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Anxiolytic-like effect of saiboku-to, an oriental herbal medicine, on histaminergics-induced anxiety in mice

Mitsutoshi Yuzurihara[a,*], Yasushi Ikarashi[b], Atsushi Ishige[a], Hiroshi Sasaki[a], Yuji Maruyama[b]

[a]*Kampo and Pharmacognosy Laboratories, Tsumura, 3586 Yoshiwara, Ami-machi, Inashiki-gun, Ibaraki 300-1192, Japan*
[b]*Department of Neuropsychopharmacology (Tsumura), Gunma University School of Medicine, 3-39-22 Showa-machi, Maebashi, Gunma 371-8511, Japan*

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Abstract

Effect of saiboku-to, an oriental herbal medicine, on anxiety in mice was investigated using a light/dark test. Anxiogenic- and anxiolytic-like effects were evaluated on the basis of shortened and prolonged time spent in the light zone of the test. Subacute administration (once a day for 7 days) of saiboku-to (0.5–2.0 g/kg, po) induced anxiolytic-like effect. To assess the effect of saiboku-to on brain histaminergic system in a state of anxiety, Compound 48/80 (1.0 μg/2 μl, icv), a non-neuronal mast cell histamine releaser, or thioperamide (10.0 mg/kg, ip), a neuronal histamine releaser possessing the inhibitory effect of histamine H_3 autoreceptors, induced decrease in the time spent in the light zone by co-injection with cimetidine (10.0 μg/2 μl, icv), a H_2 inhibitor, suggesting anxiety-like effect. These histaminergics-induced experimental anxieties were inhibited by pre-treatment with subacute administration of saiboku-to, as well as single treatment with diazepam. The results suggest that saiboku-to exhibits anxiolytic-like effect closely related to histaminergic system in the brain.

Keywords: Anxiety; Anxiolytic effect; Cimetidine; Compound 48/80; Histamine; Light/dark test; Saiboku-to; Thioperamide

1. Introduction

A traditional oriental herbal medicine "saiboku-to" has been used empirically for a long time in treatments of bronchitis and asthma [10,35] and anxiety-related disorders such as neurosis [8,18].

Anti-allergic effects in the treatment of bronchitis and asthma have been supported by various scientific evidence. For example, saiboku-to shows an inhibitory activity on Type I hypersensitivity reaction in experimentally caused asthma [22,34]. Furthermore, saiboku-to inhibits antigen-induced histamine release from sensitized lung tissue in guinea pig [22] and sensitized peritoneal mast cells in rat [11], or inhibits a mast cell degranulator, Compound 48/80-induced histamine release from peritoneal mast cells in mouse [33] and rat [12]. These findings suggest that saiboku-to is an effective herbal medicine as an anti-histamine release drug.

On the other hand, regarding anxiolytic effect of saiboku-to, although Watanabe et al. [37,38] have reported that magnoliae cortex, a constituent herb of saiboku-to, possesses central depressant effects, the scientific evidence remains insufficient. Recently, Kuribara et al. [19] have demonstrated the anxiolytic-like effect of saiboku-to using an elevated plus-maze test in mice. The authors suggest that the anxiolytic-like effect might be related to $GABA_A$ receptors, as the effect is enhanced by diazepam and antagonized by flumazenil. However, Oishi et al. [24] have demonstrated in mouse brain that diazepam produces an inhibitory effect on histamine turnover by acting on $GABA_A$ receptors. Chikai et al. [5] have also reported that several sedative drugs, $GABA_A$ receptor agonists, such as muscimol, diazepam, and pentobarbital, inhibit histamine release in rat striatum. These findings suggest a possibility that the anxiolytic-like effect of saiboku-to may be due to direct action upon brain histaminergic system. However, the effect of saiboku-to on the relationship between anxiety and histaminergic system in the brain is yet to be examined.

Brain histamine localizes in both histamine neurons and non-neuronal mast cells, with the mast cells storing approxi-

* Corresponding author. Tel.: +81-298-89-3859; fax: +81-298-89-2158.
E-mail address: yuzurihara_mitsutosh@mail.tsumura.co.jp (M. Yuzurihara).

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mately 50% of whole brain histamine levels [4,9,25,41]. Histaminergic neurons project to almost all regions of the mammalian brain from the tuberomammillary nucleus of the posterior hypothalamic region [29,36]. Clinically effective anxiolytic drugs, diazepam, benzodiazepines [5,24], and buspirone, serotonin (5-HT_{1A}) agonists [23], have been found to decrease turnover rate of brain histamine in mice and rats. These findings suggest that histaminergic system in the brain plays an important role in the regulation of anxiety. Furthermore, Imaizumi and Onodera [14] have demonstrated that anxiety-like behavioral activity is induced or enhanced by the combined administration of thioperamide, a neuronal histamine releaser having inhibitory effect of histamine H_3 autoreceptors, with zolanitidine, a histamine H_2 receptor antagonist. In addition, we have demonstrated that anxiety-like behavioral activity is also induced by co-injection of non-neuronal selective mast cell histamine releaser, Compound 48/80, with a histamine H_2 receptor antagonist, cimetidine [43]. These neuronal and non-neuronal histaminergics-induced experimental anxiety models in mice are useful for assessing the effect of saiboku-to on brain histaminergic system in a state of anxiety.

In the present study, we investigated the effect of saiboku-to on the above histaminergics-induced experimental anxieties in mice, using a light/dark test, which was shown appropriate for the evaluation of anxiety [15,42,43].

2. Method

2.1. Animals

Male ddY mice were obtained from SLC (Hamamatsu, Japan). Five animals were housed in a cage and allowed ad lib access to water and standard laboratory food (MF, Oriental Yeast, Tokyo, Japan). The animals were housed in a facility at a temperature of $24 \pm 1°C$, relative humidity of $55 \pm 5\%$, and controlled lighting with light on from 07:00 to 19:00 hours daily.

Experimental protocols met the ''Guidelines for Animal Experimentation'' approved by the Japanese Association of Laboratory Animal Science and the Japanese Pharmacological Society.

2.2. Drugs

Compound 48/80 and cimetidine were purchased from Sigma (St. Louis, MO, USA). Thioperamide maleate was purchased from Research Biochemicals International (Natick, MA, USA). Diazepam (Cercine injection, 5 mg/ml/ampoule) was purchased from Takeda Chemical Industries (Osaka, Japan).

Doses of Compound 48/80 (1.0 μg/2 μl/mouse) and thioperamide (10 mg/10 ml/kg body weight) were prepared in saline. Dose of cimetidine (10 μg/2 μl/mouse) was dissolved in 0.1 N HCl, and subsequently adjusted to the pH at 7.0 with 0.1 N NaOH. Diazepam (0.1, 0.5, and 1.0 mg/10 ml/kg) was diluted with saline before use.

Saiboku-to was obtained in the form of dried powder extract from Tsumura (Tokyo, Japan). The quality of the drug was assured by maintaining the prescribed range of index components. This drug was manufactured from a mixture of bupleuri radix (7.0 g), pinelliae tuber (5.0 g), hoelen (5.0 g), scutellariae radix (3.0 g), magnoliae cortex (3.0 g), zizyphi fructus (3.0 g), ginseng radix (3.0 g), glycyrrhizae radix (2.0 g), perillae herba (2.0 g), and zingiberis rhizoma (1.0 g). The yield was 14.7% for saiboku-to. The doses of the drug used in this study (0.5–2.0 g/10 ml/kg) were prepared in distilled water.

2.3. Apparatus for a light/dark test and experimental procedure [43]

The apparatus (model 111, Tsumura) for a light/dark test consisted of two compartments: one light zone (30 L × 27 W × 27 H cm, 1000 lx) illuminated by fluorescent light, and the other-dark zone (15 L × 27 W × 27 H cm, 5 lx) illuminated by dim red light. The two compartments were separated by a partition with an opening (7.5 × 7.5 cm). The mice were not habituated to the apparatus and the measurement began immediately after the animals were placed in the center of the light zone. The two parameters of total locomotion activity in light and dark zones and the time spent in the light zone were measured for each animal for a period of 5 min; the locomotion activity was assessed using Animex (model MK-110, Muromachi Kikai, Tokyo, Japan) and the time spent in the light zone of the light/dark test was analyzed from video recording.

2.4. Experimental schedule

2.4.1. Effect of single injection of saiboku-to or diazepam in the light/dark test

Saiboku-to (0.5–2.0 g/kg, po) and the vehicle (10 ml/kg water, po) or diazepam (0.5–2.0 mg/kg, sc) and the vehicle (10 ml/kg saline, sc) were administered. To assess the anxiolytic-like effect, a light/dark test was carried out 60 min later in saiboku-to or the vehicle-treated mice and 30 min later in diazepam or the vehicle-treated mice.

2.4.2. Effect of subacute administration (once a day for 7 days) of saiboku-to in the light/dark test and the effect of diazepam on saiboku-to-treated mice

Saiboku-to (0.5–2.0 g/kg, po) or the vehicle (10 ml/kg water, po) was administered once a day for 7 days. The light/dark test was carried out on day 8, 24 h after the last administration of saiboku-to or the vehicle. In order to examine the interaction with diazepam in the saiboku-to or vehicle-treated mice, diazepam (0.5 mg/kg, sc) was injected 30 min before the light/dark test.

Table 1
Effects of single injection of saiboku-to and diazepam on each parameter of the light/dark test

Group	Dose	Locomotion (counts/5 min)	Time spent in the light zone (s/5 min)
Saiboku-to (g/kg)	0 (Water)	355.5 ± 16.6	65.6 ± 3.2 (Control)
	0.5	347.2 ± 13.3	69.5 ± 3.3 (5.9%)
	1.0	327.3 ± 24.5	66.8 ± 7.4 (1.8%)
	2.0	369.6 ± 11.9	70.8 ± 3.2 (7.9%)
Diazepam (mg/kg)	0 (Saline)	334.4 ± 14.7	50.5 ± 4.8 (Control)
	0.5	347.4 ± 12.5	53.0 ± 5.5 (5.0%)
	1.0	361.4 ± 21.5	67.4 ± 4.2 (33.5%) *
	2.0	367.8 ± 11.4	74.3 ± 4.1 (47.1%) **

Saiboku-to (0.5–2.0 g/kg) and the vehicle (10 ml/kg water) were orally administered 60 min prior to the light/dark test. Diazepam (0.5–2.0 mg/kg) and the vehicle (10 ml/kg saline) were subcutaneously injected 30 min prior to the test. Each value is expressed as mean ± S.E. ($n = 10$). Percent changes of drug-treated groups vs. the corresponding control are given in parentheses.

* $P < .05$ vs. the corresponding control.

** $P < .01$ vs. the corresponding control.

2.4.3. Effect of subacute administration of saiboku-to or single injection of diazepam on histaminergics-induced anxiety-like behavioral activity in the light/dark test

In saiboku-to (0.5–2.0 g/kg, po, once a day for 7 days) or vehicle (10 ml/kg water, po, once a day for 7 days)-treated mice, the histaminergics-induced anxiety-like behavioral activity (decrease in the time spent in the light zone of the light/dark test) was induced by co-injection of either Compound 48/80 (1.0 μg/2 μl, icv) or thioperamide (10.0 mg/kg, ip) with cimetidine (10.0 μg/2 μl, icv) 60 min before the light/dark test on day 8, 24 h after the last administration of saiboku-to or the vehicle. Intracerebroventricular injection was carried out following the procedure described by Klemm [17] and Yuzurihara et al. [43]. Sham-operation (intracerebroventricular injection of 2 μl saline in Compound 48/80 with cimetidine-treated experiment, and intracerebroventricular injection of 2 μl saline and intraperitoneal injection of 10 ml/kg saline in thioperamide with cimetidine-treated experiment) was carried out on vehicle-treated mice for 7 days as control group for histaminergics-induced experimental anxiety.

In the other set of experiments, in order to examine the effect of diazepam on the histaminergics-induced experimental anxiety, diazepam (0.1–1.0 mg/kg, sc) was injected in mice 30 min prior to the light/dark test. As control group, sham-operated mice were used and saline (10 ml/kg saline, sc) was injected in the mice in place of diazepam.

2.5. Statistics

Data were expressed as means ± standard error (S.E.). Statistical significance was evaluated by a one-way analysis of variance (ANOVA) followed by Dunnett least significant difference procedure. Significance was accepted at $P < .05$.

3. Results

3.1. Effect of single injection of saiboku-to or diazepam in the light/dark test

Effects of single injection of saiboku-to (0.5–2.0 g/kg, po) or diazepam (0.5–2.0 mg/kg, sc) on each parameter of the light/dark test are shown in Table 1. In comparison with the corresponding control in each group, saiboku-to did not affect the locomotion activity and time spent in the light zone during the light/dark test. On the other hand, diazepams at 1.0 and 2.0 mg/kg, but not 0.5 mg/kg, significantly prolonged the time spent in the light zone without significantly altering the locomotion activity.

3.2. Effect of subacute administration of saiboku-to in the light/dark test and the effect of diazepam on saiboku-to-treated mice

Changes in each parameter of the light/dark test in mice treated with saiboku-to (0.5–2.0 g/kg, po) for 7 days, and the effect of diazepam on saiboku-to-treated mice are shown in Table 2. In saiboku-to-treated groups, saiboku-to dose-dependently prolonged the time spent in the light zone. Significant prolongation of the time (44.8%) was observed with the administration of a high-dose of saiboku-to (2.0 g/kg, po). However, no significant difference in locomotion activity was observed in all groups. Similar results were observed in diazepam-combined groups. Although a significant prolongation of time in the light zone was observed following an injection of diazepam in middle-dose (1.0 g/kg) group of saiboku-to-treated animals, this may contribute to a reduced value of standard error for control group. Comparing saiboku-to and diazepam-combined groups in

Table 2
Effect of subacute administration of saiboku-to on each parameter of the light/dark test and the effect of diazepam on the saiboku-to-treated mice

Group	Saiboku-to (g/kg)	Locomotion (counts/5 min)	Time spent in the light zone (s/5 min)
Saiboku-to	0 (Water)	283.5 ± 25.5	53.3 ± 7.1 (Control)
	0.5	265.0 ± 7.5	60.9 ± 7.2 (14.3%)
	1.0	307.7 ± 18.0	71.0 ± 4.1 (33.2%)
	2.0	290.9 ± 16.8	77.2 ± 5.1 (44.8%) *
Saibiku-to + DZ	0 (Water)	362.3 ± 15.4	52.9 ± 3.2 (Control)
	0.5	390.1 ± 10.9	67.7 ± 4.1 (28.0%)
	1.0	373.5 ± 9.7	71.4 ± 4.9 (35.0%) *
	2.0	394.5 ± 21.4	76.3 ± 5.5 (44.2%) **

Saiboku-to (0.5–2.0 g/kg) and the vehicle (10 ml/kg water) as control were orally administered once a day for 7 days. The light/dark test was carried out on the following day. In diazepam (DZ)-combined groups, diazepam (0.5 mg/kg) was subcutaneously injected 30 min prior to the light/dark test, in all saiboku-to-treated groups, as well as in vehicle control group. Each value is expressed as mean ± S.E. ($n = 10$). Percent changes of drug-treated groups vs. the corresponding control are given in parentheses.

* $P < .05$ vs. the corresponding control.

** $P < .01$ vs. the corresponding control.

1.0 mg/kg dose, since the increase rates (33.2% in saiboku-to-treated group and 35.0% in diazepam-combined group) of the time spent in the light zone was not significant difference, saiboku-to-induced behavioral changes were not enhanced by injection of diazepam.

3.3. Effect of subacute administration of saiboku-to or single injection of diazepam on histaminergics-induced decrease in the time spent in the dark zone

Effects of saiboku-to (0.5–2.0 g/kg, po, administered once a day for 7 days) on histaminergics-induced anxiety-like behavioral activities are shown in Table 3. Both Compound 48/80 (1.0 μg/2 μl, icv) and thioperamide (10 mg/kg, ip) induced a marked decrease (−48.5% and −37%, respectively) in the time spent in the light zone by co-injection with cimetidine (10.0 μg/2 μl, icv), when the value was compared to that in the corresponding sham-operated control group. These histaminergics-induced changes were significantly antagonized or inhibited by pre-treatment with saiboku-to for 7 days, in a dose-dependent manner.

Table 3
Effect of subacute administration of saiboku-to on Compound 48/80 or thioperamide with cimetidine-induced decrease in the time spent in the light zone

Group	Saiboku-to (g/kg)	Locomotion (counts/5 min)	Time spent in the light zone (s/5 min)
Sham-operated control	0 (Water)	323.1 ± 15.2	71.7 ± 3.5 (Control)
Compound 48/80 with cimetidine	0 (Water)	304.1 ± 9.5	36.9 ± 2.4 (−48.5%)***
	0.5	292.7 ± 17.6	43.8 ± 3.1 (−38.9%)***
	1.0	309.2 ± 12.5	55.0 ± 3.8 (−23.3%)†
	2.0	307.1 ± 10.9	62.0 ± 10.6 (−13.5%)‡
Sham-operated control	0 (Water)	284.2 ± 17.4	59.7 ± 4.9 (Control)
Thioperamide with cimetidine	0 (Water)	293.1 ± 14.0	37.6 ± 2.9 (−37.0%)**
	0.5	271.0 ± 12.2	37.4 ± 2.9 (−37.4%)**
	1.0	288.8 ± 10.6	46.2 ± 4.6 (−22.6%)
	2.0	278.6 ± 10.0	54.6 ± 4.1 (−8.5%)†

Saiboku-to (0.5–2.0 g/kg) and the vehicle (10 ml/kg water) as control were orally administered once a day for 7 days. The light/dark test was carried out on the following day. Compound 48/80 (1.0 μg/2 μl, icv) or thioperamide (10.0 mg/kg, ip) was injected with cimetidine (10.0 μg/2 μl, icv) 60 min prior to the test, in saiboku-to and the vehicle-treated mice. Sham operation (intracerebroventricular injection of 2 μl saline in sham-operated group for Compound 48/80 with cimetidine-treated experiment, and intracerebroventricular injection of 2 μl saline and intraperitoneal injection of 10 ml/kg saline in sham-operated group for thioperamide with cimetidine-treated experiment) was also carried out 60 min prior to the test, in vechicle-treated mice for 7 days. Each value is expressed as mean ± S.E. ($n = 10$). Percent changes of drug-treated groups to the corresponding sham-operated control are given in parentheses.

** $P < .01$ vs. the corresponding sham-operated control.

*** $P < .001$ vs. the corresponding sham-operated control.

† $P < .05$ vs. the corresponding control in each histaminergics-induced experiment.

‡ $P < .01$ vs. the corresponding control in each histaminergics-induced experiment.

Table 4
Effect of single injection of diazepam on Compound 48/80 or thioperamide with cimetidine-induced decrease in the time spent in the light zone

Group	Diazepam (mg/kg)	Locomotion (counts/5 min)	Time spent in the light zone (s/5 min)
Sham-operated control	0 (Saline)	340.9 ± 15.0	71.4 ± 4.8 (Control)
Compound 48/80 with cimetidine	0 (Saline)	322.9 ± 22.6	44.4 ± 5.7 (−37.8%)**
	0.1	355.5 ± 15.9	49.3 ± 3.6 (−31.0%)
	0.5	342.1 ± 12.1	70.0 ± 4.5 (−2.0%)††
	1.0	365.3 ± 18.7	79.7 ± 6.4 (11.6%)†††
Sham-operated control	0 (Saline)	302.5 ± 13.6	63.9 ± 4.8 (Control)
Thioperamide with cimetidine	0 (Saline)	268.5 ± 10.3	40.1 ± 4.3 (−37.2%)**
	0.1	291.5 ± 14.9	43.6 ± 3.0 (−31.8%)**
	0.5	248.8 ± 15.2	63.1 ± 6.9 (−1.3%)
	1.0	224.6 ± 15.2*	64.2 ± 7.7 (4.7%)†

Sham operation (intracerebroventricular injection of 2 μl saline in sham-operated group for Compound 48/80 with cimetidine-treated experiment, and intracerebroventricular injection of 2 μl saline and intraperitoneal injection of 10 ml/kg saline in sham-operated group for thioperamide with cimetidine-treated experiment) was carried out 60 min prior to the light/dark test, and 10 ml/kg saline was also subcutaneously injected 30 min prior to the test. In the histaminergics-induced experiments, Compound 48/80 (1.0 μg/2 μl, icv) or thioperamide (10.0 mg/kg, ip) was injected with cimetidine (10.0 μg/2 μl, icv) 60 min prior to the test. Diazepam (0.1–1.0 mg/kg) and the vehicle (10 ml/kg saline) were subcutaneously injected 30 min before the test. Each value is expressed as mean ± S.E. ($n = 10$). Percent changes of drug-treated groups vs. the corresponding sham-operated control are given in parentheses.

* $P < .05$ vs. the corresponding sham-operated control.

** $P < .01$ vs. the corresponding sham-operated control.

† $P < .05$ vs. the corresponding control in each histaminergics-induced experiment.

†† $P < .01$ vs. the corresponding control in each histaminergics-induced experiment.

††† $P < .001$ vs. the corresponding control in each histaminergics-induced experiment.

Table 4 shows the effects of diazepam (0.1–1.0 mg/kg, sc) on histaminergics-induced anxiety-like behavioral activities. Diazepam also antagonized both Compound 48/80 and thioperamide with cimetidine-induced decreases (−38.4% and −37.2%, respectively) in the time spent in the light zone during the light/dark test.

4. Discussion

The time spent in the light zone during the light/dark test is evaluated as a behavioral parameter of anxiety; anxiolytics have been found to prolong the time spent in the light zone while anxiogenics depress this parameter [6,15]. In the present study, subacute administration of saiboku-to for 7 days prolonged the time spent in the light zone, suggesting that saiboku-to possesses anxiolytic effect. The prolongation

of time was not due to the behavioral toxicity such as motor incoordination or an activation of motor behavior, as locomotion activity was not different from that of the control group. Anxiolytic-like effect of saiboku-to in the light/dark test was in agreement with that observed in the other behavioral test for evaluation of anxiety — an elevated plus-maze test, reported by Kuribara et al. [19].

It has been demonstrated that saiboku-to possesses anti-histamine release effect from peritoneal mast cells [11,12,33]. Furthermore, it has been reported that histaminergic system in the brain plays an important role in the regulation of anxiety [13,14,43] as described in the Introduction. These findings suggest a possibility that the anxiolytic-like effect by saiboku-to might be related to brain histaminergic system. In order to examine the effect of saiboku-to on the relationship between anxiety and histaminergic system in the brain, the effect of saiboku-to on histaminergics-induced experimental anxiety was examined in the present study. It has been demonstrated that anxiety-like effect (decrease in the time spent in the light zone in the light/dark test) is induced by injection of not only thiperamide, a histamine H_3 receptor antagonist [13,14], but also Compound 48/80, a mast cell degranulator [43], with H_2 antagonist. In our previous report [43], regarding the mechanism inducing experimental anxiety, we advanced following hypothesis. Both Compound 48/80 and thioperamide enhance endogenous cerebral histamine release via different mechanisms: degranulation from non-neuronal mast cells in the case of Compound 48/80 [27] and inhibition of H_3 presynaptic autoreceptors inhibitory-regulating neuronal histamine release in the case of thioperamide [21]. It has been demonstrated that brain histamine localizes in both histamine neurons and non-neuronal mast cells and half of the entire brain histamine localizes in mast cells [4,9,25,41]. Therefore, released histamine induced by Compound 48/80 or thioperamine is likely to stimulate both postsynaptic H_1 and H_2 receptors. The experimental definite anxiety-like effect in mice was induced by co-injection of H_2 blocker and each histamine releaser, and these combined treatment-induced experimental anxieties were antagonized by H_1 antagonist, mepyramine [43]. Imaizumi and Onodera [14] have also demonstrated that experimental anxiety is induced by co-injection of H_1 agonist and H_3 antagonist. Taken together, these findings suggest that H_1 receptors have anziogenic effect, while H_2 receptors anxiolytic effect. Thus, it is believed that inhibition of H_2 receptors may enhance the anxiogenetic effect of postsynaptic H_1 receptors. As shown in Table 3, in the present study, saiboku-to inhibited Compound 48/80 or thioperamide with cimetidine-induced experimental anxiety, suggesting that saiboku-to has an anxiolytic-like effect closely related to histaminergic system in the brain.

With regard to the mechanism underlying anxiolytic-like effect of saiboku-to, we have not investigated yet the effect of saiboku-to on the affinity to the histamine receptors. Therefore, the possibility of the effects of saiboku-to on the receptors, for example, antagonistic effect on H_1 receptors or agonistic effects on H_2 and H_3 receptors, should not be ruled out. However, as previously described, saiboku-to has proved to possess anti-histamine release effect from mast cells. Therefore, we assume the possibility that anxiolytic-like effect of saiboku-to is closer related to the mechanism of non-neuronal and neuronal histamine release than to the effects on histamine receptors in the brain. Recent studies showed that Compound 48/80-induced histamine release from mast cell is stimulated by activation of phosphatidilinositol (PI) turnover and Ca^{2+} mobilization through guanine nucleotide-binding regulatory protein [3,16,39]. Thus, the consecutive process of G protein — PI turnover — Ca^{2+} mobilization for histamine release may be involved in the mechanism of anxiolytic-like effect of saiboku-to. On the other hand, thioperamide, a selective antagonist of histamine H_3 receptors, increased histamine release by blocking the inhibitory-regulating histamine release of presynaptic histamine H_3 autoreceptors [21]. In general, the physiological release of neurotransmitters from depolarized nerve endings is Ca^{2+}-dependent process [30]. Histamine release of H_3 receptor antagonism by thioperamide is also mediated by Ca^{2+}-dependent exocytosis [2]. We assume that saiboku-to may inhibit a common factor of histamine secretion processes in both Compound 48/80-induced and thioperamide-induced histamine releases. Ca^{2+}-dependent exocytosis is thought to be one of the candidates for their common mechanisms. In fact, Dobashi et al. [7] have demonstrated that saiboku-to suppresses antigen-induced influx of Ca^{2+} into the mast cells of the mouse in a concentration-dependent manner. Magnolol and honokiol, which are biphenyl ingredients of Magnoliae Cortex, as one of the constituent herbal medicines of saiboku-to, antagonize voltage-sensitive Ca^{2+} channels [32,40]. Saiboku-to might inhibit histamine release by interfering with the influx of Ca^{2+} into the mast cells and histamine nerve terminals, although further studies are necessary to confirm this hypothesis.

Kuribara et al. [19] suggest that the anxiolytic-like effect of saiboku-to might be related to $GABA_A$ receptors, as the effect is enhanced by $GABA_A$ agonist, diazepam, and antagonized by the antagonist, flumazenil. However, diazepam has an inhibitory effect on histamine turnover [5] and release [24] by acting on $GABA_A$ receptors. Takeda et al. [31] and Airaksinen et al. [1] suggest the coexistence of glutamate decarboxylase and histidine decarboxylase in neurons located in the hypothalamus. Thus, it is possible that GABA released from these neurons may regulate histaminergic activity of the same neurons. It is likely that $GABA_A$ receptors in histaminergic nerve terminals are involved in the regulation of histamine release. These hypotheses are supported by the results of the present study (Table 4), strongly suggesting that diazepam inhibits histaminergics-induced experimental anxiety. However, the anxiolytic-like effect of diazepam was not enhanced by

subacute-treated saiboku-to (Table 2). This result is different from previous report [19], which suggests enhancement of anxiolytic effect of diazepam by saiboku-to, in an elevated plus-maze test. Thus, the fact that the enhancement effect could not be reproduced by another behavioral test may imply a weakness of interaction between saiboku-to and diazepam.

Saiboku-to consists of 10 herbal medicines. Recently, we demonstrated that anti-histamine release effect of saiboku-to was mainly due to the effect of Magnoliae Cortex as one of the constituent herbs [12]. The exact nature of the anti-histamine release effect of the active ingredients of Magnoliae Cortex remains to be investigated. However, various ingredients have been isolated from Magnoliae Cortex and identified. They are: β-eudesmol, α- and β-pinenes, camphene, and limonene as essential oils; magnocurarine and magnoflorine as the alkaloids; and magnolol and honokiol as the biphenyl compounds [20,26,28]. In particular, it has been demonstrated that magnolol and honokiol posses the central depressant effect [37,38]. Recently, Maruyama et al. [20] reported that both ingredients are anxiolytic agents. Thus, biphenyl compounds, magnolol and honokiol are likely to be responsible as active compounds for anti-histamine release effect of saiboku-to.

A traditional oriental herbal medicine "saiboku-to" has been used empirically for a long time in psychiatric field for treatments of anxiety-related disorders such as neurosis. Our present study in which experimentally caused anxieties in mice were inhibited by subacute treatment with saiboku-to, would support clinical application of saiboku-to as an anxiolytic.

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