of SRBT are related to its anti-depressive actions. This hypothesis is supported by a finding that glucocorticoid-related drugs (e.g., mifepristone) are sometimes used successfully as anti-depressants (Belanoff et al., 2001).

Although the active ingredients in SRBT for chronic stress-induced abnormality of the neuroendocrine system have not yet been identified, the major components of the crude drugs comprising this drug have been isolated and are shown in Table 1. Among them, several active ingredients that influence the glucocorticoid secretion system in particular have been isolated. For example, oral administration of saikosaponins increases plasma adrenocorticotropic hormone (ACTH) and CORT levels in rats (Hiai et al., 1981; Yokoyama et al., 1981), and this CORT elevation is clearly blocked by DEX (Hiai et al., 1981). Also, ginseng saponins similarly increase adrenal cyclic adenosine 3′, 5′-monophosphate (cAMP), a second messenger of ACTH, and thereby stimulate synthesis and secretion of corticosteroids in normal rats, but not in hypophysectomized rats (Hiai et al., 1979). These findings have suggested that some kinds of saponins contained in SRBT elevate plasma CORT levels due to activation of the HPA system. Alternatively, ginseng saponins act as a functional ligand for GRs (Lee et al., 1997). Although long-term exposure to excessive glucocorticoids, e.g., during chronic stress, causes several neuronal degenerations such as neuronal atrophy in the PFC (Cerqueira et al., 2005) and hippocampus (Mizoguchi et al., 1992; Watanabe et al., 1992; Sousa and Almeida, 2002; McEwen, 2005), optimal stimulation of GRs is also essential for maintaining the neuronal functions in both regions (Sloviter et al., 1989; Mizoguchi et al., 2004). In addition, glucocorticoids are the most important hormones for overcoming acute physical stress. Therefore, some ingredients having an activation action for the HPA system may facilitate the resistance, coping, and adaptation responses of organisms to stressors through modulation of the glucocorticoid secretion system, which may be involved in an anti-stress action of SRBT. The active ingredients of this drug should be identified by the detailed studies.

In conclusion, our present results revealed that SRBT could prevent chronic stress-induced dysfunction of glucocorticoid negative feedback system because of modification of GR levels in the PFC and hippocampus. This mechanism may underlie an anti-depressive action of SRBT. This finding provides information important for the prevention and treatment of depression.

Acknowledgements

This work was supported by grants from the Ministry of Education, Culture, Sports, Science and Technology of Japan (18590663) and by the Research Grant for Longevity Sciences (18C-8) from the Ministry of Health, Labor and Welfare of Japan. We thank deeply Ms. Yayoi Tanaka for her technical assistance.

References

Arana GW, Baldessarini RJ, Ornsteen M. The dexamethasone suppression test for diagnosis and prognosis in psychiatry. Arch Gen Psychiatry 1985;42:1193–204.

Beck CA, Estes PA, Bona BJ, Muro-Cacho CA, Nordeen SK, Edwards DP. The steroid antagonist RU486 exerts different effects on the glucocorticoid and progesterone receptors. Endocrinology 1993;133:728–40.

Belanoff JK, Flores BH, Kalezhan M, Sund B, Schatzberg AF. Rapid reversal of psychotic depression using mifepristone. J Clin Psychopharmacol 2001;21:516–21.

Carroll BJ, Feinberg M, Greden JF, Tarika J, Albala AA, Haskett RF, et al. A specific laboratory test for the diagnosis of melancholia: standardization, validation and clinical utility. Arch Gen Psychiatry 1981;38:15–22.

Cerqueira JJ, Pego JM, Taipa R, Bessa JM, Almeida OF, Sousa N. Morphological correlates of corticosteroid-induced changes in prefrontal cortex-dependent behaviors. J Neurosci 2005;25:7792–800.

Dauvois S, White R, Parker MG. The antiestrogen ICI 182780 disrupts estrogen receptor nucleocytoplasmic shuttling. J Cell Sci 1993;106:1377–88.

Diorio D, Viau V, Meaney MJ. The role of the medial prefrontal cortex (cingulate gyrus) in the regulation of hypothalamic–pituitary–adrenal responses to stress. J Neurosci 1993;13:3839–47.

Feldman S, Conforti N. Modifications of adrenocortical responses following frontal cortex simulation in rats with hypothalamic deafferentations and medial forebrain bundle lesions. Neuroscience 1985;15:1045–7.

Feldman S, Weidenfeld J. Glucocorticoid receptor antagonists in the hippocampus modify the negative feedback following neural stimuli. Brain Res 1999;821:33–7.

Galigniana MD, Housley PR, DeFranco DB, Pratt WB. Inhibition of glucocorticoid receptor nucleocytoplasmic shuttling by okadaic acid requires intact cytoskeleton. J Biol Chem 1999;274:16222–7.

Guiochon-Mantel A, Lescop P, Christin-Maitre S, Loosfelt H, Perrot-Applanat M, Malgrom E. Nucleocytoplasmic shuttling of the progesterone receptor. EMBO J 1991;10:3851–9.

Hiai S, Sasaki S, Oura H. Effect of ginseng saponin on rat adrenal cyclic AMP. Planta Med 1979;37:15–9.

Hiai S, Yokoyama H, Nagasawa T, Oura H. Stimulation of the pituitary–adrenocortical axis by saikosaponin of *Bupleuri radix*. Chem Pharm Bull 1981;29:495–9.

Holsboer F. The dexamethasone suppression test in depressed patients: clinical and biochemical aspects. J Steroid Biochem 1983;19:251–7.

Iizuka S, Ishige A, Komatsu Y, Matsumiya T, Tsuji M, Takeda H. Effects of *saiko-ka-ryukotsu-borei-to* on irritable characteristics in El mice. Methods Find Exp Clin Pharmacol 1998;20:19–26.

Kalin NH, Weiler SJ, Shelton SE. Plasma ACTH and cortisol concentration before and after dexamethasone. Psychiatry Res 1982;7:87–92.

Kanba S, Yamada K, Mizushima H, Asai M. Use of herbal medicine for treating psychiatric disorders in Japan. Psychiatry Clin Neurosci 1998;52(Suppl): S331–3.

Kikutani T. Treatment of insomnia by ethical kampo drugs, applied to health insurance system. J Tradit Sino–Japanese Med 1984;5:23–7.

Koshikawa N, Imai T, Takahashi I, Yamauchi M, Sawada S, Kansaku A. Effects of *hochu-ekki-to*, *yoku-kan-san* and *saiko-ka-ryukotsu-borei-to* on behavioral despair and acetic acid-induced writhing in mice. Methods Find Exp Clin Pharmacol 1998;20:47–51.

Kovács KJ, Makara GB. Corticosterone and dexamethasone act at different brain sites to inhibit adrenalectomy-induced adrenocorticotropin hypersecretion. Brain Res 1988;474:205–10.

Lee YJ, Chung E, Lee KY, Lee YH, Huh B, Lee SK. Ginsenoside-Rg1, one of the major active molecules from *Panax ginseng*, is a functional ligand of glucocorticoid receptor. Mol Cell Endocrinol 1997;133:135–40.

Lowry OH, Rosebrough NJ, Farr AL, Randall RJ. Protein measurement with the foline phenol reagent. J Biol Chem 1951;193:265–75.

Lowy MT. Corticosterone regulation of brain and lymphoid corticosteroid receptors. J Steroid Biochem Mol Biol 1991;39:147–54.

Madan AP, DeFranco DB. Bidirectional transport of glucocorticoid receptors across the nuclear envelope. Proc Natl Acad Sci U S A 1993;90:3588–92.

Magarinos AM, Somoza G, De Nicola AF. Glucocorticoid negative feedback and glucocorticoid receptors after hippocampectomy in rats. Horm Metab Res 1987;19:105–9.

Mazure CM. Does stress cause psychiatric illness? In: Spiegel D, editor. Progress in psychiatry, vol. 46. Washington DC: American Psychiatric Press; 1995. p. 270–98.

McEwen BS. Glucocorticoids, depression, and mood disorders: structural remodeling in the brain. Metabolism 2005;54(5 Suppl 1):20–3.

Miller AH, Spencer RL, Pulera M, Kang S, McEwen BS, Stein M. Adrenal steroid receptor activation in rat brain and pituitary following dexamethasone: implications for the dexamethasone suppression test. Biol Psychiatry 1992;32:850–69.

Mizoguchi K, Kunishita T, Chui DH, Tabira T. Stress induces neuronal death in the hippocampus of castrated rats. Neurosci Lett 1992;138:157–60.

Mizoguchi K, Yuzurihara M, Ishige A, Sasaki H, Chui DH, Tabira T. Chronic stress induces impairment of spatial working memory because of prefrontal dopaminergic dysfunction. J Neurosci 2000;20:1568–74.

Mizoguchi K, Yuzurihara M, Ishige A, Sasaki H, Chui DH, Tabira T. Chronic stress differentially regulates glucocorticoid negative feedback response in rats. Psychoneuroendocrinology 2001;26:443–59.

Mizoguchi K, Yuzurihara M, Ishige A, Sasaki H, Tabira T. *Saiko-ka-ryukotsu-borei-to*, an herbal medicine, prevents chronic stress-induced disruption of glucocorticoid negative feedback in rats. Life Sci 2002;72:67–77.

Mizoguchi K, Ishige A, Aburada M, Tabira T. Chronic stress attenuates glucocorticoid negative feedback: involvement of the prefrontal cortex and hippocampus. Neuroscience 2003a;119:887–97.

Mizoguchi K, Yuzurihara M, Ishige A, Aburada M, Tabira T. *Saiko-ka-ryukotsu-borei-to*, a herbal medicine, ameliorates chronic stress-induced depressive state in rotarod performance. Pharmacol Biochem Behav 2003b;75:419–25.

Mizoguchi K, Ishige A, Takeda S, Aburada M, Tabira T. Endogenous glucocorticoids are essential for maintaining prefrontal cortical cognitive function. J Neurosci 2004;24:5492–9.

O'Donnell D, Francis D, Weaver S, Meaney MJ. Effects of adrenalectomy and corticosterone replacement on glucocorticoid receptor levels in rat brain tissue: a comparison between western blotting and receptor binding assays. Brain Res 1995;687:133–42.

Paxinos G, Watson C. The rat brain in stereotaxic coordinates. New York: Academic Press; 1982.

Rosewicz S, McDonald AR, Maddux BA, Goldfine ID, Miesfeld RL, Logsdon CD. Mechanism of glucocorticoid receptor down-regulation by glucocorticoids. J Biol Chem 1988;263:2581–4.

Sarai K. Oriental medicine as therapy for resistant depression: use of some herbal drugs in the Far East (Japan). Prog Neuropsychopharmacol Biol Psychiatry 1992;16:171–80.

Sasaki K, Suzuki K, Yoshizaki F, Ando T. Effect of *saiko-ka-ryukotsu-borei-to* on the stress-induced increase of serum corticosterone in mice. Biol Pharm Bull 1995;18:563–5.

Sasaki K, Suzuki K, Ueno M, Takako K, Yoshizaki F. Increase in monoamine levels caused by emotional stress in mice brain regions is attenuated by *saiko-ka-ryukotsu-borei-to*. Methods Find Exp Clin Pharmacol 1998;20: 27–30.

Scherrer LC, Dalman FC, Massa E, Meshinchi S, Pratt WB. Structural and functional reconstitution of the glucocorticoid receptor-hsp90 complex. J Biol Chem 1990;265:21397–400.

Sloviter RS, Valiquette G, Abrams GM, Ronk EC, Sollas AL, Paul LA, et al. Selective loss of hippocampal granule cells in the mature rat brain after adrenalectomy. Science 1989;243:535–8.

Sousa N, Almeida OF. Corticosteroids: sculptors of the hippocampal formation. Rev Neurosci 2002;13:59–84.

Tamano H, Takeda A. Suppressive effect of saiko-ka-ryukotsu-borei-to, a herbal medicine, on excessive release of glutamate in the hippocampus. Brain Res Bull 2004;64:273–7.

Watanabe Y, Gould E, McEwen BS. Stress induces atrophy of apical dendrites of hippocampal CA3 pyramidal neurons. Brain Res 1992;588:341–5.

Yokoyama H, Hiai S, Oura H. Chemical structures and corticosterone secretion-inducing activities of saikosaponins. Chem Pharm Bull 1981;29:500–4.