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Inhibition of Gastric Acid Secretion by Saiboku-to, an Oriental Herbal Medicine, in Rats

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Intraduodenal saiboku-to (250–1000 mg/kg) dose-dependently reduced gastric acid secretion and histamine output, without altering acetylcholine output in pylorus-ligated rats. Saiboku-to also inhibited subcutaneous bethanechol (1 mg/kg) and tetragastrin (0.3 mg/kg) -induced increases in gastric acid secretion in vagotomized pylorus-ligated rats; however, it did not inhibit subcutaneous histamine (20 mg/kg) -induced increase in acid secretion. These results, taken together, suggest a possibility that saiboku-to may inhibit histamine release. Thus, the effect of saiboku-to on histamine release was directly investigated by using anti-dinitrophenyl IgE-sensitized rat peritoneal mast cells. Antigen (dinitrophenyl)-induced histamine release from the mast cells was clearly dose-dependently inhibited by saiboku-to at concentrations of 0.1–1.0 mg/ml. These results suggest that the inhibited gastric acid secretion with saiboku-to is due to inhibited histamine release.

KEY WORDS: gastric acid; histamine; mast cells; pylorus-ligated rats; saiboku-to.

Saiboku-to, an Oriental herbal medicine, is a traditional Chinese medicine called Kampo medicine in Japan. It is a mixture of 10 medical herbs and has been used for treatment of bronchial asthma (1) and anxiety-related disorders such as neurosis (2). Recently, we demonstrated that saiboku-to inhibited gastric erosion induced by restraint water-immersion stress or by ethanol treatment (3). The antierosion effect involved the inhibition of gastric acid secretion, one of aggressive factors. However, acid secretion is regulated by many factors including anxietic effect in the central nervous system (CNS); vagal activity; cholinergic, histaminergic, and gastrinergic neurotransmissions; and the activities of various postsynaptic receptors such as muscarine, histamine H_2 and gastrin receptors; and proton pump (4). On the other hand, Nishiyori et al (5) and Toda et al (6) have suggested that saiboku-to inhibits type I hypersensitivity reaction through the suppression of histamine release. We, thus, hypothesized in a previous paper (3) that the inhibition of gastric acid secretion might be also related to the release of gastric histamine. However, the specifics of the inhibitory mechanism of saiboku-to on gastric acid remain to be fully demonstrated.

The purpose of the present study is to clarify the effect of the inhibitory mechanism of saiboku-to on gastric acid secretion.

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MATERIALS AND METHODS

Animals. Male Wistar rats purchased from SLC Japan (Hamamatsu, Japan) were housed in a facility at a temperature of 24 ± 1°C, relative humidity of 55 ± 5%, and controlled lighting with lights on from 07:00 to 19:00 hr

daily. The animals were allowed free access to water and standard laboratory food (MF, Oriental Yeast, Tokyo, Japan).

Experimental protocols were approved by the Committee of Animal Experimentation at Gunma University School of Medicine and met the guidelines of the Japanese Association for Laboratory Animal Science.

Reagents and Drugs. Bethanechol chloride, proglumide, anti-dinitrophenyl (DNP) IgE, phosphatidylserine, spermidine trihydrochloride, *o*-phthalaldehyde (OPA), and bovine serum albumin (BSA) were purchased from Sigma (St. Louis, Missouri, USA). Cimetidine, atropine sulfate monohydrate, eserine sulfate, *N*-2-hydroxyethylpiperazine-*N'*-ethanesulfonic acid (HEPES), phenolphthalein, and toluidine blue were purchased from Wako (Osaka, Japan). The remaining drugs were: histamine dihydrochloride (Nacalai, Kyoto, Japan), tetragastrin potassium iodide (Boehringer Ingelheim, Tokyo, Japan), DNP-BSA (LSL, Tokyo, Japan), 16,16-dimethyl prostaglandin E_2 (dmPGE$_2$; Cayman Chemical, Ann Arbor, Michigan, USA), and heparin sodium injection (Green Cross, Osaka, Japan). Ethylhomocholine iodide (EHC) was synthesized from 3-diamino-1-propanol and iodoethane purchased from Sigma.

Saiboku-to is composed of bupleuri radix (7 g), pinelliae tuber (5 g), hoelen (5 g), scutellariae radix (3 g), magnoliae cortex (3 g), zizyphi fructus (3 g), ginseng radix (3 g), glycyrrhizae radix (2 g), perillae herba (2 g) and zingiberis rhizoma (1 g). The 10 dried medical herbs were boiled gently in 500 ml of water for 60 min, filtered, and the resultant decoction freeze-dried to obtain saiboku-to extract. The yield of the extract was 14.7%. Saiboku-to used in the present study 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.

In the experiments of gastric acid secretion, doses of bethanechol, histamine, tetragastrin, atropine, cimetidine, and dmPGE$_2$ were prepared in saline (2 ml). Doses of saiboku-to were prepared in distilled water (10 ml) and proglumide in 1% Tween 80 (10 ml).

Experimental Schedules for Determination of Gastric Acid, Histamine and ACh Levels in Gastric Juice. The rats weighing 240–260 g after a 24-hr fast were pylorus-ligated with or without gastric vagotomy under 2.0% halothane anesthesia performed using a small animal anesthetizer (model TK-4, Bio Machinery, Funabashi, Japan). Vagotomy was carried out by cutting bilateral (anterior and posterior) gastric branches of the celiac vagus (7). Drugs were intraduodenally or subcutaneously administered immediately after pylorus-ligation. The rats awaked within 5 min after the surgical anesthesia stopped. In the experiment for determination of ACh level in gastric juice, 1 ml of 1 mM eserine solution was orally administered to the awakened rats. Four hours following pylorus ligation, the stomachs of the rats were removed after the cardia was ligated under the same halothane anesthesia. Gastric juice was collected and centrifuged at 17,300*g* at 4°C for 10 min. After measuring the volume of gastric juice, an aliquot was used for the determination of acidity, histamine, or ACh levels.

Determination of Acidity in Gastric Juice. As an indicator, 10 μl of 1% phenolphthalein in ethanol was added to 1.0 ml of diluted gastric juice in water (gastric juice–water 1:9). Acidity was measured by titration with 0.01 M NaOH and expressed as milliequivalents per liter. Acid output was calculated as follows: Acid output (μeq/hr) = volume (ml/4 hr) × acidity (meq/liter/sampling time (4 hr).

Determination of Histamine Level in Gastric Juice. One hundred microliters of 1.0 mg/ml spermidine as an internal standard of histamine, 120 μl of 5M NaOH, 400 mg of NaCl, and 3.5 ml of *n*-butanol were added to 1.0 ml of gastric juice. The mixture was centrifuged at 1300*g* at 4°C for 5 min. After the lower layer was removed, an upper layer was washed twice with 2 ml of NaCl-saturated 0.1 M NaOH. Three milliliters of the butanol layer (an upper layer) was transferred to the other test tube, and 4 ml of *n*-heptane as well as 2.5 ml of 0.1 M HCl were added to it and mixed. After 5 min, the upper layer was removed by aspiration. An aliquot (100 μl) of the lower layer was injected into a high-performance liquid chromatography (HPLC) system for the determination of histamine level.

The HPLC system is equipped with fluorescent detection (LC-10AD, Shimazu, Kyoto, Japan) as well as postcolumn derivatization with OPA (8). A STR ODS-II reversed-phase column (4.6 mm ID × 150 mm length, Shimazu) was used for separation of histamine and spermidine. The mobile phase consisted of a mixture of solution A (100 mM sodium tartaric acid buffer, pH 4.4, containing 10 mM sodium 1-octanesulfonate) and solution B (99.7% methanol for HPLC) (A/B 2:1). The flow-rate was maintained at 1.0 ml/min. The effluent from the column was mixed with a postcolumn solution (400 mM sodium borate buffer, pH 9.2, and 10 mM OPA in methanol; 2:1) at a flow rate of 0.5 ml/min. The mixture of the effluent and the postcolumn solution flowed to a reaction coil of stainless steel tubing (0.5 mm ID × 4.0 m length) at a final flow rate of 1.5 ml/min and passed through a fluorescent detector where the OPA derivatives of histamine and spermidine were detected at excitation of 360 nm and emission of 440 nm. Temperatures of the separation column and the reaction coil were maintained at 50°C. A Shimazu LC-10A workstation was employed for data collection and processing. The retention times were 4.40 min for histamine and 7.89 min for spermidine. The detection limit of histamine in the injected sample was 10 ng.

Histamine output was calculated as follows: Histamine output (ng/hr) = volume (ml/4 hr) × histamine level (ng/ml)/sampling time (4 hr).

Determination of ACh Level in Gastric Juice. One milliliter of the gastric juice containing eserine was neutralized at pH 7.4 by 1 M NaOH. EHC (1 μmol/20 μl), an internal standard of ACh, was added to it. The mixture was centrifuged at 17,300*g* at 4°C for 15 min. The supernatant was purified by passage through a 0.45-μm millipore filter. An aliquot, typically 10 μl of the filtrate, was injected into HPLC system for determination of the ACh level.

The HPLC system (Nanospace, Shiseido, Tokyo, Japan) is equipped with electrochemical detection (9). An ACh Separation analytical column [3 μm, 4 × 60 mm, polymeric styrene-based packing materials, Bioanalytical Systems (BAS) Tokyo, Japan] was used for separation of EHC and ACh. An immobilized column (5 × 4 mm, BAS) containing acetylcholinesterase and choline oxidase was used for postcolumn reaction. A glassy carbon precolumn (4 × 10 mm, Irica, Kyoto, Japan) was set up between the injector and the

analytical column to trap the interfering compounds. The mobile phase was 0.05 M phosphate buffer, pH 8.4, containing 1.0 mM disodium EDTA and 0.4 mM sodium 1-octanesulfonate. The flow rate was set at 0.70 ml/min. The potential of a platinum working electrode was set to +0.50 V vs a Ag–AgCl reference electrode. Temperatures of the precolumn, separation column and postcolumn were all maintained at 35°C. A Shiseido Nanospace workstation was employed for data collection and processing. The retention times were 4.82 min for EHC and 9.32 min for ACh. The detection limit of ACh in the injected sample was 0.5 pmol.

ACh output was calculated as follows: ACh output (pmol/hr) = volume (ml/4 hr) × ACh level (pmol/ml)/ sampling time (4 hr).

Experimental Schedules for Antigen-Induced Histamine Release from Rat Peritoneal Mast Cells. The experiment was conducted using the method described by Sakaguchi et al (10). The rats weighing 200–250 g were injected intraperitoneally 1.0 ml monoclonal anti-DNP IgE (titer: 1000). Twenty-four hours later, the animals were decapitated and 10 ml of Tyrode's solution (in mM: NaCl 137.0, KCl 1.0, $MgCl_2$ 1.0, $CaCl_2$ 1.6, NaH_2PO_4 0.41, and 1% glucose, pH 7.4) containing 0.3% BSA and 5.0 units/ml heparin was injected intraperitoneally. After gently massaging the abdomen for 2–3 min, the collected peritoneal solution containing mast cells was centrifuged at 70*g* for 5 min at 4°C. The residue containing mast cells was washed three times with 10 ml of Tyrode's solution and resuspended in 10 mM HEPES–Tyrode's buffer, pH 7.4, containing 0.1% BSA. For the determination of concentration of mast cells in the suspended solution, 50 μl of 0.05% toluidine blue in saline was added to an equal volume of the cell suspension. The mast cells stained with toluidine blue were counted microscopically using Burker-Turk counting chamber. Finally, the concentration of mast cells in the suspension was adjusted to 1×10^5 cells/ml.

Fifty microliters of saiboku-to solution (final concentration: 0.1, 0.3, and 1.0 mg/ml) was added to 400 μl of the cell suspension preincubated for 5 min at 37°C. After incubation at 37°C for 10 min, the release of histamine from mast cells was elicited by the addition of 50 μl of antigen solution, a mixture of DNP-BSA (final concentration: 10 μg/ml) and phosphatidylserine (final concentration: 10 μg/ml). The phosphatidylserine was used for maintaining a stable antigen–antibody reaction. After additional incubation at 37°C for 10 min, the reacted suspension was centrifuged at 70*g* at 4°C for 5 min to separate the supernatant and the residue (mast cells). Five hundred microliters of 3% $HClO_4$ was added to the residue. Ten microliters of 33% $HClO_4$ or 3% $HClO_4$ was added to 100 μl of the supernatant or the residue-derived supernatant, respectively, and then 20 μl of spermidine (1.0 mg/ml) dissolved in distilled water as the internal standard was added and filtered. An aliquot, typically 5 μl of the filtrate, was injected into HPLC system for determination of histamine level.

The histamine release was expressed as the percentage of total cellular content of histamine in each experiment. The total cellular content is the sum of the levels in the supernatant and in the residue measured after antigen challenge.

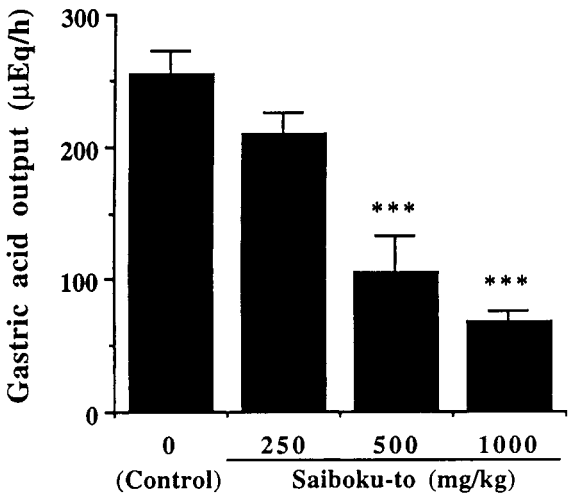

Fig 1. Effect of saiboku-to on gastric acid secretion in pylorus-ligated rats. Animals were killed 4 hr after pylorus ligation for gastric juice collection. Saiboku-to (250, 500, and 1000 mg/kg) or vehicle (10 ml/kg distilled water in control) was intraduodenally injected immediately after pylorus ligation. Data are mean ± SEM ($N = 6$). Statistical significance was assayed by a one-way ANOVA, followed by Bonferroni multiple-comparison test: $^{***}P < 0.001$ compared to control.

RESULTS

Effect of Saiboku-to on Outputs of Gastric Acid, ACh, and Histamine into Gastric Juice in Pylorus-Ligated Rats. Effects of saiboku-to (250, 500, and 1000 mg/kg, intraduodenally) on the outputs of gastric acid, ACh, and histamine into gastric juice are shown in Figures 1, 2, and 3, respectively.

Control gastric acid output was 250 ± 25 μeq/hr. Saiboku-to dose-dependently inhibited the acid output. Significant inhibitions were observed at 500 mg/kg ($P < 0.05$) and 1000 mg/kg ($P < 0.01$).

Control ACh output was 103 ± 28 pmol/hr. No significant changes in ACh output were observed in saiboku-to (250, 500, and 1000 mg/kg, intraduodenally) -treated rats.

Control histamine output was 120 ± 11 ng/hr. Saiboku-to dose-dependently reduced the histamine output. Significant reductions were observed at 500 mg/kg ($P < 0.05$) and 1000 mg/kg ($P < 0.001$).

Effects of Saiboku-to and Various Gastric Acid Inhibitors on Histamine-, Bethanechol-, and Tetragastrin-Induced Gastric Acid Secretion in Vagotomized Pylorus-Ligated Rats. In preliminary studies (data not shown), effects of secretagogues (bethanechol, histamine, and tetragastrin) on gastric acid secretion of pylorus-ligated rats were examined. Gastric acid secretion was completely inhibited by vagotomy. In vagotomized rats, all secretagogues dose-depen-

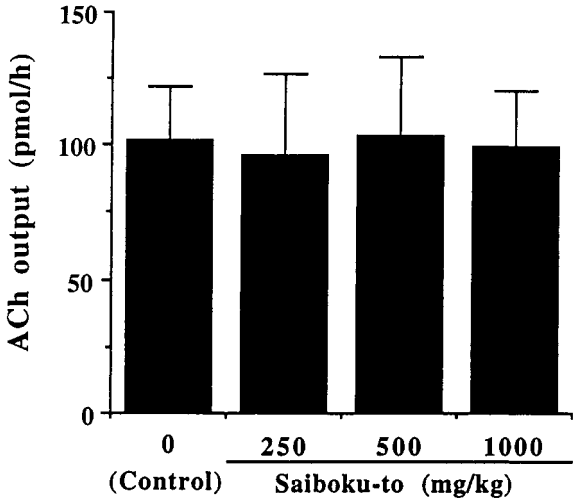

Fig 2. Effect of saiboku-to on ACh output into gastric juice in pylorus-ligated rats. Animals were killed 4 hr after pylorus ligation for gastric juice collection. Saiboku-to (250, 500, and 1000 mg/kg) or vehicle (10 ml/kg water in control) was intraduodenally injected immediately after pylorus ligation. Data are mean ± SEM (N = 10). Statistical significance was assayed by a one-way ANOVA, followed by Bonferroni multiple-comparison test. No significant differences were observed.

dently stimulated gastric acid secretion. We confirmed that the equivalent dose of each secretagogue (subcutaneous injection) on gastric acid secretion was 1 mg/kg for bethanechol, 20 mg/kg for histamine, and 0.3 mg/kg for tetragastrin. Effects of gastric acid inhibitors (atropine, cimetidine, $dmPGE_2$, and proglumide) on gastric acid secretion were also examined in earlier studies using pylorus-ligated rats. We confirmed that the equivalent dose of each inhibitor on gastric acid secretion was 0.1 mg/kg (subcutaneously) for atropine, 20 mg/kg (subcutaneously) for cimetidine, 80 μg/kg (subcutaneously) for $dmPGE_2$, and 500 mg/kg (intraduodenally) for proglumide. From these results, we determined the dose of each secretagogue and inhibitor in the following experiments.

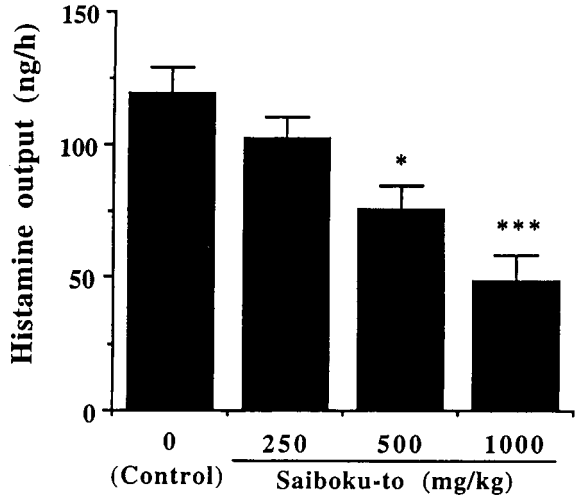

Fig 3. Effect of saiboku-to on histamine output into gastric juice in pylorus-ligated rats. Animals were killed 4 hr after pylorus ligation for gastric juice collection. Saiboku-to (250, 500, and 1000 mg/kg) or vehicle (10 ml/kg in control) was intraduodenally injected immediately after pylorus ligation. Data are mean ± SEM (N = 10). Statistical significance was assayed by a one-way ANOVA, followed by Bonferroni multiple-comparison test: $^{*}P < 0.05$ and $^{***}P < 0.001$ compared to control.

Figure 4 shows the effects of saiboku-to and various gastric acid inhibitors on gastric acid secretion stimulated by each secretagogue in vagotomized rats. The bethanechol (1 mg/kg, subcutaneously) -induced increase in gastric acid output (51.57 ± 5.72 μeq/hr) was significantly inhibited by 0.1 mg/kg atropine (−65%, $P < 0.001$), 20 mg/kg cimetidine (−55%, $P < 0.01$), 80 μg/kg $dmPGE_2$ (−65%, $P < 0.001$), 500 mg/kg proglumide (−35%, $P < 0.05$), and 1000 mg/kg saiboku-to (−40%, $P < 0.01$), as shown in Figure 4A.

Histamine (20 mg/kg, subcutaneously) -induced increase in acid output (51.73 ± 7.93 μeq/hr) was significantly inhibited by cimetidine (−88%, $P < 0.001$) and $dmPGE_2$ (−64%, $P < 0.01$), but was not affected by atropine, proglumide and saiboku-to, as shown in Figure 4B.

Tetragastrin (0.3 mg/kg, subcutaneously) -induced acid output (53.56 ± 8.13 μeq/hr) was significantly inhibited by cimetidine (−86%, $P < 0.001$), $dmPGE_2$ (−84%, $P < 0.001$), proglumide (−42%, $P < 0.05$) and saiboku-to (−35%, $P < 0.05$), but was not affected by atropine, as shown in Figure 4C.

Effect of Saiboku-to on Histamine Release from Rat Peritoneal Mast Cells. Effects of saiboku-to (0.1, 0.3, and 1.0 mg/ml) on antigen-induced histamine release from mast cells sensitized with anti-DNP-IgE are shown in Figure 5. In the control group, a 30% histamine release from the mast cells was induced by the addition of 10 μg/ml of the antigen, DNP-BSA. Antigen-induced histamine release was dose-dependently inhibited by saiboku-to at 0.1 (−53% of control level, $P < 0.01$), 0.3 (−73%, $P < 0.001$), and 1.0 mg/ml (−90%, $P < 0.001$).

DISCUSSION

In the present study, we first reconfirmed our previous finding (3) that saiboku-to inhibits gastric acid secretion as shown in Figure 1. Our aim was to investigate the mechanism of this gastric acid inhibition with saiboku-to. Many factors are thought to be

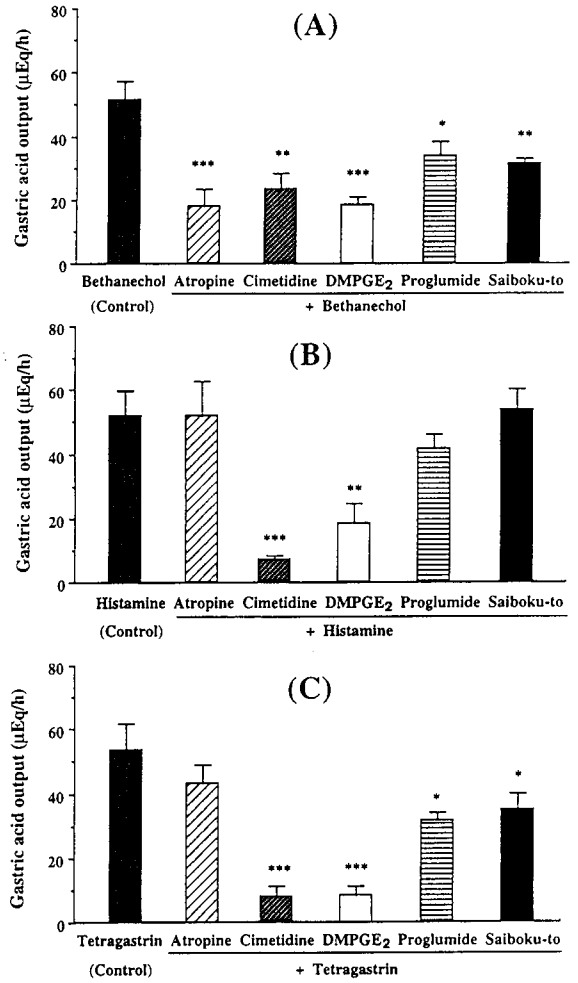

Fig 4. Effect of saiboku-to and various gastric acid inhibitors on bethanechol- (A), histamine- (B), and tetragastrin- (C) induced gastric acid secretion in vagotomized pylorus-ligated rats. Atropine (0.1 mg/kg, subcutaneously), cimetidine (20 mg/kg, subcutaneously), $dmPGE_2$ (0.080 mg/kg), proglumide (500 mg/kg), or saiboku-to (1000 mg/kg) was injected following pylorus ligation after vagotomy. Each secretagogue (1 mg/kg bethanechol, 20 mg/kg histamine, or 0.3 mg/kg tetragastrin) was subcutaneously injected immediately after drug treatments. Animals were killed 4 hr after pylorus ligation for gastric juice collection. Data are mean ± SEM ($N = 6$). The effect of the drug was assessed using Cochran-Cox test: $^{*}P < 0.05$, $^{**}P < 0.01$, and $^{***}P < 0.001$ compared to corresponding control.

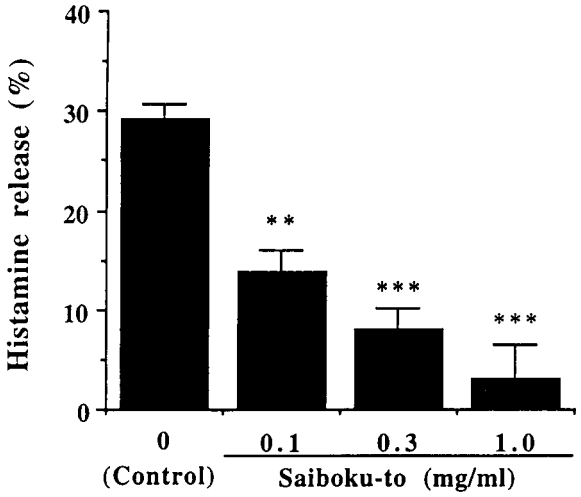

Fig 5. Effect of saiboku-to on antigen-induced histamine release from rat peritoneal mast cells. Data are mean ± SEM ($N = 4$). Statistical significance was assayed by a one-way ANOVA, followed by Bonferroni multiple-comparison test: $^{**}P < 0.01$ and $^{***}P < 0.001$ compared to control.

responsible for acid inhibition. They include: (1) anxiolytic effect on the CNS; (2) anti-vagal nerve activity; (3) inhibition of cholinergic, histaminergic, or gastrinergic neurotransmission; (4) inhibition of the activities of various postsynaptic gastric receptors such as histamine H_2, muscarine, and gastric receptors located on the basolateral membrane of the acid-secreting parietal cell; and (5) inhibition of the activity of the final common acid-secreting pump, H^+/K^+-ATPase located on the canalicular surface of the cell (4).

We eliminated the effect of saiboku-to on efferent activity in gastric vagus via CNS by using vagotomized rats. Under the condition of vagotomy, cimetidine, a histamine H_2 receptor antagonist, inhibited gastric acid secretion stimulated by bethanechol and tetragastrin as well as histamine, suggesting that histamine plays a pivotal role in stimulation of the parietal cell, as proposed by Hirschowitz et al (11). This hypothesis was also supported by the data showing that $dmPGE_2$ antagonized gastric acid secretion, which was stimulated by three secretagogues. Histamine increases the level of intracellular cAMP by binding to a stimulatory membrane receptor (R_s or H_2 receptor) that is coupled via a stimulatory GTP-binding protein (G_s), which activates adenylate cyclase stimulating gastric acid secretion. Prostaglandin E decreases the level of cAMP by binding to an inhibitory membrane receptor (R_i) that is coupled via an inhibitory GTP-binding protein (G_i), which inhibits of adenylate cyclase (12). In addition, atropine, a muscarinic receptor inhibitor, or proglumide, a gastrinic receptor inhibitor, inhibited gastric acid secretion stimulated by the respective secretagogues, bethanechol or tetragastrin, but did not antagonize the acid secretion stimulated by other secretagogues. Taken together, these results suggest not only that receptors for histamine, ACh, and gastrin are present on the parietal cell but also that ACh and gastrin are capable of releasing histamine from

mucosal histamine-storing cells such as, presumably, enterochromaffine-like cells and mast cells (13).

In vagotomized pylorus-ligated rats, saiboku-to inhibited gastric acid secretion induced by both bethanechol and tetragastrin, but not by histamine. This result is in line with those obtained by investigation of the effect of certain mast cell stabilizers such as sodium cromoglycate and FPL52694 (14) on gastric acid secretion in rats. Tabuchi and Furuhama (15) have also demonstrated that DS-4574, a mast cell stabilizer, inhibits not only gastric acid secretion induced by both carbachol and pentagastrin (but not by histamine), but also histamine leakage into the gastric juice produced by carbachol or pentagastrin, suggesting that the inhibitory effect of these drugs on gastric acid secretion is mediated by inhibition of endogenous histamine release from histamine-storing cells in the stomach. Recently, we demonstrated that histamine and ACh levels in the gastric juice of pylorus-ligated rat reflected the activities of histaminergic and cholinergic neurotransmissions (16). The present study shows that saiboku-to inhibited histamine output, without affecting ACh output into gastric juice, suggesting that saiboku-to may inhibit histamine release from histamine-storing cells in the stomach, yet not affect vagal activity. Furthermore, the inhibitory effect of saiboku-to on histamine release was directly investigated by using anti-dinitrophenyl (DNP) -IgE-sensitized rat peritoneal mast cells. Antigen (DNP) -induced histamine release from the mast cells was clearly dose-dependently inhibited by saiboku-to at 10^{-4} to 10^{-3} g/ml. These results strongly suggest that inhibited histamine release is responsible for inhibition of gastric acid secretion by saiboku-to.

Nishiyori et al (5) have reported that saiboku-to shows an inhibitory activity on the type I hypersensitivity reaction in experimentally caused asthma. The authors conclude that the inhibition is via the suppression of histamine release as antigen-induced histamine release from sensitized guinea pig lung tissue was inhibited by 10^{-6} to 10^{-4} g/ml of saiboku-to in dose-dependent fashion. Toda et al (6) also reported that saiboku-to is useful in the treatment of type I allergic reactions as saiboku-to (10^{-4}–10^{-2} g/ml) inhibits histamine release from and degranulation of mouse peritoneal mast cells induced by the polymeric amine compound 48/80, a very potent mast cell degranulator. Taken together with these findings, the present results showing an inhibitory effect of saiboku-to on histamine release from mast cells not only support previous allergic studies but also suggest that inhibition of gastric acid secretion by saiboku-to is caused by the same mechanism as an effective inhibitor of histamine release.

Saiboku-to consists of 10 medical herbs. Some constitutional herbs in saiboku-to—bupleuri radix, scutellariae radix, zizyphi fructus, and glycyrrhizae radix—have been shown to inhibit 48-hr homologous passive cutaneous anaphylaxis in rat as a typical model of type I reaction using anti-DNP-ascaris-IgE serum (17). Magnolol in magnoliae cortex inhibits stress-induced lesions and gastric secretion (18). It is also known that glycyrrhizin in glycyrrizae radix and the derivative, carbenoxolone, protect restraint water-immersion stress-induced ulcer (18). Polysaccharides prepared from bupleuri radix have been reported to protect gastric mucosa (19). Thus, some substances in saiboku-to may be thought to be the candidates for anti-histamine release. We have also identified at least 16 compounds including the known compounds such as magnolol, honokiol, glycyrrhizin, and saikosaponin from the extract of saiboku-to (unpublished data). However, there have been no reports investigating the effects of these compounds on histamine release. The relationship between the identified substances and histamine release warrants further study.

In conclusion, the present results suggest that an inhibitory effect of saiboku-to on gastric acid secretion is likely mediated by inhibition of endogenous histamine release from histamine-storing cells in the stomach. These findings will be useful for identifying substances in saiboku-to which are effective as anti-histamine release drugs.

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