Oral administration of *Cimicifuga racemosa* extract affects immobilization stress-induced changes in murine cerebral monoamine metabolism

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**ABSTRACT**

We investigated the effects of *Cimicifuga racemosa* (CR) plant extracts on the changes in levels of the cerebral monoamines norepinephrine (NE), dopamine (DA), and serotonin (5-HT), the respective metabolites 3-methoxy-4-hydroxyphenylglycol (MHPG), 3,4-dihydroxyphenylacetic acid (DOPAC), and 5-hydroxyindoleacetic acid (5-HIAA), and plasma corticosterone in mice subjected to acute immobilization stress. Single oral administration of the CR extract (1,000 mg/kg) significantly attenuated plasma corticosterone levels that had been increased as a result of enforced immobilization. Acute immobilization stress caused significant changes in the corresponding amine-to-metabolite ratios in the hypothalamus, hippocampus, and cortex; however, CR-extract treatment significantly attenuated the MHPG/NE change in the hypothalamus, and the 5-HIAA/5-HT changes in each region of the brain. Our results suggest that the CR extract interacts not only with the hypothalamic-pituitary-adrenal (HPA) axis but also with the sympathetic adrenomedullary (SAM) system under stress conditions. Thus the CR extract can alleviate acute stress responses by suppressing the changes of amine-to-metabolite ratio in brain.

*Cimicifuga racemosa* (CR) is a member of the Ranunculaceae family that is indigenous to eastern North America, with a range that extends as far as south Florida. Dried CR rhizomes are known as black cohosh and have been widely used as herbal dietary supplements for almost five decades. Historically, Native American women ingested CR water extract for pain relief during menstruation and childbirth. In recent years, ethanolic and isopropanolic extracts of CR have been used for the treatment of general menopause symptoms including hot flushes, profuse sweating, irritability, and anxiety, and its popularity has grown among women hoping to avoid the potential toxicity of classical hormone-replacement therapy (2).

We previously showed that CR extracts had anti-stress activities in mice in which plasma corticosterone and aspartate aminotransferase (AST) levels were increased as a result of enforced immobilization: a single oral administration of CR extract (1,000 mg/kg) significantly attenuated these increases (23). Previous studies have reported that acute immobilization stress not only elicits activation of the hypothalamic-pituitary-adrenal (HPA) axis but also affects the metabolism of monoamines such as norepinephrine (NE), dopamine (DA), and serotonin (5-HT) in the brain. And these amine-to-metabolite ratios are increased by acute immobilization stress. Furthermore, the relationships among NE, DA, and 5-HT play an important role in regulation of the sympathetic adrenomedullary (SAM) system under stress conditions (5, 20, 31).

Several studies have reported that the neuronal activities of serotonergic and dopaminergic pathways innervating the striatum, hippocampus, hypo-
thalamus, cerebral cortex, and amygdala are altered by exposure to acute stress (10, 17, 19). Other studies have found that the CR extract interacts with neurotransmitters such as 5-HT (3, 25) and DA (2, 21); it is thus possible that these neurotransmitter systems are responsible for the CR extract-induced reduction of acute stress responses. The present study investigated the effects of CR extract on changes in cerebral monoamine metabolism induced by acute immobilization stress in mice.

Pathogen-free 7-week-old male BALB/c mice (SLC, Shizuoka, Japan) were used for immobilization-stress experiments. All animals were housed under the following controlled conditions: temperature, 22 ± 1°C; relative humidity, 50 ± 10%; and light/dark cycle, 0700–1900 h/1900–0700 h. The animals were fed a commercial diet (CRF-1; Oriental Yeast, Tokyo, Japan) and were given water ad libitum. They were handled and sacrificed in accordance with the procedures outlined in the Guidelines for Animal Experimentation of Asahi Group Holdings Ltd., compiled from the Guidelines for Animal Experimentation of the Japanese Association for Laboratory Animal Science.

We used dried powder of an ethanolic extract of CR rhizomes (CR extract powder) purchased from Nippon Funmatsu Yakuhin (Osaka, Japan). The mice were divided into three groups, each containing five animals; the normal and control groups were orally administered distilled water, and the CR extract group was orally administered 1,000 mg/kg CR extract as an aqueous suspension. At 30 min after administration, the control and CR extract groups underwent enforced immobilization, being confined to an adjustable 30 mL plastic syringe for 60 min. Immediately after this step, the mice were decapitated and blood was collected to assay the levels of plasma corticosterone. The hypothalamus, hippocampus, and cortex regions were separated on ice, and the weight of each was determined. The samples were then frozen.

The frozen tissues were fractured in 0.2 M perchloric acid containing 0.1 mM disodium ethylenediaminetetraacetic acid (EDTA) and isoproterenol as an internal standard. The homogenate was then centrifuged (20,000 × g for 15 min). The supernatant was adjusted to pH 3.0 with 1 M sodium acetate and then passed through a 0.2-μm regenerated cellulose filter. An aliquot of this filtrate was injected onto a C18 reversed-phase column (Eicom Corporation, Kyoto, Japan) in a high-performance liquid chromatography (HPLC) system (Waters, MA, USA) equipped with an electrochemical detector. The mobile phase used with this aliquot (0.1 M acetate-citrate buffer with 17% methanol) allowed for the separation of the three major monoamines NE, DA, 5HT, and their respective metabolites 3-methoxy-4-hydroxyphenylglycol (MHPG), 3,4-dihydroxyphenylacetic acid (DOPAC), and 5-hydroxyindoleacetic acid (5-HIAA) (28). Sodium octyl sulfate (190 mg/L) was added as an ion-pairing agent, and EDTA (5 mg/L) was added as an antioxidant. Each peak area was normalized to isoproterenol, and the tissue concentration of the three monoamines and their metabolites, and the amine-to-metabolite ratios were calculated. Plasma corticosterone was measured using a commercial enzyme-linked immunosorbent assay (ELISA) kit (IBL, Hamburg, Germany).

All results are expressed as the mean ± standard error of the mean (SEM). The data were analyzed by a one-way analysis of variance (ANOVA) followed by Tukey’s multiple comparison test. A difference of $P < 0.05$ was considered statistically significant. PASW Statistics 18 software (SPSS Inc. 2009) was used to analyze the data.

The stress response involves both the SAM system and the HPA axis. A single oral administration of CR extract (1,000 mg/kg) significantly attenuated plasma corticosterone levels that were increased as a result of enforced immobilization in mice (Fig. 1). This suggested that the CR extract interacted with the HPA axis, which was involved in the endocrine response to stress. To investigate whether the CR extract also interacted with the SAM system and autonomic responses to stress, we examined its effects on the changes in cerebral monoamine metabolism induced by acute immobilization stress in mice.
The concentrations of cerebral monoamines and metabolites are shown in Table 1. The concentration of the NE metabolite MHPG in the hypothalamus and hippocampus was significantly increased by acute immobilization stress, but CR-extract treatment significantly attenuated the changes in the hypothalamus. There were no changes in the concentration of NE in any region of the brain, and no changes to MHPG levels in the cortex. Although the concentrations of DOPAC in each region were significantly increased by stress, CR-extract treatment had no effect on DA or its metabolism. The concentration of 5-HIAA in each region of the brain was significantly increased by exposure to stress, CR-extract treatment had no effect on DA or its metabolism. The concentration of 5-HIAA in each region of the brain was significantly increased by exposure to stress, CR-extract treatment had no effect on DA or its metabolism.

Stress also caused significant changes in the corresponding amine-to-metabolite ratios in each region of the brain, with the exceptions of the 5-HIAA/5HT ratio in the hypothalamus and the MHPG/NE ratio in the hippocampus. Treatment with the CR extract significantly attenuated the changes in the hypothalamus and the 5-HIAA/5-HT change in each region (Fig. 2).

<table>
<thead>
<tr>
<th>Brain region</th>
<th>Groups</th>
<th>Monoamine and metabolite (ng/g)</th>
<th>NE</th>
<th>MHPG</th>
<th>DA</th>
<th>DOPAC</th>
<th>5-HT</th>
<th>5-HIAA</th>
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<tr>
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<tr>
<td>Hypothalamus</td>
<td>Normal</td>
<td>2373.7 ± 126.9</td>
<td>379.7 ± 23.4</td>
<td>671.5 ± 14.2</td>
<td>268.7 ± 6.6</td>
<td>1183.3 ± 52.0</td>
<td>1144.9 ± 29.6</td>
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</tr>
<tr>
<td></td>
<td>Control</td>
<td>2566.6 ± 150.8</td>
<td>703.3 ± 28.3</td>
<td>910.4 ± 111.4</td>
<td>454.7 ± 48.4°</td>
<td>1485.1 ± 52.5°</td>
<td>1540.4 ± 49.2°</td>
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<tr>
<td></td>
<td>CR extract</td>
<td>2548.7 ± 103.8</td>
<td>579.9 ± 18.9°</td>
<td>872.7 ± 44.4</td>
<td>413.2 ± 20.1°</td>
<td>1672.4 ± 49.8°</td>
<td>1303.7 ± 70.2°</td>
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<tr>
<td>Hippocampus</td>
<td>Normal</td>
<td>319.8 ± 10.5</td>
<td>203.2 ± 13.1</td>
<td>32.8 ± 4.3</td>
<td>10.9 ± 1.0</td>
<td>386.5 ± 7.1</td>
<td>350.2 ± 34.3</td>
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<td></td>
<td>Control</td>
<td>326.0 ± 13.4</td>
<td>242.4 ± 5.1°</td>
<td>46.9 ± 8.6</td>
<td>25.4 ± 5.7°</td>
<td>389.0 ± 14.2</td>
<td>454.1 ± 12.1°</td>
<td></td>
</tr>
<tr>
<td></td>
<td>CR extract</td>
<td>333.6 ± 21.2</td>
<td>225.7 ± 8.6</td>
<td>40.6 ± 2.5</td>
<td>21.9 ± 1.4</td>
<td>425.5 ± 16.6</td>
<td>378.3 ± 16.4</td>
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<tr>
<td>Cortex</td>
<td>Normal</td>
<td>371.0 ± 11.3</td>
<td>210.6 ± 7.7</td>
<td>700.1 ± 29.7</td>
<td>155.7 ± 7.5</td>
<td>384.1 ± 4.8</td>
<td>160.4 ± 5.7</td>
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<tr>
<td></td>
<td>Control</td>
<td>320.0 ± 29.8</td>
<td>225.3 ± 12.6</td>
<td>626.4 ± 59.8</td>
<td>191.5 ± 9.0°</td>
<td>332.7 ± 10.0°</td>
<td>210.5 ± 13.7°</td>
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<tr>
<td></td>
<td>CR extract</td>
<td>352.4 ± 11.6</td>
<td>214.1 ± 6.9</td>
<td>733.3 ± 46.5</td>
<td>184.9 ± 8.4</td>
<td>401.4 ± 10.2°</td>
<td>192.2 ± 6.6</td>
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</table>

Results represent the mean ± SEM of values in each group (n = 5). P-value for one-way ANOVA followed by Tukey’s test (°P < 0.05, °°P < 0.01 vs. normal mice, †P < 0.05, ††P < 0.01 vs. control mice).
Eaton et al. (13) previously reported that both DA receptor D1 and D2 agonists activate corticotrophin-releasing factor (CRF) neurons in the paraventricular nuclei (PVN) of the hypothalamus. Lohning et al. (21) demonstrated that CR-extract activity is mediated by central D2-receptors. In their study, the effects of orally administered CR extract (25–100 mg/kg) were shown to cause both a dose-dependent fall in body temperature and a significant prolongation of ketamine-induced sleeping time. These effects were inhibited by pretreatment with a D2-receptor antagonist. In our current study, we investigated an effect of the CR extract at a dose of 1,000 mg/kg on cerebral monoamine metabolism during stress because it had been observed that the CR extract (1,000 mg/kg) attenuated an increase of plasma corticosterone level during stress in our previous study (23). However, treatment with the CR extract did not affect changes in DA release or DA metabolism related to immobilization stress. Our results indicated that the alleviative effect of the CR extract on immobilization-stress-induced corticosterone production was not related to its dopaminergic activity.

The hippocampus has the highest density of corticosteroid receptors in the brain. It also receives major 5-HT innervations, expresses various receptor subtypes, and its serotonergic transmissions are regulated by glucocorticoids (16, 18, 24). Repeated stress has been shown to decrease 5-HT<sub>1A</sub> receptor binding in several brain regions (15), whereas glucocorticoid was reported to decrease 5-HT<sub>1A</sub> receptor mRNA expression in cultured hippocampal cells (24) and the rat hippocampus (4). Moreover, 5-HT was found to increase the number of glucocorticoid receptors and their binding in cultured hippocampal cells (22). This suggests that the increased serotonergic activity observed as an increased 5-HIAA/5-HT ratio in the present study might have been related to the mediation of a glucocorticoid feedback mechanism on the HPA axis.

The noradrenergic system, with cell bodies originating within the locus coeruleus (LC), is a principal stress-response system of the body, together with the CRF system (6). NE is a potent stimulator of CRF release, particularly in the PVN of the hypothalamus. The relationship between CRF and NE is complex; however, the two systems appear to be cross-linked to form a functional means of responding to stress based on homeostasis. When circulating levels of glucocorticoid are high, the negative-feedback pathway serves to decrease CRF as well as NE synthesis in the PVN. Elevated glucocorticoid levels also act to inhibit the effects of NE on CRF release from the PVN. Additionally, serotonergic projections from the dorsal raphe nucleus inhibit firing of the LC, whereas noradrenergic projections from the LC have an excitatory effect on cell bodies in the dorsal raphe (19). Thus the relationship between noradrenergic system, CRF system, and serotonergic system appears to regulate the response to stress.

Agonists of the 5-HT<sub>1A</sub> receptor and selective 5-HT reuptake inhibitors (SSRIs) are clinically useful in the treatment of various anxiety disorders (8, 9). 5-HT<sub>1A</sub> as well as other receptors such as 5-HT<sub>1D</sub> and 5-HT<sub>1D</sub>, bind with affinity to the CR extract, which also demonstrates receptor-mediated functional activity such as the observed induction of cyclic AMP in human embryonic kidney cells stably overexpressing the 5-HT<sub>7</sub> receptor (3). In our current study, CR-extract treatment attenuated the production of 5-HIAA and MHPG, and changes in the 5-HIAA/5-HT and MHPG/NE ratios, in comparison with control mice. We propose that the CR extract behaves as a 5-HT<sub>1A</sub> agonist that is susceptible to glucocorticoid feedback during exposure to stress conditions, thus attenuating increases in plasma corticosterone production.

It was previously reported that the CR extract behaves as a competitive ligand and partial agonist for opiate receptors (26, 27), similar to 5-HT receptors. A competitive binding assay using a Chinese hamster ovary (CHO) cell line stably transfected with human μ-opioid receptors reported a half-maximal inhibitory concentration (IC<sub>50</sub>) of 170 μg/mL (27), whereas another found that the CR extract and isolated cycloartane glycosides had γ-aminobutyric acid A receptor (GABA<sub>A</sub>) modulating effects (7), which might be relevant to our present results. Although little is known about the pharmacokinetics and metabolites of the CR extract, evidence was recently reported of the bioavailability of actein, which is a triterpene glycoside included in the CR extract, after its oral administration to rats (14). It remains to be clarified whether these compounds penetrate the blood brain barrier for efficient DA, 5-HT, opiod, and GABA<sub>A</sub> modulation in vivo.

In summary, our results suggest that the CR extract interacts not only with the HPA axis but also with the SAM system under stress conditions. Further studies concerning noradrenergic, dopaminergic, and serotonergic systems in the brain are necessary to understand its possible effects on acute immobilization stress.
Acknowledgments
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REFERENCES