Neuroscience 207 (2012) 124–136

GEISSOSCHIZINE METHYL ETHER, AN ALKALOID IN UNCARIA HOOK, IS A POTENT SEROTONIN$_{1A}$ RECEPTOR AGONIST AND CANDIDATE FOR AMELIORATION OF AGGRESSIVENESS AND SOCIALITY BY YOKUKANSAN

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Abstract—Yokukansan (YKS), a traditional Japanese medicine, is composed of seven kinds of dried herbs. It is widely prescribed in clinical situation for treating psychiatric disorders such as aggressiveness in patients with dementia. We previously demonstrated that YKS and Uncaria hook (UH), which is a constituent herb of YKS, had a partial agonistic effect to 5-HT$_{1A}$ receptors *in vitro*. However, it has still been unclear whether this *in vitro* effect is reflected in *in vivo*, and what the active ingredients are. The purpose of the present study is to find the active ingredient in YKS and to demonstrate the effect in *in vivo*. In the present study, we first studied the effect of YKS and UH on aggressiveness and sociality in socially isolated mice. YKS and UH ameliorated the isolation-induced increased aggressiveness and decreased sociality, and these ameliorative effects were counteracted by coadministration of 5-HT$_{1A}$ receptor antagonist WAY-100635, or disappeared by eliminating UH from YKS. These results suggest that the effect of YKS is mainly attributed to UH, and the active ingredient is contained in UH. To find the candidate ingredients, we examined competitive binding assay and [^{35}S] guanosine 5′-O-(3-thiotriphosphate) (GTPγS) binding assay of seven major alkaloids in UH using Chinese hamster ovary cells expressing 5-HT$_{1A}$ receptors artificially. Only geissoschizine methyl ether (GM) among seven alkaloids potently bound to 5-HT$_{1A}$ receptors and acted as a partial agonist. This *in vitro* result on GM was further demonstrated in the socially isolated mice. As did YKS and UH, GM ameliorated the isolation-induced increased aggressiveness and decreased sociality, and the effect was counteracted by coadministration of WAY-100635. These lines of results suggest that GM in UH is potent 5-HT$_{1A}$ receptor agonist and a candidate for pharmacological effect of YKS on aggressiveness and sociality in socially isolated mice.

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Abbreviations: AD, Alzheimer's disease; ANOVA, analysis of variance; BBB, blood–brain barrier; BPSD, behavioral and psychological symptoms of dementia; CHO-h5-HT$_{1A}$, Chinese hamster ovary cells stably expressing human recombinant 5-HT$_{1A}$ receptors; DMSO, dimethyl sulfoxide; DTT, dithiothreitol; EDTA, ethylenediaminetetraacetic acid; GDP, guanosine-5′-diphosphate; GM, geissoschizine methyl ether; GTPγS, guanosine 5′-O-(3-thiotriphosphate); HEPES, 4-(2-hydroxyethyl)piperazine-1-ethanesulfonic acid; PCA, para-chloroamphetamine; UH, Uncaria hook; YKS, yokukansan; YKS-UH, YKS analog eliminating UH; 8-OH-DPAT, 8-hydroxy-2-(di-n-propylamino)tetralin.

Key words: aggressiveness, geissoschizine methyl ether, serotonin$_{1A}$ receptor, sociality, Uncaria hook, yokukansan.

The traditional Japanese medicine yokukansan (YKS), which is referred to as a "*kampo* medicine", is composed of seven kinds of dried medical herbs. It has been approved by the Ministry of Health, Labour and Welfare of Japan as a remedy for neurosis, insomnia, or night crying and irritability in children. Clinical studies recently reported that YKS improves behavioral and psychological symptoms of dementia (BPSD) and it notably affects hallucinations, agitation, and aggressiveness in patients with Alzheimer's disease (AD), dementia with Lewy bodies, and other forms of senile dementia (Iwasaki et al., 2005a,b; Shinno et al., 2007; Mizukami et al., 2009). Although the pathogenesis of BPSD such as aggressiveness and agitation is not fully understood yet, it is suggested that the dysfunction of serotonergic mechanism is one of the important pathogenesis for the aggressiveness. In animal model studies, it is shown that aggressiveness is closely related to dysfunction of brain serotonergic mechanisms (Popova, 2006; Takahashi et al., 2011). Furthermore, in clinical investigations, a serotonergic deficit in raphe nuclei and reduced 5-HT$_{1A}$ receptor densities in raphe nuclei and hippocampus were reported in patients with AD (Yamamoto and Hirano, 1985; Aletrino et al., 1992; Halliday et al., 1992; Kepe et al., 2006). Lai et al. (2003) reported that the reduced 5-HT$_{1A}$ receptor binding in the temporal cortex correlates with aggressive behavior in AD.

Recently, various basic studies were performed by many groups including us to clarify the pharmacological property of YKS. We previously demonstrated that YKS ameliorated a 5-HT neurotoxin para-chloroamphetamine (PCA)-induced aggressive behavior and social behavior in rats, and the ameliorative effect was counteracted by coadministration of 5-HT$_{1A}$ receptor antagonist WAY-100635 (Kanno et al., 2009). A further *in vitro* binding study demonstrated that YKS and Uncaria hook (UH), which is a constituent herb of YKS, had a partial agonistic effect to 5-HT$_{1A}$ receptors (Terawaki et al., 2010). From these results, we hypothesized that the psychotropic effect of YKS might be due to UH showing a partial agonistic effect to 5-HT$_{1A}$ receptors. However, it has still been unclear whether this *in vitro* effect is reflected in *in vivo*, and what the active ingredients are.

In this study, we experimented to identify candidate active ingredient, which affects aggressive behavior and

0306-4522/12 $36.00
doi:10.1016/j.neuroscience.2012.01.037

social behavior using the socially isolated mouse model. Social isolation causes enhanced aggressive behavior with alterations in 5-HT metabolism and densities of 5-HT_{1A} and 5-HT_{1B} receptors in rodents (Rilke et al., 1998; Schiller et al., 2003). This aggressiveness in social isolation is significantly rescued by 5-HT_{1A} and 5-HT_{1B} receptor agonist, 5-HT_{2A} receptor antagonist, and serotonin reuptake inhibitor (Sánchez et al., 1993; Sánchez and Hyttel, 1994; Sánchez and Meier, 1997; Rilke et al., 2001; Sakaue et al., 2002; Pinna et al., 2003; Koike et al., 2009). These findings suggest that the socially isolated mice are a valuable tool for developing new drug for BPSD.

The purpose of the present study is to find the active ingredient in YKS and to demonstrate the effect *in vivo*. To clarify the purpose, we demonstrated the effect of UH after verification of the effect of YKS using socially isolated mice. Next, the binding activity of ingredients in YKS on 5-HT_{1A} receptors was screened using *in vitro* binding assay. Finally, *in vivo* effect of a screened active ingredient was demonstrated in the socially isolated mice.

EXPERIMENTAL PROCEDURES

Animals

Male ddY mice aged 4 weeks were obtained from Japan SLC, Inc. (Shizuoka, Japan). After habituation for 1 week, the animals were housed individually in transparent cages (11.5×31×15.5 cm^3) during the experimental period. As group-housed controls and counterparts for the social interaction test, five animals were housed in a cage (23×31×15.5 cm^3). All mice were housed at a temperature of 23±2 °C, relative humidity of 55%±10%, and a 12-h light/dark cycle, with lights on from 07:00 to 19:00 daily, and allowed *ad libitum* access to water and regular chow (MF, Oriental Yeast Co., Ltd., Tokyo, Japan) during the habituation and experimental periods.

All experimental procedures were performed according to the "Guidelines for the care and use of laboratory animals" approved by the Laboratory Animal Committee of Tsumura & Co. and the guidelines of the Japanese Association for Laboratory Animal Science.

Drugs and reagents

The dry powdered extract of YKS is composed of the following seven dried medicinal herbs: Atractylodes lancea rhizome (4.0 g, rhizome of Atractylodes lancea De Candolle), Poria sclerotium (4.0 g, sclerotium of *Poria cocos* Wolf), Cnidium rhizoma (3.0 g, rhizome of *Cnidium officinale* Makino), UH (3.0 g, thorn of *Uncaria rhynchophylla* Miquel), Japanese Angelica root (3.0 g, root of *Angelica acutiloba* Kitagawa), Bupleurum root (2.0 g, root of *Bupleurum falcatum* Linné), and Glycyrrhiza (1.5 g, root and stolon of *Glycyrrhiza uralensis* Fisher). YKS, YKS analog eliminating UH (YKS-UH), UH, and seven UH-derived alkaloids (rhynchophylline, isorhynchophylline, corynoxeine, isocorynoxeine, hirsuteine, hirsutine, and geissoschizine methyl ether [GM]) used in the present study were supplied by the Botanical Raw Materials Research Department of Tsumura & Co. (Ibaraki, Japan). YKS, YKS-UH, and UH were dissolved in distilled water, and GM was dissolved in 0.5% Tween-80. A 5-HT_{1A} receptor antagonist, WAY-100635 maleate salt (Sigma, St. Louis, MO, USA), was dissolved in saline.

The membranes of Chinese hamster ovary cells stably expressing human recombinant 5-HT_{1A} receptors (CHO-h5-HT_{1A}) and [^{3}H] 8-hydroxy-2-(di-n-propylamino)tetralin (8-OH-DPAT) (NET929, 170.2 Ci/mmol) were purchased from PerkinElmer (Waltham, MA, USA). [^{35}S] guanosine 5′-O-(3-thiotriphosphate) (GTPγS) (SJ-1308, 1033 Ci/mmol) was purchased from GE Healthcare UK Ltd (Buckinghamshire, UK). Dithiothreitol (DTT), metergoline, 5-HT, guanosine-5′-diphosphate (GDP), GTPγS, and HEPES were purchased from Sigma. Other reagents used for the binding analysis were purchased from commercial sources.

Social interaction test

The social interaction test was performed according to procedures reported previously (Ikarashi et al., 2009; Uchida et al., 2009). In brief, a subject mouse and a naive untreated age-matched group-housed mouse were placed together in an open-field apparatus (50×50×40 cm^3; Neuroscience, Inc., Tokyo, Japan). Interactions between the two animals were monitored with a video camera for 10 min. The total number of aggressive behaviors (aggressive grooming, tail rattling, chasing, and attacking) and social behaviors (sniffing, following, and contacting) by the subject animal toward the other animal were counted. The total travel distance (cm) during the social interaction was analyzed by using software (analyzing behavior system, Viewer II: Bioserve, Bonn, Germany) as motor activity. In the test, there were low-response animals that seldom contacted their counterpart, and the individuals were excluded from the following evaluation.

Changes in aggressive and social behaviors observed in socially isolated mice

To clarify the relationship between the development of social interactive behaviors and the isolation-period, changes in aggressive behavior, social behavior, and motor activity in the isolated mice and group-housed mice (control) were evaluated by social interaction test on the 0th, 3rd, 4th, and 6th week of this experiment.

Effects of a single administration of YKS, UH, and GM on aggressive and social behaviors in socially isolated mice

YKS (0.5 and 1.0 g/kg), UH (75 and 150 mg/kg), or GM (75, 150, and 300 μg/kg) was orally administered in a single dose to the mice that had been isolated for 4 weeks. Distilled water (10.0 ml/kg) for the tests of YKS and UH, or 0.5% Tween-80 (10.0 ml/kg) for the test of GM was orally administered to the corresponding isolated control or group-housed mice by the same schedule. The social interaction test was performed 60 min after the drug administration.

Effects of repeated administration of YKS, UH, and GM on aggressive and social behaviors in socially isolated mice

YKS (0.5 and 1.0 g/kg), YKS-UH (1.0 g/kg), UH (75 and 150 mg/kg), or GM (150 and 300 μg/kg) was orally administered once a day for 14 days from the 4th week to 6th week to the isolated mice. Distilled water (10.0 ml/kg) for the tests of YKS and UH or 0.5% Tween-80 (10.0 ml/kg) for the test of GM was orally administered to the corresponding isolated control or group-housed mice by the same schedule. In this experiment, WAY-100635 (0.1 mg/kg) or saline (10.0 ml/kg) was administered i.p. once 30 min after administration of YKS (1.0 g/kg), UH (150 mg/kg), or GM (300 μg/kg) on the 14th day. The social interaction test was performed 60 min after the final administration of test substance on the 14th day.

Coadministration effects of WAY-100635 on repeated administration effects of GM

GM (300 μg/kg) was orally administered once a day for 15 days from the 4th to 6th week to the isolated mice, and WAY-100635