Regular Article

Yokukansan, a Herbal Medicine in Japan, Buffers Social Crowding Stress via Ameliorating Glucocorticoid Secretion Response to Vasopressin

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On the basis of the data that yokukansan (YKS), a herbal medicine, ameliorates aggressive behavior and abnormal glucocorticoid secretion of socially isolated mice under zinc deficiency, we tested whether YKS preventively buffers crowding stress-induced attenuations of glucocorticoid secretion response and long-term potentiation (LTP), an index of cognition. YKS-containing water was administered during the period of exposure to social crowding stress for 3 weeks. Serum corticosterone level was not significantly modified by administration of YKS-containing water and was also not increased after social-crowding stress. When vasopressin was injected into crowding-stressed rats to assess corticosterone secretion via pituitary–adrenocortical axis activation, vasopressin-induced increase in serum corticosterone was significantly attenuated compared to non-stressed control rats, indicating that the pituitary–adrenocortical response to vasopressin is affected after exposure to crowding stress. Interestingly, administration of YKS-containing water rescued attenuation of vasopressin-induced increase in serum corticosterone. LTP at Schaffer collateral-CA1 pyramidal cells synapses was attenuated in the hippocampal slices from crowding-stressed rats, while administration of YKS-containing water rescued the attenuation. The present study demonstrates that intake of YKS rescues crowding stress-induced impairments of glucocorticoid secretion response to vasopressin and hippocampal LTP. The intake of YKS may be benefit to buffering chronic stress.

Key words  Yokukansan; social-crowding stress; hippocampus; pituitary–adrenocortical response; vasopressin; herbal medicine

Yokukansan (YKS), a herbal medicine in Japan, is effective for medical treatment for behavioral and psychological symptoms of dementia (BPSD) such as agitation/aggression, irritability, hallucinations, depression and anxiety, which may be aggravated by environmental stress. The clinical effectiveness of YKS on BPSD was reported by Iwasaki et al. After that, other clinical studies have demonstrated additional effectiveness of YKS on Alzheimer’s-type and Parkinsonia dementia in regard with behavioral and psychological symptoms. Zinc deficiency is closely related with behavioral and psychological symptoms. Isolation-induced aggressive behavior under zinc deficiency is ameliorated by intake of YKS. Administration of YKS-containing water ameliorates the increase in the basal serum corticosterone concentration of zinc-deficient mice, while serum corticosterone is almost the same concentration between the control and zinc-deficient mice after the resident–intruder (aggressive behavior) test, suggesting that zinc deficiency affects the response of corticosterone secretion induced by the test. On the other hand, administration of YKS significantly increases serum corticosterone concentration in zinc-deficient mice after the resident–intruder test and YKS ameliorates the decreased rate (%) of serum corticosterone concentration after the test to the basal concentration in zinc-deficient mice. These findings suggest that the intake of YKS is effective for buffering social isolation stress under zinc deficiency via ameliorating the response of glucocorticoid secretion.

Glucocorticoids (corticosterone in mice and rats) are released from the adrenal cortex via activation of the hypothalamic–pituitary–adrenal (HPA) axis after exposure to stress. The hippocampus is enriched with glucocorticoid receptors and is a major target area for glucocorticoids. Hippocampal function is linked with stress response and is involved in the regulation of the HPA axis activity. Stress-induced excess secretion of glucocorticoids affects excitatory glutamatergic neurotransmission in the hippocampus, which plays a key role for not only abnormal behavior but also cognitive disturbance. YKS and its ingredients ameliorates enhanced glutamate neurotransmission in the hippocampus under stressful circumstances. It is possible that the enhanced glutamate neurotransmission irreversibly affects hippocampal function, followed by the dysregulation of the HPA axis activity. Here we tested whether YKS preventively buffers crowding stress-induced attenuations of glucocorticoid secretion response and long-term potentiation (LTP), an index of cognition.

MATERIALS AND METHODS

Animals and Drugs  Three-week-old Wistar rats (male) were obtained from Japan SLC (Hamamatsu, Japan). The laboratory condition for the rats is 23±1°C and 55±5% humidity. The rats freely access water and standard chew diet. The experiments were done in accordance with the Guidelines for the Care and Use of Laboratory Animals of the University of Shizuoka. The guidelines refer to American Association for Laboratory Animals Science and the guidelines laid down by the NIH. [Arg8]-vasopressin acetate salt was obtained from Sigma-Aldrich Co. (Saint Louis, MO, U.S.A.).

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Yokukansan (YKS)  YKS consists of seven dried medicinal herbs, which are Poria Sclerotium (PS: 4.0 g, sclerotium of *Poria cocos* RYVARDEN et GILBERTSON), Atractylodes Lancea Rhizome (ALR: 4.0 g, rhizome of *Atractylodes lancea* DE CANDOLLE), Uncaria Hook (UH: 3.0 g, hook of *Uncaria rhynchophylla* MIQUEL), Cnidium Rhizome (CR: 3.0 g, rhizome of *Cnidium of cinale MAKINO*), Japanese Angelica Root (JAR: 3.0 g, root of *Angelica acutiloba KITAGAWA*), Bupleurum Root (BR: 2.0 g, root of *Bupleurum falcatum LINNEI*), and Glycyrrhiza (GR: 1.5 g, root and stolon of *Glycyrrhiza uralensis FISHER*). YKS was prepared as a dry powdered extract by Tsumura & Co. (Tokyo, Japan). Therefore, each plant material was identified by its external morphology and authenticated by marker compounds of plant specimens according to the procedures of the Japanese Pharmacopoeia and Tsumura company’s standards. In brief, a mixture of seven component herbs was extracted for 1 h with purified hot water (95°C). The extracted solution was separated from insoluble waste and concentrated by evaporating under reduced pressure. Spray drying was used to produce a dried extract powder. The quality was standardized based on the Good Manufacturing Practices defined by the Japanese Ministry of Health, Labour and Welfare. The components of a YKS extract were previously confirmed by the three-dimensional HPLC analysis.19

**Exposure to Crowding Stress and Drug Administration** Three-week-old rats were divided into unstressed and social crowding-stressed groups. In unstressed group, the control rats were housed in 3 per a standard cage (30×40×20 cm) for 3 weeks. In crowding-stressed group, the rats were housed 6 per a small cage (15×25×15 cm) for 3 weeks. To administer YKS as a drinking water at the averaged dose of 300 mg/kg body weight/d during the housing, YKS dissolved in water (2 mg/mL) was given to the rats. YKS concentration in water was determined from the averaged intake volume of the drinking water. YKS did not influence the intake of water. The intake of the drinking water was not also influenced by exposure to crowding stress.

**Measurement of Serum Corticosterone Level** Saline or vasopressin in saline (25 µg/kg body weight) was intraperitoneally (i.p.) injected into unstressed and crowding-stressed rats. One hour later, blood samples were collected from the rats via the common carotid arteries under diethyl ether anesthesia. We collected blood samples in the morning (AM8:00–11:00) and completed the correction within 2 min. Zardooz et al. report that nearly 4-min ether anesthesia does not significantly increase serum corticosterone level in rats compared to that in unanesthetized rats.20 Serum was obtained by centrifuging (6000 rpm) blood samples for 10 min at 4°C. Serum corticosterone concentration was measured by using corticosterone enzyme-linked immunosorbent assay (ELISA) kit.

**Hippocampal Slice Preparation and CA1 Long-Term Potentiation (LTP) Induction** Unstressed and stressed rats were decapitated under anesthesia condition with diethyl ether. The brain was quickly excised from the rats and the brain was bathed in ice-cold artificial cerebrospinal fluid (ACSF), which is composed of 119 mM NaCl, 2.5 mM KCl, 1.3 mM MgSO4, 1.0 mM NaH2PO4, 2.5 mM CaCl2, 26.2 mM NaHCO3, and 11 mM D-glucose (pH 7.3). Transverse hippocampal slices (400 µm) were prepared in an ice-cold ACSF using a vibratome ZERO-1 (Dosaka Kyoto, Japan) and then maintained in a chamber at room temperature for at least 1 h. All solutions were used in the experiments under bubbling with 95% O2 and 5% CO2.

The hippocampal slices, which were transferred to a recording chamber, were submerged beneath continuously superfusing ACSF at 26–27°C. To confirm synaptic response, the Schaffer collateral/commisural pathway was stimulated with a bipolar tungsten electrode. Extracellular record was performed using a glass micropipette, which is filled with 3 M NaCl (2–10 Ω). The recording electrode was inserted along the trajectory of Schaffer collateral/commisural pathway to assess CA1 pyramidal cell response. Test stimuli (0.033 Hz) at 200 µs/pulse were given to the Schaffer collateral/commisural pathway every 30 s. The stimulus intensity was set for producing approximately 40% of the maximum field excitatory postsynaptic potential (fEPSP). LTP was induced by delivery of high-frequency stimulation at 100 Hz for 1 s. Field EPSP amplitudes were averaged over 180-s intervals and expressed as the rates (%) of the averaged fEPSP amplitude measured during the 30-min baseline that was shown as 100%. Averaged fEPSP amplitudes for the last 15 min after LTP induction were represented as the magnitude of LTP.

**Data Analysis** For multiple comparisons, differences between treatments were assessed by two-way ANOVA followed by post hoc testing using the Bonferroni multiple comparisons test (the statistical software, GraphPad Prism 5). A value of *p*<0.05 was considered significant.

**RESULTS**

**Changes in Body Weight and YKS Administration under Crowding Stress** The mean body weight was 76.0±1.3 g at the start of the experiments. Increase in body weight was significantly suppressed in rats subjected to crowding stress for 3 weeks (unstressed/water, 196.6±5.9 g; stressed/water, 158.9±4.0 g) (Fig. 1). Even in rats administered YKS for 3 weeks, significant reduction of body weights was observed in crowding-stressed group in comparison with unstressed group (unstressd/YKS, 194.9±4.0 g; stressd/YKS, 156.0±3.9 g). The mean body weight in unstressed group (unstressed/water, 196.6±5.9 g; stressed/water, 158.9±4.0 g) (Fig. 1). Even in rats administered YKS for 3 weeks, significant reduction of body weights was observed in crowding-stressed group in comparison with unstressed group (unstressd/YKS, 194.9±4.0 g; stressd/YKS, 156.0±3.9 g). Rats were also subjected to crowding stress in small cages (6 rats/cage) and water containing YKS (2 mg/mL) (*n*=6) or water containing YKS (*n*=6) were administered in the same manner. Two-way ANOVA followed by Bonferroni post-hoc test: *** *p*<0.001, vs. unstressed rats.

![Fig. 1. Body Weights after Administration of YKS to Crowding-Stressed Rats](image-url)
stressed/YKS, 171.9±4.3 g) (Fig. 1). The intake of YKS did not ameliorate the reduction of body weights under crowding stress. The intake of YKS was approximately 200 mg/kg body weight/d in stressed rats 3 weeks after the start of crowding stress, while it was approximately 180 mg/kg body weight/d in unstressed rats. Three weeks after the start of crowding stress, in any case, the intake of YKS was reduced from the initial estimation (300 mg/kg body weight) because of the differential increase in body weight.

Effect of YKS Administration on Vasopressin-Induced Corticosterone Secretion in Crowding-Stressed Rats

The intake of YKS had no significant effect on serum corticosterone level in unstressed rats, which were injected with saline (Fig. 2: saline/unstressed, 117±42 ng/mL; YKS/unstressed, 203±58 ng/mL). Serum corticosterone level was not significantly increased in crowding-stressed rats, which were injected with saline (saline/stressed, 159±16 ng/mL). The intake of YKS had no significant effect on serum corticosterone level in crowding-stressed rats (YKS/stressed, 166±54 ng/mL).

To assess corticosterone secretion via pituitary–adrenocortical axis activation,21) vasopressin was i.p. injected into crowding-stressed rats. Vasopressin markedly increased serum corticosterone level in water-administered unstressed rats, while the increase was significantly suppressed in water-administered stressed rats (Fig. 2: vasopressin/unstressed, 601±19 ng/mL; vasopressin/stressed, 455±13 ng/mL). Vasopressin also markedly increased serum corticosterone level in YKS-administered unstressed rats and the increase was not suppressed in YKS-administered stressed rats (Fig. 2: vasopressin/unstressed, 718±45 ng/mL; vasopressin/stressed, 795±34 ng/mL).

Effect of YKS Administration on Hippocampal LTP in Crowding-Stressed Rats

In the hippocampal slices prepared from unstressed rats, CA1 LTP was almost the same magnitude between water- and YKS-administered rats (Fig. 3: water, 145.1±6.8%; YKS, 145.6±10.8%). In contrast, CA1 LTP was remarkably attenuated in hippocampal slices prepared from water-administered stressed rats, but not in hippocampal slices prepared from YKS-administered stressed rats (water, 108.7±4.2%; YKS, 136.1±6.9%).

DISCUSSION

Glucocorticoid secretion after exposure to stress can buffer stress. However, excess secretion of glucocorticoids readily facilitates glutamate release from hippocampal neuron terminals via activation of membrane-associated mineralocorticoid receptors.22) In the hippocampus, corticosterone-mediated blockade of glutamate transporter activity induces glutamate accumulation in the extracellular compartment.23) Extracellular glutamate accumulation after exposure to acute stress induces cognitive decline via attenuated LTP.13,15) The hippocampus is vulnerable to glucocorticoid-induced accumulation of extracellular glutamate. Glucocorticoid secretion via HPA axis activation is regulated by the hippocampus through the negative feedback mechanism.12,24) Chronic stress affects both hippocampal function and the HPA axis activity.25-27) The decrease in volumes of the hippocampus are linked with abnormal secretion of cortisol, which is induced by disturbed negative feedback mechanism in human depressives.28-30)
Therefore, it is possible that glucocorticoid-induced enhancement of glutamate neurotransmission irreversibly affects hippocampal function, followed by the dysregulation of the HPA axis activity. Because the intake of YKS is effective for attenuating glucocorticoid-induced enhancement of glutamate neurotransmission, we examined whether intake of YKS ameliorates the impairments of glucocorticoid secretion response and hippocampal LTP under chronic stress.

YKS-containing water was administered during the period of exposure to crowding stress for 3 weeks. Serum corticosterone level was not significantly modified by administration of YKS-containing water to unstressed rats, while crowding stress did not increase serum corticosterone level even in rats administered YKS-containing water. A short-term crowding stress for 3d does not affect the pituitary–adrenocortical response to the corticotropin-releasing hormone (CRH) but affects the pituitary–adrenocortical response to the vasopressin. Vasopressin and CRH have complementary roles in the secretion of adrenocorticotropic hormone (ACTH) after exposure to different stress, which stimulates glucocorticoid secretion. ACTH secretion via vasopressin and CRH is important for buffering stress. It is possible that the pituitary–adrenocortical response to the vasopressin is vulnerable to crowding stress followed by hippocampal dysfunction. In the present study, vasopressin was injected into crowding-stressed rats to assess corticosterone secretion response via pituitary–adrenocortical axis activation. Vasopressin-included increase in serum corticosterone was significantly attenuated compared to non-stressed control rats, in agreement with Bugajski’s paper, indicating that crowding stress for 3 weeks affects the pituitary–adrenocortical response to vasopressin. Interestingly, the intake of YKS rescued the attenuation of vasopressin-included increase in serum corticosterone. These results suggest that the intake of YKS is effective for buffering chronic stress via ameliorating pituitary–adrenocortical response to vasopressin. This amelioration implies that the intake of YKS is effective for buffering chronic stress.

In conclusion, the present paper demonstrates that intake of YKS rescues crowding stress-induced impairments of pituitary–adrenocortical response to vasopressin and hippocampal LTP. The intake of YKS may be benefit to buffering chronic stress.

Conflict of Interest
The authors declare no conflict of interest.

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