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Research Paper

Effect of yokukansan, a traditional Japanese medicine, on social and aggressive behaviour of *para*-chloroamphetamine-injected rats

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Abstract

Objectives Yokukansan, a traditional Japanese medicine, 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. It has recently been reported to improve behavioural and psychological symptoms of dementia, such as hallucinations, agitation, and aggressiveness in patients with some forms of senile dementia. Little is known about the mechanism underlying the effectiveness of yokukansan. Our aim was to clarify the involvement of yokukansan in serotonergic function in *para*-chloroamphetamine (PCA)-induced aggressive behaviour in rats.

Methods The effect of yokukansan on social interactions, including social and aggressive behaviour, was examined in PCA-injected rats. Concentration and release level of serotonin (5-HT) in the hypothalamus were measured.

Key findings PCA reduced not only the 5-HT concentration but also the high K^+-induced 5-HT release in the rat hypothalamus. Social interaction tests showed a significant decrease in social behaviour and a significant increase in aggressive behaviour in the PCA-treated rats. The decrease in social behaviour was ameliorated by the 5-HT1A agonist buspirone and further decreased by a 5-HT1A antagonist, *N*-[2-[4-(2-methoxyphenyl)-1-piperazinyl]ethyl]-*N*-(2-pyridinyl)cyclo-hexanecarboxamide trihydrochloride (WAY-100635), whereas it was further decreased by the 5-HT2A agonist, 2,5-dimethoxy-4-iodoamphetamine (DOI), and ameliorated by the 5-HT2A antagonist ketanserin. On the other hand, the increase in aggressive behaviour was ameliorated by buspirone but not affected by WAY-100635, whereas it was enhanced by DOI and ameliorated by ketanserin. A single injection of yokukansan ameliorated the PCA-induced decrease in social behaviour but not aggressive behaviour. Chronic treatment for 14 days with yokukansan ameliorated PCA-induced abnormal behaviour, decreased social behaviour and increased aggressive behaviour, but it did not ameliorate PCA-induced decreases in the cerebral 5-HT concentration and 5-HT release. The ameliorative effects of chronic yokukansan on behaviour were counteracted by co-administration of WAY-100635.

Conclusions These results suggest that yokukansan might have two different effects: an acute effect on social behaviour and a chronic effect on aggressive behaviour. One of the mechanisms of these effects of yokukansan may be related to the agonistic effect on 5-HT1A receptors.

Keywords aggressive behaviour; *p*-chloroamphetamine; social behaviour; yokukansan

Introduction

Behavioural and psychological symptoms of dementia (BPSD), such as aggression, anxiety and hallucinations, often occur in patients with Alzheimer's disease and dementia with Lewy bodies, and BPSD decreases the quality of life of patients and their care-givers. The severity of BPSD and the care burden show a positive correlation,[1,2] and are frequently the primary cause of hospitalization or institutionalization.[3] To date, antipsychotics have been used for BPSD therapy. However, such drugs induce extrapyramidal symptoms and other adverse events and, in consequence, they decrease the quality of life or increase the difficulty of maintaining activities of daily living. In addition, in 2005 the US Food and

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Drug Administration warned that mortality was increased in elderly patients with dementia who used atypical antipsychotics.[4] Thus, new remedies without adverse effects have been sought.

Recently, yokukansan, a traditional Japanese medicine referred to as a 'kampo medicine' in Japan, was reported to improve BPSD, such as hallucinations, agitation and aggressiveness, in patients with Alzheimer's disease, Lewy-body dementia, and other forms of senile dementia.[5–8] Yokukansan 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, but little is known about the mechanism underlying the effectiveness of yokukansan.

Serotonergic deficits have been reported in raphe nuclei of the brain in patients with Alzheimer's disease.[9–11] Recently, Lai *et al*.[12] reported that reduced serotonin 5-HT1A receptor binding in the temporal cortex correlates with aggressive behaviour in Alzheimer's disease. In addition, hallucinogens, such as lysergic acid diethylamide (LSD-25), *N*,*N*-dimethyltriptamine, bufotenine, psilocin, harmine and harmaline, which have an indoleamine structure resembling the serotonin structure, induce not only abnormal behaviour such as a head-twitch response or aggressive behaviour but also decreases in cerebral serotonergic activity, including serotonin (5-HT) release and 5-HT turnover in rats and mice.[13] Aggressive behaviour is reported to be induced by treatment causing cerebral 5-HT depletion such as *para* (*p*)-chloroamphetamine (PCA), *p*-chlorophenylalanine, 5,7-dihydroxytryptamine, 3,4-methylenedioxy methamphetamine and a tryptophan-free diet.[14–19] These findings suggest that a hypofunction of the cerebral serotonergic system is closely related to behavioural and psychological symptoms such as aggression and hallucination.

More recently, we demonstrated in rats[20] that yokukansan ameliorates abnormal social interaction behaviour, such as enhanced aggressive behaviour and decreased social behaviour induced by PCA, which is well-known to be a potent serotonergic neurotoxin.[21] In addition, an in-vitro binding assay showed that yokukansan possesses a partial agonistic effect on 5-HT1A receptors.[20] These results suggest the possibility that the effectiveness of yokukansan against impaired social interaction behaviour in PCA-injected rats may be mediated through improving serotonergic neurotransmission. However, sufficient animal experiments to confirm these hypotheses have not been performed yet.

In this study, to further clarify the involvement of yokukansan in serotonergic function in the abnormal social interaction behaviour induced by PCA, the effect of yokukansan on cerebral concentration and release of 5-HT and social interaction behaviour were investigated in PCA-injected rats.

Materials and Methods

Animals

Seven-week-old male Wistar rats, 230 ± 30 g, were obtained from Charles River Laboratories (Yokohama, Japan). The rats were group-housed (five rats in a cage) during habituation for one week, and thereafter housed individually in stainless steel cages (RBC-12 type, 260 × 380 × 200 mm; Ishihara Co. Ltd, Tokyo, Japan) during the experimental period. They were housed at a temperature of 23 ± 2°C, relative humidity of 55 ± 10% and a 12-h light–dark cycle (lights on at 0700 h) daily, and allowed free access to water and standard laboratory food during the habituation and experimental periods.

Conspecific male rats were also obtained from Charles River to evaluate aggression of subject rats in a social interaction test. They were group-housed (five rats in a cage) in the breeding environment described above until used in the tests.

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.

Drugs and reagents

Yokukansan is composed of seven dried medicinal herbs: *Atractylodes lancea* rhizome (4.0 g), *Poria sclerotium* (4.0 g), *Cnidium* rhizome (3.0 g), Japanese angelica root (3.0 g), *Bupleurum* root (2.0 g), *Glycyrrhiza* root (1.5 g) and *Uncaria* hook (3.0 g). The dry powdered extract of yokukansan used in this study was supplied by Tsumura & Co. (Tokyo, Japan). Regarding the ingredients of yokukansan extract, we previously performed a three-dimensional high-performance liquid chromatographic analysis.[7] In brief, the dried extract (1.0 g) of yokukansan was dissolved in 20 ml methanol under ultrasonication for 30 min and centrifuged at 3000 rev/min for 5 min. The supernatant was filtered through a 0.45-μm membrane filter. A 30-μl volume of the filtrate was injected into an HPLC system (Shimadzu SPD-M10AVP; Shimadzu Co., Kyoto, Japan) to obtain a three-dimensional chromatogram. The chromatographic conditions were: column, TSK-gel ODS-80TS (4.6Φmm × 250 mm long; Tosoh Co., Tokyo, Japan); mobile phase, a linear gradient for 60 min with 0.05 M $AcONH_4$, pH 3.6 (90%→0%) and 100% CH_3CN (10%→100%); column temperature, 40°C; flow rate, 1.0 ml/min; detector, diode array; and scan range, UV 200–400 nm. In the chromatogram, at least 25 compounds have been identified in yokukansan extract.

The dosages (0.5–1.0 g/kg) of yokukansan used in this study are equivalent to approximately 10–15 times the clinical dose. The dosage was decided based on the results of our previous study demonstrating that yokukansan ameliorated aggressive behaviour observed in amyloid β-injected mice.[22]

PCA, buspirone, *N*-[2-[4-(2-methoxyphenyl)-1-piperazinyl] ethyl]-*N*-(2-pyridinyl)cyclo-hexanecarboxamide trihydrochloride (WAY-100635), 2,5-dimethoxy-4-iodoamphetamine (DOI), ketanserin and isoproterenol were purchased from Sigma (St Louis, MO, USA). Other reagents used for the analysis were purchased from commercial sources.

Determination of 5-HT and 5-hydroxyindoleacetic acid concentrations

Yokukansan (1.0 g/kg, p.o., $n = 8$) or distilled water (10 ml/kg, p.o., $n = 8$) was administered once a day to rats for 14 days after one injection of PCA (5 mg/kg, i.p.). As a control, distilled

water (10 ml/kg, p.o., $n = 8$) was administered for 14 days to rats injected with saline (1.0 ml/kg, i.p.) instead of PCA.

All rats were sacrificed by decapitation, 60 min after the final injection of each test substance on the 14th day, for determination of the hypothalamic 5-HT concentration. In brief, the dissected hypothalamus was homogenized using an ultrahomogenizer (SM-50W; SMT Co. Ltd, Tokyo, Japan) for 30 s in 1.0 ml of ice-cold 0.1 M perchloric acid containing 800 μM isoproterenol as an internal standard. The homogenate was left on ice for 30 min and centrifuged at 15 000*g* and 4°C for 15 min. The supernatant was passed through a 0.45-μm Millipore filter. Samples (10 μl) of the filtrate were injected into an HPLC column with electrochemical detection (ECD) for determination of 5-HT and 5-hydroxyindoleacetic acid (5-HIAA).[23]

Microdialysis experiment

Yokukansan (1.0 g/kg, p.o., $n = 4$) or distilled water (10 ml/kg, p.o., $n = 4$) was administered once a day to rats for 14 days after one injection of PCA (5 mg/kg, i.p.). Distilled water (10 ml/kg, p.o., $n = 4$) was administered to rats injected once with saline (1.0 ml/kg, i.p.) for 14 days as a control.

On day 7 after injection of PCA or saline, rats were anaesthetized with 50 mg/kg sodium pentobarbital intraperitoneally, and a guide cannula (CMA/12; CMA, Solna, Sweden) was implanted into the hypothalamus (coordinates: anterior 2.3 mm and right lateral 1.1 mm from the bregma, and ventral 7.0 mm from the dura) according to a rat brain atlas.[24] On day 14, a vertical-type microdialysis probe (membrane, 0.5 mm o.d. × 2.0 mm long; CMA) was inserted into the implanted guide cannula of unanaesthetized freely-moving rats. The probe was perfused at a constant flow rate of 2.0 μl/min with Ringer's solution (in mM: 147 Na^+, 4 K^+, 1.2 Ca^{2+}, 153.4 Cl^-). The dialysate (40 μl) was sampled at 20-min intervals and directly injected into an HPLC-ECD for determination of 5-HT.[25] After confirmation that the basal level was stable, high-K^+ stimulation (40, 60 and 100 mM KCl) for 20 min was carried out at 100-min intervals.

After completion of the experiments, rats were killed by decapitation and placement of the probe in the hypothalamus was verified.

Social interaction test

For assessment of the acute effect, a single injection of buspirone (0.5 and 1.0 mg/kg, i.p., $n = 9$), WAY-100635 (0.03 and 0.1 mg/kg, i.p., $n = 9$), DOI (0.03 and 0.1 mg/kg, i.p., $n = 9$), ketanserin (0.1 and 0.5 mg/kg, i.p., $n = 9$), yokukansan (0.5 and 1.0 g/kg, p.o., $n = 12$) or distilled water (10 ml/kg, p.o., $n = 14$) was given to rats on the 14th day after injection of PCA (5 mg/kg, i.p.). Distilled water (10.0 ml/kg, p.o., $n = 13$) was administered to rats injected with saline (1.0 ml/kg, i.p.) as a control.

For assessment of the chronic effect, yokukansan (0.5 and 1.0 g/kg, p.o., $n = 12$), yokukansan (1.0 g/kg, p.o.) plus WAY-100635 (0.1 mg/kg, i.p., $n = 12$), or distilled water (1.0 ml/kg, p.o., $n = 22$) was administered once a day to rats for 14 days after injection of PCA. As a control, distilled water (10 ml/kg, p.o., $n = 21$) was administered for 14 days to rats injected with saline.

The social interaction test was performed on the 14th day, 60 min after the final injection of each test substance, according to a procedure reported previously.[20] In brief, a subject rat and an untreated rat were placed together in an open-field apparatus (90 cm L × 90 cm W × 50 cm H). Interactive behaviours between the two rats were monitored with a video camera for 10 min. The total number of aggressive acts, including aggressive grooming, tail rattling, chasing and attacking, and social acts, including sniffing, following and contacting, of the subject rat toward the other rat, were counted. The total distance travelled (cm) of each rat in the open-field apparatus was analysed by using software (analysing behaviour system, Viewer II; Bioserve, Bonn, Germany), as motor activity.

Statistics

All data are expressed as the mean ± SEM. The statistical significance of differences between groups in the social interaction test was analysed by one-way analysis of variance, followed by Tukey–Kramer's test. Biochemical data (5-HT concentration or 5-HT release) were analysed by one-way analysis of variance, followed by Bonferroni multiple comparisons test. The significance level in each statistical analysis was accepted at $P < 0.05$.

Results

Effect of yokukansan on hypothalamic concentrations of 5-HT and 5-hydroxyindoleacetic acid in *p*-chloramphetamine-injected rats

The effect of yokukansan on the hypothalamic concentrations of 5-HT and its metabolite 5-HIAA in PCA-injected rats is shown in Figure 1. Concentrations of both 5-HT and 5-HIAA were significantly decreased in PCA-injected rats on the 14th day after PCA injection compared with those in saline-injected rats. Chronic administration of yokukansan (1.0 g/kg, p.o., for 14 days) did not affect the decrease in concentrations of 5-HT and 5-HIAA.

Effect of yokukansan on high-K^+-induced hypothalamic 5-HT release in *p*-chloramphetamine-injected rats

Figure 2 shows the effect of yokukansan on the hypothalamic 5-HT release (extracellular concentration) elicited by solutions of various K^+ concentrations. In control rats, high-K^+ stimulation (40, 60 and 100 mM) increased 5-HT release in a concentration-dependent manner. However, the 5-HT release due to high-K^+ stimulation did not increase in PCA-injected rats as much as that in controls, and a significant decrease was observed at 100 mM KCl. Chronic administration of yokukansan (1.0 g/kg, p.o., for 14 days) did not alter the decrease in the level of 5-HT released.

Effect of agonists or antagonists of 5-HT1A and 5-HT2A receptors on social interaction behaviour in *p*-chloramphetamine-injected rats

Figure 3 shows acute effects (single injection) of agonists or antagonists for 5-HT1A and 5-HT2A receptors on social interaction behaviour, including social behaviour (Figure 3a),

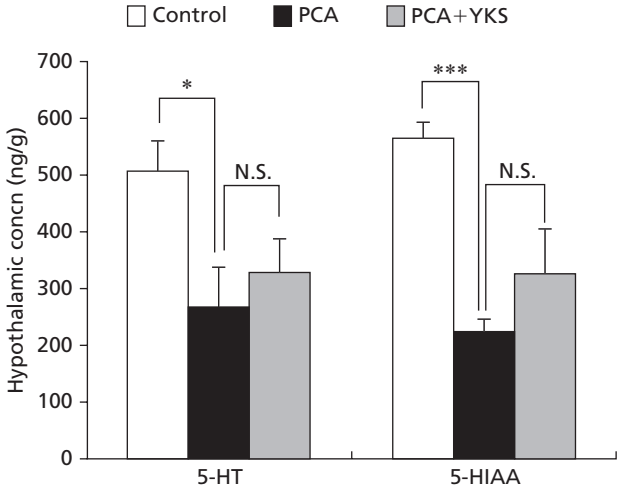

Figure 1 Effect of yokukansan on depletion of 5-HT and 5-hydroxyindoleacetic acid concentrations in the hypothalamus of *p*-chloroamphetamine-injected rats. Yokukansan (YKS) (1.0 g/kg, p.o., $n = 8$) was administered to rats once a day for 14 days after one injection of *p*-chloramphetamine (PCA) (5 mg/kg, i.p.). Control or PCA rats ($n = 8$) were administered distilled water (10 ml/kg, p.o.) for 14 days after injection of saline (1.0 ml/kg, i.p.) or PCA. All rats were sacrificed by decapitation on the 14th day 60 min after the final injection of each test substance for determination of hypothalamic concentrations of 5-HT and 5-hydroxyindoleacetic acid (5-HIAA). The concentrations of 5-HT and 5-HIAA (ng/g wet weight) are expressed as the mean ± SEM. $*P < 0.05$, $***P < 0.001$ vs control (one-way analysis of variance, followed by post-hoc Bonferroni multiple comparisons test). N.S., non-significant.

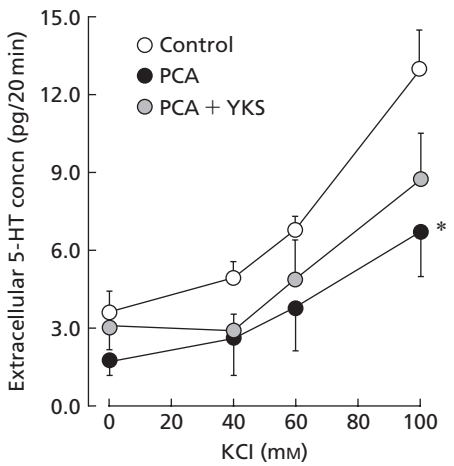

Figure 2 Effect of yokukansan on the decrease in high-K^+-induced 5-HT release in the hypothalamus of *p*-chloroamphetamine-injected rats. Control or *p*-chloramphetamine (PCA) rats ($n = 4$) were administered distilled water (10 ml/kg, p.o.) for 14 days after injection of saline (1.0 ml/kg, i.p.) or PCA. Yokukansan (YKS) (1.0 g/kg, p.o., $n = 4$) was administered once a day to rats for 14 days after injection of PCA (5 mg/kg, i.p.). Microdialysis for determination of extracellular 5-HT concentrations was performed on day 14 on unanaesthetized freely-moving rats: high-K^+ stimulation (40, 60, and 100 mM KCl) for 20 min each was carried out at 100-min intervals after confirmation that the basal level was stable. The extracellular concentrations of 5-HT (pg/20 min) are expressed as the mean ± SEM. $*P < 0.05$ vs corresponding control (one-way analysis of variance followed by post-hoc Bonferroni multiple comparisons test).

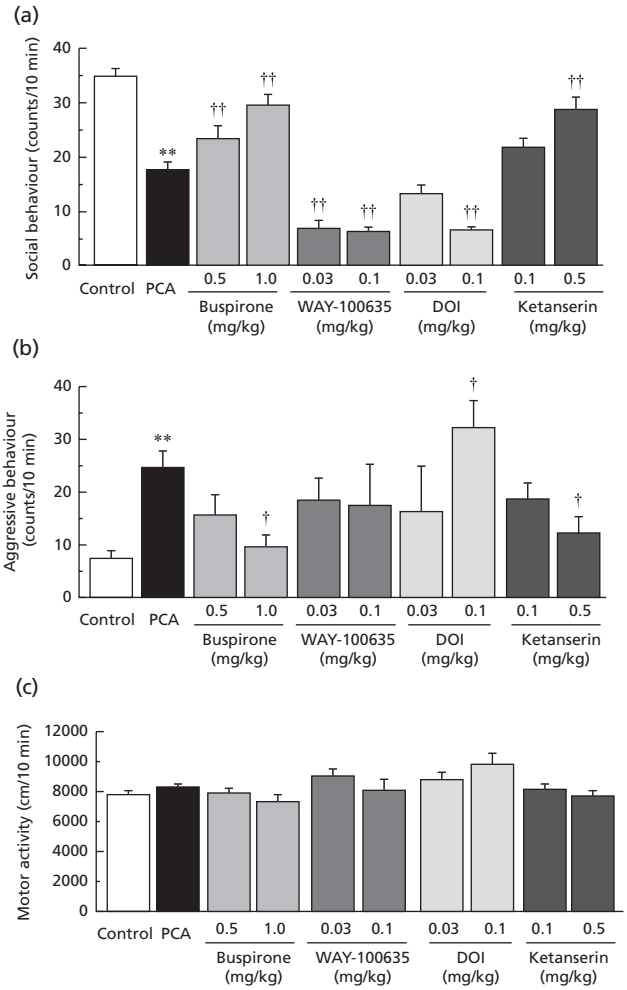

Figure 3 Effect of buspirone, WAY-100635, DOI and ketanserin on social behaviour (a), aggressive behaviour (b) and motor activity (c) of *p*-chloroamphetamine-injected rats. Control or *p*-chloramphetamine (PCA) rats were administered distilled water (10.0 ml/kg, i.p.) on the 14th day after injection of saline (1.0 ml/kg, i.p., $n = 13$) or PCA (5 mg/kg, i.p., $n = 14$). Buspirone (0.5 and 1.0 mg/kg, i.p., $n = 9$), WAY-100635 (0.03 and 0.1 mg/kg, i.p., $n = 9$), DOI (0.03 and 0.1 mg/kg, i.p., $n = 9$) or ketanserin (0.1 and 0.5 mg/kg, i.p., $n = 9$) was administered to rats on the 14th day after injection of PCA. A social interaction test was performed 60 min after the final injection of each test substance on the 14th day. Each data point represents as the mean ± SEM. $**P < 0.01$ vs control group; $†P < 0.05$, $††P < 0.01$ vs PCA group (one-way analysis of variance followed by post-hoc Tukey–Kramer's test).

aggressive behaviour (Figure 3b) and motor activity (Figure 3c), in PCA-injected rats.

Treatment with PCA significantly decreased the number of social acts. The decreased social behaviour in the PCA-injected rats was significantly ameliorated by the 5-HT1A agonist buspirone (0.5 and 1.0 mg/kg, i.p.) and significantly worsened by the 5-HT1A antagonist WAY-100635 (0.5 and 0.1 mg/kg, i.p.) in a dose-dependent manner. In contrast, the PCA-induced decrease in social behaviour was significantly worsened by the 5-HT2A agonist DOI (0.03 and 0.1 mg/kg, i.p.) and significantly ameliorated by the 5-HT2A antagonist ketanserin (0.1 and 0.5 mg/kg, i.p.) in a dose-dependent manner.

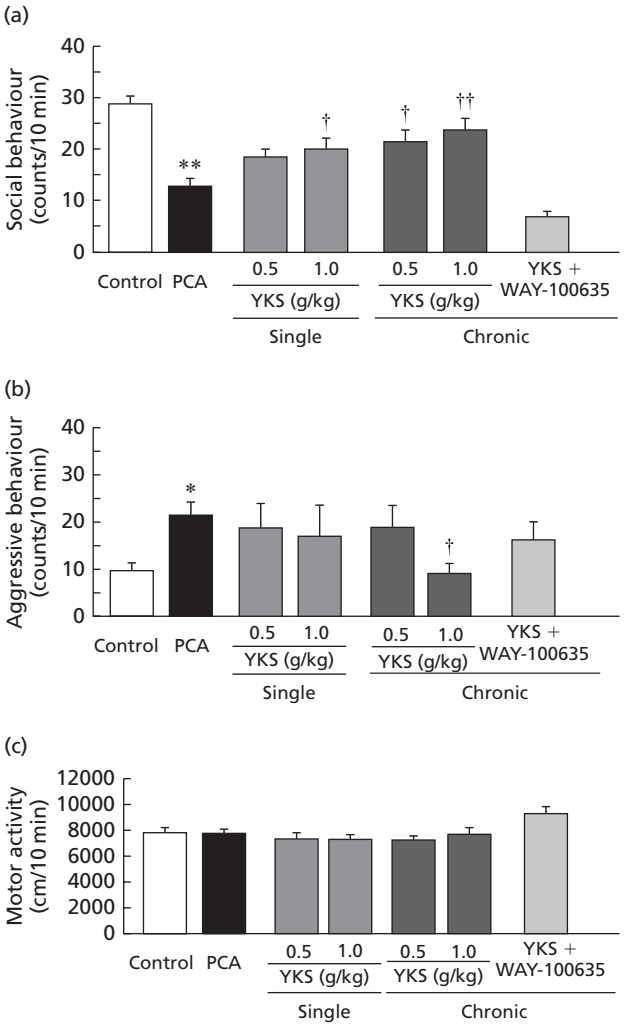

Figure 4 Effect of yokukansan on social behaviour (a), aggressive behaviour (b) and motor activity (c) in *p*-chloroamphetamine-injected rats. Control or *p*-chloramphetamine (PCA) rats were administered distilled water (10.0 ml/kg, p.o.) for 14 days after injection of saline (1.0 ml/kg, i.p., $n = 21$) or PCA (5 mg/kg, i.p., $n = 22$). Single treatment: yokukansan (YKS) (0.5 and 1.0 g/kg, p.o., $n = 12$) was administered to rats on the 14th day after injection of PCA. Chronic treatment: YKS (0.5 and 1.0 g/kg, p.o., $n = 12$), or YKS (1.0 g/kg, p.o.) + WAY-100635 (0.1 mg/kg, i.p., $n = 12$) was administered once a day to rats for 14 days after injection of PCA. The social interaction test was performed on the 14th day 60 min after the final injection of each test substance. Each data point represents the mean ± SEM. $^{*}P < 0.05$, $^{**}P < 0.01$ vs control group; and $\dagger P < 0.05$, $\dagger\dagger P < 0.01$ vs PCA group (one-way analysis of variance followed by post-hoc Tukey–Kramer's test).

The number of aggressive acts significantly increased in PCA-injected rats compared with control rats. The increased aggressive behaviour was significantly ameliorated by the 5-HT1A agonist buspirone (0.5 and 1.0 mg/kg, i.p.) in a dose-dependent manner but not affected by the 5-HT1A antagonist WAY-100635 (0.03 and 0.1 mg/kg, i.p.), and significantly enhanced by the 5-HT2A agonist DOI (0.1 mg/kg, i.p.) and significantly inhibited by the 5-HT2A antagonist ketanserin (0.5 mg/kg, i.p.).

No significant difference was observed in the motor activity in buspirone, WAY-100635, DOI and ketanserin groups compared with the control or PCA group.

Effect of yokukansan and yokukansan + WAY-100635 on social interaction behaviour in *p*-chloramphetamine-injected rats

As shown in Figure 4a, single injection of yokukansan (0.5 and 1.0 g/kg, p.o.) significantly ameliorated the PCA-induced decrease in social behaviour. Chronic treatment of yokukansan (0.5 and 1.0 g/kg, p.o., for 14 days) also ameliorated the decrease in social behaviour, and the ameliorative effect of yokukansan (1.0 g/kg, p.o.) was completely counteracted by concomitant administration of WAY-100635 (0.1 mg/kg, i.p.) for the same period.

Figure 4b shows the effect of yokukansan on PCA-induced aggressive behaviour. A single injection of yokukansan (0.5 and 1.0 g/kg, p.o.) did not alter the PCA-induced increase in aggressive behaviour. However, chronic administration of yokukansan (0.5 and 1.0 g/kg, p.o.) for 14 days significantly ameliorated the PCA-induced aggressive behaviour in a dose-dependent manner. The ameliorative effect of chronic treatment with yokukansan (1.0 g/kg, p.o.) was counteracted by concomitant administration of WAY-100635 (0.1 mg/kg, i.p.).

No significant difference was observed in the motor activity in single or chronic-treated yokukansan (1.0 and 0.5 g/kg) groups compared with the control or PCA group (Figure 4c).

Discussion

PCA is well known to be a potent serotonergic neurotoxin[21]; this neurotoxin specifically reduces concentrations of 5-HT and its metabolite 5-HIAA in several brain regions by inhibiting tryptophan hydroxylase, a rate-limiting enzyme of 5-HT synthesis.[26,27] We previously demonstrated that 5-HT and 5-HIAA concentrations are decreased in most regions of the brain by a single injection of PCA.[20] In this study, the 5-HT concentration and high-K^+-elicited 5-HT release were examined in the hypothalamus as a representative of the terminal areas of serotonergic innervation, because the hypothalamus is known to be important in regulating aggressive response and emotional behaviour[28,29] as well as an area of the brain rich in 5-HT.[30] PCA decreased both the 5-HT concentration and 5-HT release in the hypothalamus. In addition, chronic treatment with 1.0 g/kg yokukansan (an effective dose for improvement of social and aggressive behaviour) did not ameliorate the PCA-induced decreases in 5-HT concentration and 5-HT release. These results suggest not only that the experimental conditions for induction of cerebral 5-HT depletion were appropriate but also that the effect of yokukansan was not due to amelioration of presynaptic 5-HT depletion or decreased 5-HT release.

In the rats with the PCA-induced 5-HT depletion described above, a significant decrease in social behaviour was observed, and this decrease in social behaviour was ameliorated by the 5-HT1A receptor agonist buspirone or the 5-HT2A receptor antagonist ketanserin. These ameliorative

effects were completely counteracted by a 5-HT1A receptor antagonist, WAY-100635, or a 5-HT2A receptor agonist, DOI, respectively. These results support previous evidence that 5-HT1A and 5-HT2A receptors mediate an opposing or compensatory function in a variety of cellular and behavioural events.[31–35] Because single or repeated (chronic) treatment with yokukansan ameliorated the PCA-induced decrease in social behaviour as well as buspirone or ketanserin, the ameliorating effects of yokukansan may be mediated via agonism or antagonism of 5-HT1A or 5-HT2A receptors like buspirone or ketanserin. However, we previously demonstrated that yokukansan binds to 5-HT1A receptors as a partial agonist but does not bind to 5-HT2A receptors.[20] Taken together, the ameliorating effect on social behaviour by yokukansan is thought to be due to its direct partial agonistic effect on 5-HT1A receptors but not 5-HT2A receptors.

Social behaviour, such as sniffing, following and contacting, observed between two rats has been evaluated as an index of anxiety or anxiolytic effect because benzodiazepine- and 5-HT-related anxiolytic drugs increase social behaviour, whereas anxiogenic agents decrease it.[36] Kuribara and Maruyama[37] demonstrated the anxiolytic effect of yokukansan treatment (0.25–2.0 g/kg, p.o. for 7 days) by using a plus-maze test, which is often used for evaluation of anxiety in mice. Taken together, our data regarding social behaviour in social interaction tests might suggest that yokukansan has an anxiolytic effect.

On the other hand, although PCA-induced aggressive behaviour was ameliorated by the 5-HT1A agonist buspirone, the behaviour was not affected by the 5-HT1A antagonist WAY-100635 at 1.0 mg/kg, which is an effective dose for amelioration of impaired social behaviour or anxiety. In contrast, the 5-HT2A agonist DOI enhanced the PCA-induced aggressive behaviour and the 5-HT2A antagonist ketanserin ameliorated it, suggesting that regulation of the activity of mainly 5-HT2A receptors seems to regulate the aggressive response in PCA-injected rats. In fact, PCA-induced aggressive behaviour was not ameliorated by a single injection of yokukansan, but it was ameliorated by chronic treatment (1.0 g/kg yokukansan) for 14 days. A similar effect of yokukansan regarding improvement of a head-twitch response has been reported by Egashira *et al*.[38] the DOI-induced head-twitch response was reduced by chronic administration of yokukansan for 14 days but not by a single administration. The authors suggest that down-regulation of the 5-HT2A receptor protein by chronic administration of yokukansan is closely related to the ameliorative effect. However, as already described, yokukansan did not bind to 5-HT2A receptors, although it did bind to 5-HT1A receptors.[20] This result suggests that the improvement brought about by yokukansan in aggressive behaviour or head-twitch response is not due to its direct antagonism of 5-HT2A receptors. Recently, Carrasco *et al*.[29] and Wieland *et al*.[35] demonstrated that activation of 5-HT1A receptors produces desensitization of 5-HT2A receptors, suggesting that 5-HT2A receptors might interact with 5-HT1A receptors. Thus, yokukansan also may diminish 5-HT2A receptor function (aggressive behaviour or head-twitch response) via its agonistic binding to 5-HT1A receptors. Because behavioural changes, such as aggressive behaviour and the head-twitch response, were not abolished by a single injection of yokukansan, it is inferred that chronic treatment with yokukansan may be necessary to induce down-regulation of the 5-HT2A receptor protein by its partial agonistic stimulation of 5-HT1A receptors, although detailed studies are needed to confirm this hypothesis. In this study, to verify whether the chronic effect of yokukansan is actually related to the agonism of 5-HT1A receptors, a 5-HT1A antagonist, WAY-100635, was administered together with yokukansan for 14 days. Amelioration of PCA-induced aggressive behaviour by chronic yokukansan treatment was completely counteracted by co-administration of WAY-100635. This result may suggest that the improvement in aggressive behaviour occurs via the 5-HT1A receptor-binding activity of yokukansan. In this study, because the treatment with buspirone, WAY-100635, DOI, ketanserin, yokukansan or yokukansan + WAY-100635 did not affect motor activity as shown in Figures 3c and 4c, it suggests that the behavioural changes (aggressive and social behaviour) in these drug-treated rats were not due to changes in the motor activity (i.e. these changes were selective).

From the results of these behavioural experiments, we suggest the possibility that yokukansan may have two different effects: an acute effect against anxiety and a chronic effect against aggressive behaviour. The former may be due directly to its partial agonism of 5-HT1A receptors, and the later may involve down-regulation of 5-HT2A receptors by its partial agonistic stimulation of 5-HT1A receptors.

We previously demonstrated that *Uncaria* hook, which is a constituent herb of yokukansan that possesses a partial agonistic binding to 5-HT1A receptors, is the essential component of yokukansan in 5-HT1A receptor activity.[20] Zhu *et al*.[39] demonstrated binding of *Uncaria* hook to 5-HT1A receptors using guinea-pig brain membranes. Jung *et al*.[40] reported that administration of *Uncaria* hook to rodents produces anxiolytic-like effects in the elevated plus maze and hole-board test and that the effects are abolished by WAY-100635, a 5-HT1A receptor antagonist. A three-dimensional chromatogram[7] shows that yokukansan contains at least 25 compounds, including oxyindole alkaloids such as corynoxeine, isocorynoxeine, rhynchophiline, isorhynchophiline, and indole alkaloids such as geissoshizine methyl ether, hirsuteine and hirsutine, which are contained in *Uncaria* hook. Geissoshizine methyl ether has been demonstrated to possess agonistic effect on 5-HT1A receptors[41,42] Thus, alkaloids may play an important role in the action of yokukansan, although the details on the active compounds must be clarified in future studies.

Conclusions

A single injection of yokukansan ameliorated PCA-induced decrease in social behaviour but not aggressive behaviour in rats. Chronic treatment for 14 days with yokukansan ameliorated aggressive behaviour as well as social behaviour, suggesting that yokukansan might have two different effects: an acute effect on social behaviour and a chronic effect on aggressive behaviour. The ameliorative effects of yokukansan

were counteracted by co-administration of the 5-HT1A receptor antagonist WAY-100635, suggesting that one of the mechanisms of yokukansan may be an agonistic effect on 5-HT1A receptors.

Declarations

Conflict of interest

The Author(s) declare(s) that they have no conflicts of interest to disclose.

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