Potentiation of $\beta$-Adrenergic Function by Saiboku-To and Bakumondo-To in Canine Bronchial Smooth Muscle

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ABSTRACT—To determine the effects of the Kampo drugs Saiboku-to, Bakumondo-to and Orengedoku-to on $\beta$-adrenergic function in airway smooth muscle, we studied isolated canine bronchial segments under isometric conditions in vitro. Incubation of tissues with these drugs did not alter the contractile responses to acetylcholine and histamine. The relaxation of tissues precontracted with acetylcholine induced by isoproterenol was augmented by Saiboku-to and Bakumondo-to, so that the concentration of isoproterenol required to produce a half-maximal effect (IC50) was decreased from $4.6\pm0.5\times10^{-8}\text{M}$ to $1.9\pm1.0\times10^{-8}\text{M}$ ($P<0.05$) and to $1.0\pm0.8\times10^{-8}\text{M}$ ($P<0.01$), respectively, whereas Orengedoku-to had no effect. These effects were concentration-dependent with the threshold concentrations being 0.3 mg/ml for Saiboku-to and 0.1 mg/ml for Bakumondo-to. Saiboku-to and Bakumondo-to did not affect cyclic AMP levels in airway smooth muscle per se but potentiated the isoproterenol-induced cyclic AMP accumulation. These results suggest that Saiboku-to and Bakumondo-to but not Orengedoku-to potentiate $\beta$-adrenergic function in airway smooth muscle, which may reflect the efficacy of these drugs on airway hyperresponsiveness and asthma.

Keywords: Saiboku-to, Bakumondo-to, $\beta$-Adrenoceptor, Airway smooth muscle, Asthma

The Kampo drugs Saiboku-to and Bakumondo-to are traditional herbal medicines that have been widely used in the treatment of asthma and bronchitis. There is increasing evidence that Saiboku-to exerts its inhibitory effect on bronchoconstriction and airway hyperresponsiveness by attenuating both type I and type IV allergic reactions (1, 2), and by reducing the IgE-dependent release of several chemical mediators including histamine from basophils and platelet-activating factor from neutrophils (3). Bakumondo-to, another antiasthmatic agent consisting of crude drugs with some homology to Saiboku-to, is also effective in inhibiting cough and airway hyperresponsiveness (4), although its mechanism of action remains uncertain. Orengedoku-to is an antihypertensive Kampo drug, which causes a potent relaxation of vascular smooth muscle, but its effect on airway smooth muscle is unknown.

The autonomic nervous system plays an important role in the regulation of bronchomotor tone (5), and it has been proposed that the impairment of $\beta$-adrenergic receptor function is one of the characteristic features of airway hyperresponsiveness and exacerbation of asthmatic symptoms (6). Therefore, upregulation of airway $\beta$-adrenergic receptors would benefit prevention and treatment of asthma. In the present study, to determine whether these Kampo drugs can affect $\beta$-adrenergic function and, if so, what the mechanism of action is, we studied canine bronchial smooth muscle segments under isometric conditions in vitro.

MATERIALS AND METHODS

Preparation of bronchial segments

Mongrel dogs, weighing 22–34 kg, were anesthetized with sodium pentobarbital (30 mg/kg, i.v.). Both lungs were removed and immersed in oxygenated Krebs-Henseleit solution of the following composition: 118 mM NaCl, 5.9 mM KCl, 2.5 mM CaCl$_2$, 1.2 mM MgSO$_4$, 1.2 mM NaH$_2$PO$_4$, 25.5 mM NaHCO$_3$ and 5.6 mM glucose. Ring segments of intrapulmonary lobar or segmental bronchi were dissected free of connective tissue and mounted in organ chambers filled with 14 ml of Krebs-Henseleit solution maintained at 37°C and at pH 7.4 and continuously bubbled with 95% O$_2$–5% CO$_2$. The bronchial segments were connected to strain gauges (TB-652T,
Nihon Kohden, Tokyo) for continuous recording of isometric tension by a pen recorder (WT-685G, Nihon Kohden). The tissues were then allowed to equilibrate for 60 min while they were washed with Krebs-Henseleit solution every 15 min, and the resting tension was adjusted to 4 g (7). A contractile response was measured as the difference between peak tension developed and resting tension.

**Effects of Kampo drugs on contractions induced by acetylcholine and histamine**

To assess whether Kampo drugs alter the responses to bronchospasmogenic agonists, the effects of Saiboku-to, Bakumondo-to and Orengedoku-to (1.0 mg/ml for each) on the contractions induced by acetylcholine and histamine were determined. Cumulative concentration-response curves for acetylcholine (10⁻⁹ to 10⁻³ M) were generated in the same tissues before and 30 min after the addition of each Kampo drug. Response to a given concentration was allowed to reach a plateau before the next higher concentration. Contractile responses to histamine (10⁻⁸ to 3 × 10⁻³ M) were likewise assessed in the presence and absence of the Kampo drug by using different tissues to avoid tachyphylaxis. At the end of these experiments, each bronchial ring was blotted on a gauze pad and weighed. Maximal contractions (E_max) were normalized for tissue weight and expressed as grams per gram of tissue weight. The concentrations of acetylcholine and histamine required to produce half-maximal contractions (EC₅₀) were determined by linear regression analysis.

**Effects of Kampo drugs on ß-adrenergic function**

To determine whether Kampo drugs alter ß-adrenergic function in airway smooth muscle, after treatment of tissues with either Saiboku-to, Bakumondo-to or Orengedoku-to (1.0 mg/ml) for 30 min, we added acetylcholine (10⁻¹ M) to the chamber, and when the contractile response reached a plateau, the ß-adrenergic receptor agonist isoproterenol was cumulatively added in half-molar increments, final concentrations ranging from 10⁻¹⁰ to 10⁻⁵ M, at 10-min intervals or 2 min after a stable plateau was achieved, whichever was the longer period. In the control experiment, tissues were preincubated for 30 min in the absence of Kampo drugs and the relaxant response to isoproterenol was determined in a similar manner. The concentration of isoproterenol that produced a half-maximal effect (IC₅₀) was determined by linear regression analysis.

In evaluating the concentration-dependent effects of Kampo drugs, tissues were incubated with Saiboku-to, Bakumondo-to or Orengedoku-to at various concentrations (0.01 – 1.0 mg/ml) for 30 min, precontracted with 10⁻⁵ M acetylcholine, and then the muscle relaxation produced by isoproterenol at a concentration near its EC₅₀ (5 × 10⁻⁸ M) was determined.

**Measurement of intracellular cyclic AMP levels**

To assess whether the effects of Kampo drugs were associated with cyclic AMP production, we measured intracellular levels of cyclic AMP. Tissues were pretreated for 30 min with the phosphodiesterase inhibitor 3-isobutyl-1-methylxanthine (10⁻³ M) to prevent cyclic AMP degradation and then incubated for 30 min in the absence and presence of isoproterenol (5 × 10⁻⁸ M) alone or isoproterenol plus Saiboku-to, Bakumondo-to or Orengedoku-to (1.0 mg/ml). After the incubation, they were homogenized in a glass-homogenizer in 10% trichloroacetic acid with [³H]cyclic AMP (New England Nuclear, Boston, MA, USA) added as a tracer for recovery determinations. After the extraction of trichloroacetic acid with ether, the residue was dissolved in acetate buffer. The cyclic AMP levels were determined in duplicate by radioimmunoassay (8), and normalized for protein content of the tissue as determined by the Lowry method (9).

**Drugs**

The following drugs were used: Saiboku-to, Bakumondo-to, and Orengedoku-to (freeze-dried extract, Tsumura & Co., Tokyo) and acetylcholine chloride, histamine diphosphate, and isoproterenol hydrochloride (Sigma Chemicals, St. Louis, MO, USA). For each day’s experiment, the Kampo drugs were each dissolved in Dulbecco’s modified Eagle’s medium (Sigma Chemicals) at a concentration of 100 mg/ml, vortexed, and sonicated in a bath-type sonicator. This solution was then passed through a syringe filter (pore size, 0.8 µm; Iwaki Glass, Tokyo) and subsequently diluted by Krebs-Henseleit solution.

**Statistics**

All values are expressed as means ± S.E. Statistical analyses were performed by ANOVA or the Newman-Keuls multiple comparison test, and a P value of less than 0.05 was considered significant.

**RESULTS**

Incubation of canine bronchial smooth muscle segments with Saiboku-to, Bakumondo-to and Orengedoku-to at 1.0 mg/ml did not alter the resting tension or the contractile responses to acetylcholine and histamine (Table 1). On the other hand, the isoproterenol-induced relaxant responses of the tissues precontracted with acetylcholine were potentiated by the same concentrations of Saiboku-to and Bakumondo-to but not by Orengedoku-to (Fig. 1), so that the concentration-response curves for isoproterenol were shifted to the left and the IC₅₀ values were...
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### Table 1. Effects of Saiboku-to, Bakumondo-to and Orengedoku-to on contractile responses of canine bronchial smooth muscle to acetylcholine

|            | Acetylcholine | Histamine |
|------------|---------------|-----------|
|            | \(-\log[EC_{50}]\) | \(E_{max}\) | \(-\log[EC_{50}]\) | \(E_{max}\) |
| Control    | 5.7±0.3       | 157±9     | 5.5±0.5       | 136±11     |
| Saiboku-to | 6.0±0.4       | 146±10    | 5.2±0.6       | 122±13     |
| Bakumondo-to | 5.7±0.3     | 138±12    | 5.2±0.4       | 117±9      |
| Orengedoku-to | 6.1±0.5     | 153±13    | 5.6±0.3       | 134±14     |

Definition of abbreviations: \(EC_{50}\), concentration required to produce a half-maximal contraction; \(E_{max}\), maximal contraction in grams per gram of tissue weight. Values are means±S.E.; \(n\), number of specimens.

decreased from \(4.6\pm0.5 \times 10^{-8}\) M to \(1.9\pm1.0 \times 10^{-8}\) M by Saiboku-to (\(P<0.05\), \(n=8\)) and to \(1.0\pm0.8 \times 10^{-8}\) M by Bakumondo-to (\(P<0.01\), \(n=8\)). The isoproterenol (\(5 \times 10^{-8}\) M)-induced muscle relaxation was potentiated by each Kampo drug in a concentration-dependent manner with a threshold concentration of 0.3 and 0.1 \(\text{mg/ml}\), respectively, whereas Orengedoku-to had no effect (Fig. 2).

Incubation of bronchial smooth muscle with Saiboku-to, Bakumondo-to and Orengedoku-to at 1.0 \(\text{mg/ml}\) per se did not significantly alter intracellular cyclic AMP levels. Isoproterenol (\(5 \times 10^{-8}\) M) increased cyclic AMP contents from \(9.0\pm1.9\) to \(19.1\pm2.2\) pmole/mg protein (\(P<0.01\), \(n=10\)), an effect that was potentiated by Saiboku-to to \(27.0\pm2.6\) pmole/mg protein (\(P<0.05\), \(n=10\)) and by Bakumondo-to to \(28.7\pm2.3\) pmole/mg protein (\(P<0.01\), \(n=10\)) but not by Orengedoku-to (Fig. 3).

### DISCUSSION

Our in vitro studies demonstrate that the Kampo drugs Saiboku-to and Bakumondo-to but not Orengedoku-to...
potentiate $\beta$-adrenergic function in canine airway smooth muscle. This notion is based on the findings that incubation of bronchial segments with each drug augmented the isoproterenol-induced muscle relaxation without affecting responses to contractile agonists including acetylcholine and histamine, and that this effect was accompanied by the potentiation of intracellular cyclic AMP accumulation in response to isoproterenol.

It has been postulated that the airway hyperresponsive ness, a characteristic feature of asthma, may result from an imbalance in the autonomic nervous system control of the airways (5, 6). Szentivanyi (10) proposed that the imbalance might be due to the diminished $\beta$-adrenergic responses of the bronchial smooth muscle. Additionally, impairment of $\beta$-adrenergic receptor-mediating relaxation of airway smooth muscle has been reported in patients with asthma (11, 12), and acute exacerbation of asthmatic symptoms produced by viral infection or prior use of $\beta$-adrenergic agonists may be associated with the downregulation of $\beta$-adrenergic receptors (13, 14). In the present study, bronchodilation induced by isoproterenol was enhanced by *Saiboku-to* and *Bakumondo-to* in a concentration-dependent fashion, indicating that potentiation of $\beta$-adrenergic receptor function might have occurred in airway smooth muscle. However, further studies may be required to determine whether the local concentrations of these drugs in humans are sufficient to exert the effects.

Stimulation of $\beta$-adrenergic receptors on airway smooth muscle causes bronchodilation by elevating intracellular cyclic AMP and activating the cyclic AMP-dependent protein kinase that phosphorylates myosin light chain kinase (15). Although the mechanism of the observed effects is not clear, the potentiation of the isoproter enol-induced cyclic AMP accumulation by *Saiboku-to* and *Bakumondo-to* suggests that these drugs might have acted on a site(s) at least proximal to the cyclic AMP synthesis in the signal transduction pathway of $\beta$-adrenergic receptor stimulation in airway smooth muscle. These possibilities probably include enhancement of $\beta$-adrenergic receptor binding, stimulation of guanyl nucleotide binding protein, upregulation of adenylate cyclase, and/or inhibition of cyclic AMP phosphodiesterase activity.

Since Kampo drugs are blended herbal medicines made from several crude drugs that may possess complex interactions, it is generally uncertain what crude drugs are responsible for their biological actions. One possibility is that *Zizyphi fructus* could have played a role in the modulation of $\beta$-adrenergic functions, because this crude drug is contained in *Saiboku-to* and *Bakumondo-to* but not in *Orengedoku-to*, and because *Zizyphi fructus* has been reported to have cyclic AMP-like bioactivities (16). Another candidate would be *Glycyrrhizae radix* that is a common constituent of *Saiboku-to* and *Bakumondo-to* and possesses anti-allergic actions.

*Saiboku-to* and *Bakumondo-to* have no direct relaxant action on airway smooth muscle but are considered to be anti-asthmatic drugs. In addition to its inhibitory action on the release of chemical mediators histamine and platelet-activating factor (3), *Saiboku-to* has recently been shown to have a stimulatory effect on airway mucociliary transport (17) and a preventive effect on the downregulation of glucocorticoid and $\beta$-adrenergic receptors (18), thereby explaining its efficacy on patients with asthma. Our results may raise another possibility that the potentiation of $\beta$-adrenergic receptor functions in airway smooth muscle could also account for the clinical benefit of anti-asthmatic Kampo drugs.
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