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Research Article

# Modulation of the Pacemaker Activity by Phytochemicals (Sinomenine, Tetrandrine and Magnoflorine) of *Sinomeni Caulis et Rhizoma* in Spontaneously Beating Sino-Atrial Nodal Cells

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## Abstract

**Background:** The main constituent (crude) of Mokuboito (Kampo formulation) is *Sinomeni Caulis et Rhizoma* (SCR), which exerts the cardioprotective actions. However, the effects of its ingredients (phytochemicals) on the ionic channels and the spontaneous activity in rat sino-atrial (SA) nodal pacemaker cells are still unknown yet.

**Objective:** To examine the modulation by the phytochemicals in SCR of the chronotropic effects, the electrophysiological actions on the spontaneous action potentials and the underlying ionic currents in the SA nodal cells were investigated.

**Methods:** Whole-cell patch-clamp and current-clamp techniques were performed to examine the effects of the phytochemicals at $36^0$c in modified Tyrode solution.

**Results:** The phytochemicals such as sinomenine and tetrandrine depressed the spontaneous activity in a concentration-dependent manner. They inhibited the action potential amplitude and the maximum rate of depolarization, and increased the action potential duration and the cycle length. Some dysrhythmias and sinus arrest often occurred. The L-type $Ca^{2+}$ current ($I_{Ca}$) and the hyperpolarization-activated inward current ($I_f$) decreased by 13.4 ± 2.6% (n = 6, P < 0.05) and by 12.1 ± 2.0% (n = 7, P < 0.05) at 300 μM sinomenine, and by 61.2 ± 3.8% (n = 6, P < 0.001) and by 40.1 ± 3.5% (n = 8, P < 0.05) at 10 μM tetrandrine, respectively. The delayed rectifier $K^+$ current ($I_{Krec}$) also decreased by 20.3 ± 3.6% (n = 6, P < 0.05) at 1 mM sinomenine, and by 39.5 ± 3.5% (n = 8, P < 0.05) at 30 μM tetrandrine. But magnoflorine did not cause any effect on both the action potentials and the ionic currents to significant extent.

**Conclusion:** These results indicate that the cardiac ionic channels contributing to the pacemaker activity in rat SA nodal cells are so high sensitive to sinomenine and tetrandrine enough to modify the spontaneous activity.

***Keywords:*** *Sinomenine; Tetrandrine; Sinomeni Caulis et Rhizoma (SCR); Ionic Currents; Pacemaker Activity; Rat Sino-Atrial Nodal Cells*

## Introduction

As a kind of Kampo formulation, Mokuboito (Formula aristolochiae) is composed of four herbal drugs; *Sinomeni Caulis et Rhizoma* (SCR) (or *Sinomenium acutum Rehder et Wilson*), *Cinnamoni* cortex, *Ginseng* radix and *Gypsum*. Mokuboito has been applied for the disturbance of body fluids and Rheumatic diseases as a pain killer [1,2]. In clinical, Mokuboito has also been used for heart failure to improve the symptom, and to reduce New York Heart Association (NYHA) class and plasma brain natriuretic peptide (BNP) concentration [1,3]. In our recent reports [4-6], SCR, a main constituent (crude), exerts the vasodilating and the cardioprotective actions. SCR possesses a lot of ingredients (phytochemicals), which would affect the ionic channels of cardiomyocytes and produce ameliorative cardiovascular effects.

The main constituent of SCR is sinomenine, one of the alkaloids extracted from herbs. Sinomenine has also been used for clinical treatment of Rheumatoid arthritis (RA), due to the anti-inflammatory and immunomodulative actions [4,7,8]. On the other hand, tetrandrine is also an alkaloid to inhibit $Ca^{2+}$ channel protein [9]. It exerts antiarrhythmic actions with quinidine-like effects [10]. Furthermore, tetrandrine causes the vasodilating effect resulting in antihypertensive actions, and protects concanavalin A-induced hepatitis via inhibiting NF-κB activation [11]. On the other hand, magnoflorine has the properties on protecting human HDL against lipid peroxidation [12] and the antidiabetic activity by α-glucosidase inhibitor [13].

We have already investigated the cardiovascular pharmacological actions of Mokuboito in guinea pig ventricular myocytes and rat vascular smooth muscle [4,14]. However, the effects of sinomenine and other phytochemicals on the spontaneous activity and the ionic channels in the SA nodal pacemaker cells are still unknown yet. Aim of the present experiments was to examine the modulation of the chronotropic effects of the phytochemicals in spontaneously beating rat SA nodal cells. The electro-physiological and -pharmacological actions on the spontaneous action potentials and the underlying ionic currents were investigated using a patch-clamp technique.

## Materials and Methods

All experiments were carried out according to the guidelines laid down by the University Welfare Committee, and also under the terms of the Declaration of Helsinki.

### Cell preparation

Wistar rats, weighing 180 - 250g, were anaesthetized with sodium pentobarbital (30 mg/kg, i.p.). Rat sino-atrial nodal cells were isolated with enzyme solution, as previously described [15,16]. Under artificial respiration, the chest cavity was opened. An injection needle, connected to a perfusion line, was inserted through the right atrial wall, and Tyrode solution was directly infused into the atrial cavity at a rate of ~10 ml/min with a hydrostatic pressure of ~70 cm $H_2O$. To avoid mixing of the perfusate with venous return, and also to expand the atrial cavity by using the perfusion pressure, the superior and inferior venae were ligated. Then, the inferior vena cava was cut distal to the ligature to allow drainage of perfusate, which passed through the pulmonary and then the systemic circulation. Within several minutes, the drained perfusate became largely blood-free. The spontaneous heart beat was stopped by switching the perfusate from normal Tyrode to a nominally $Ca^{2+}$-free Tyrode solution. Then, $Ca^{2+}$-free solution containing 0.4 mg/ml trypsin (Wako Pure Chemical Industries, Osaka, Japan) was applied for 5 - 6 minutes to remove the endocardial endothelium. $Ca^{2+}$-free solution containing 0.85 mg/ml collagenase (Wako Pure Chemical) was perfused for approximately 5 minutes. Then, the heart was dissected out into fresh collagenase solution. The right atrium was opened by cutting along the atrial septum and also by cutting the ventral wall of the superior vena cava. The atrial tissue including the SA node was dissected out and was gently shaken in the collagenase plus elastase (0.1 mg/ml) solution (Boehringer Mannheim GmbH, Germany). The enzyme treatment lasted for 20 - 25 minutes, depending on the extent of tissue digestion seen under a dissection microscope. Finally, the digested tissue was put in the modified KB (Kraftbrühe) solution, and trimmed by scissors into a small SA node fragment of ~1 mm in width and ~3 - 4 mm in length. The major bundle of atrial cells running through the crista terminalis was discarded. The SA node tissue was placed in a 35-mm plastic Petri dish with a fresh KB solution and the SA node cells were dissociated by gentle puffing with KB solution to the tissue. The dissociated cells were stored in the same solution at 4°C for experimentation.

### Whole-cell voltage- and current-clamp experiments

Whole-cell voltage-clamp recordings were performed using an Axopatch patch-clamp amplifier (Axon Instruments, Burlingame, CA, U.S.A.) and standard techniques [15,16]. Patch pipettes from borosilicate glass capillaries were fabricated using a two-stage puller; they had a resistance of 5 - 7 MΩ. The series resistance was less than 10 mV, and no compensation was used. The liquid junction potential between the pipette solution and the external solution (less than 10 mV) was corrected for all membrane potential recordings. For the action potential parameters, the action potential amplitude (APA) was measured as the difference between the peak of the action potential and the maximum diastolic potential (MDP), the action potential duration (APD) as the duration at 50% repolarization, and the cycle length (CL) as the interval between the peaks of action potentials. The L-type $Ca^{2+}$ current ($I_{Ca}$) was measured as the difference between the peak current and the zero current, and the delayed rectifier $K^+$ current ($I_{Krec}$) and the hyperpolarization-activated inward current ($I_f$) were the difference between the current at the end of a 1-s test pulse (to fully activate them) and the zero current.

Experiments were carried out at 36°C. The data were stored and analyzed on an IBM-AT microcomputer, using the PCLAMP analysis program (Axon Instruments). Current traces were filtered using a cut-off frequency of 2 kHz for plotting. All the values are given as means ± S.E.M. The differences between the mean values were analyzed by Student's t-test for paired data, and analysis of variance (ANOVA) followed by Bonferroni inequality, and/or Chi square test. A P value less than 0.05 was considered significant.

### Experimental solutions

The composition of the modified Tyrode solution was (in mM): NaCl 137, KCl 5.4, $CaCl_2$ 1.8, $MgCl_2$ 1.0, $NaH_2PO_4$ 0.3, glucose 5.0, and HEPES [N-(2-hydroxyethyl)piperazine-N'-2-ethansulfonic acid] (Wako Pure Chemical) 5.0. The pH was adjusted to 7.4 with NaOH. The pipette solution (intracellular) contained (in mM): K-aspartate 110, KCl 20, $MgCl_2$ 1, EGTA 5, Mg-ATP 5, creatine phosphate 5, and HEPES 5 (pH 7.2). Drugs used were sinomenine (7, 8-didehydro-4-hydroxy-3, 7-dimethoxy-17-methyl-9α,13α,14α-morphinan-6-one), tetrandrine [(1β)-6-6', 7, 12-tetramethoxy-2, 2'-dimethyl-berbaman], and magnoflorine [(6αS)-2, 10, dimethoxy-6, 6-dimethyl-5, 6, 6α, 7-tetrahydro-4H-dibenzo [de,g] quinoline-6-ium-1, 11-diol] (Sigma Chemicals, USA). The concentrations used in this study were decided by the data from the previous reports [4,9,13,17].

## Results

To examine the effects of the phytochemicals on the spontaneous action potentials, a current-clamp configuration was carried out. Sinomenine had depressant effects on the spontaneous activity, and the depression behaved in a concentration (100 μM to 1 mM)-dependent fashion, as shown in figure 1. Sinomenine at 300 μM prolonged the cycle length (CL) by 11.6 ± 3.8% (n = 8, $P < 0.05$), shortened the action potential duration (APD) at 50% repolarization by 15.8 ± 3.3% (n = 8, $P<0.05$), and decreased the action potential amplitude (APA) by 4.5 ± 3.2% (n = 8, $P > 0.05$) (Figure 1B). The maximum diastolic potential (MDP) also decreased by 6.2 ± 2.4% (n = 8, $P < 0.05$). Sinomenine 1 mM had further depressant actions. The action potential amplitude (APA) and the maximum rate of depolarization ($V_{max}$) were inhibited. Simultaneously, the MDP also markedly decreased by 34.6 ± 3.8% (n = 8, $P < 0.01$).

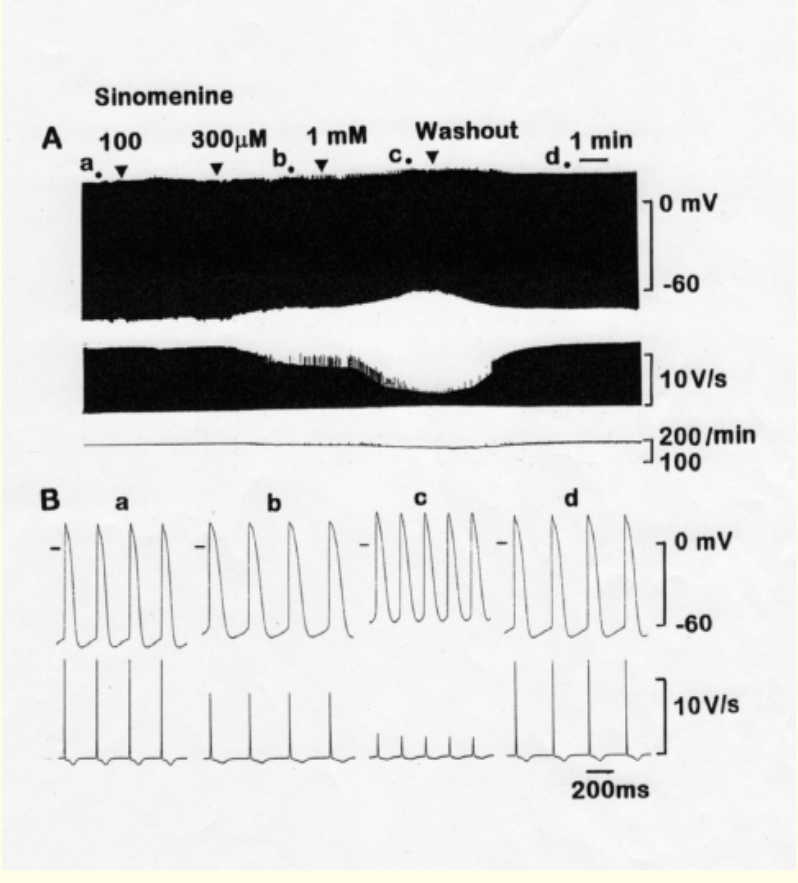



***Figure 1:*** *Concentration-dependent effects of sinomenine on the spontaneously beating sino-atrial nodal cells. A: The action potentials and the maximum rate of depolarization (Vmax). B: Recordings at different time scale of spontaneous action potentials and Vmax at small letters (a-d) on panel A.*

Sinomenine often elicited dysrhythmias. In some cells, substantially a sinus arrest (accompanied with oscillatory potentials) occurred. The incidence is summarized in table 1. The occurrence was concentration-dependent. The dysrhythmias lasted for 10 - 15 minutes after washout. However, the sinus rate with regular rhythm failed to recover completely to control level (by approximately 60 - 70%) in almost the SA nodal cells.

| Sinomenine | n | 10 μM | 30 μM | 100 μM | 300 μM | 1 μM |
|---|---|---|---|---|---|---|
| | 9 | 0 | 0 | 1 | 5 | 9 |
| Tetrandrine | n | 0.3 μM | 1 μM | 3 μM | 10 μM | 30 μM |
| | 8 | 0 | 0 | 1 | 5 | 8 |
| Magnoflorine | n | 3 μM | 10 μM | 30 μM | 100 μM | 300 μM |
| | 8 | 0 | 0 | 0 | 0 | 0 |

***Table 1:*** *Incidence of sinus arrest in the presence of phytochemicals.*

Tetrandrine at 0.3 to 30 μM caused the concentration-dependent inhibitory effects on the action potentials (Figure 2). Tetrandrine (0.3 to 30 μM) also caused the similar effects, which were greater than sinomenine-induced responses. The APA decreased by 63.6 ± 3.6% (n = 7, $P < 0.001$) at 3 μM, but not at the lower concentrations. At 3 μM, tetrandrine prolonged the CL by 1.4 ± 3.5% (n = 8, $P > 0.05$). Simultaneously the $V_{max}$ significantly decreased by 9.1 ± 2.5% (n = 7, $P < 0.05$) at 3 μM. The APD increased. As well the MDP was slightly reduced at lower concentrations (by approximately 5% at 3 μM), and at 10 μM by 20.1 ± 3.2% (n = 6, $P < 0.01$). At 30 μM, sinus arrest occurred in all the cells (n = 8) (Table 1). On the other hand, magnoflorine (0.3 to 10 μM) caused weak effects, but did not cause any effects to significant extent (Figure 3). Magnoflorine never caused the sinus arrest by any concentrations.

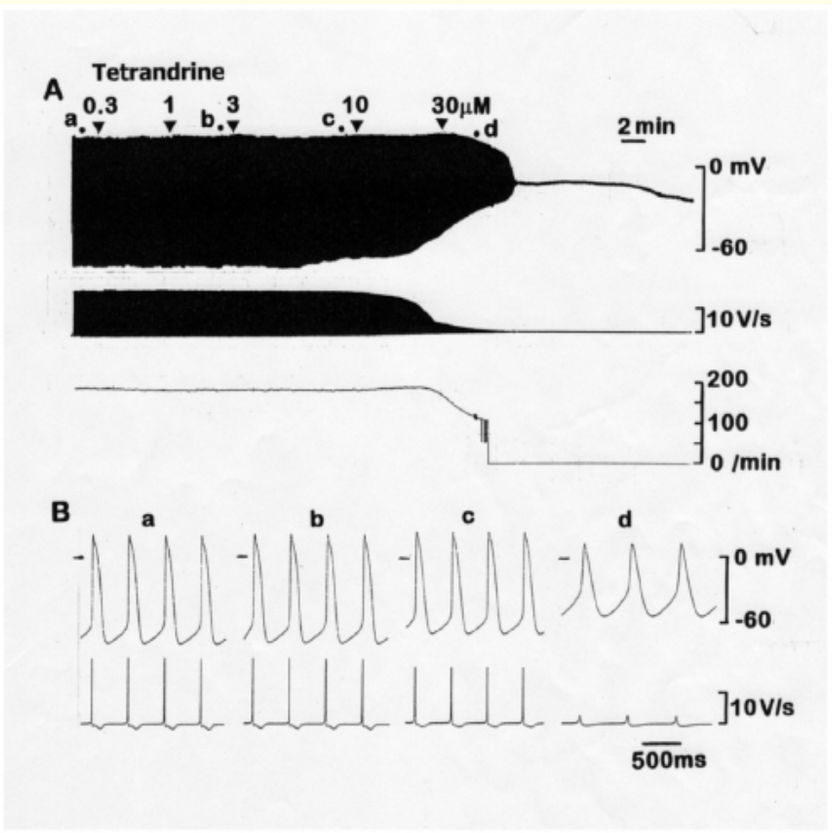



***Figure 2:*** *Modulation by tetrandrine of the spontaneously beating sino-atrial nodal cells. A: The action potentials and the maximum rate of depolarization (Vmax). Sinus arrest occurred at 30 μM. B: Recordings at different time scale of spontaneous action potentials and Vmax at small letters (a-d) on panel A.*

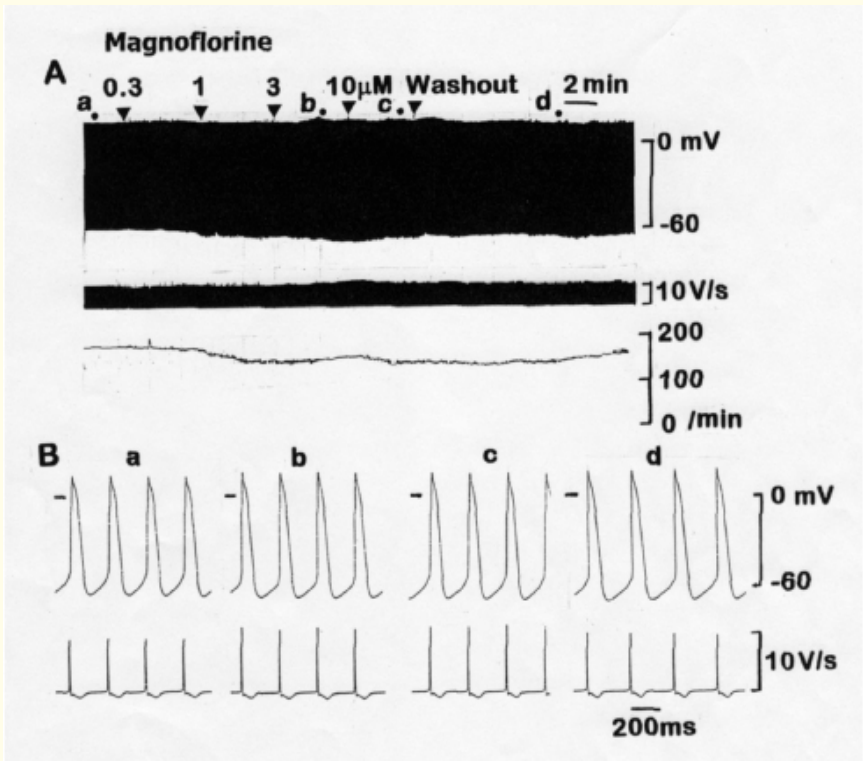
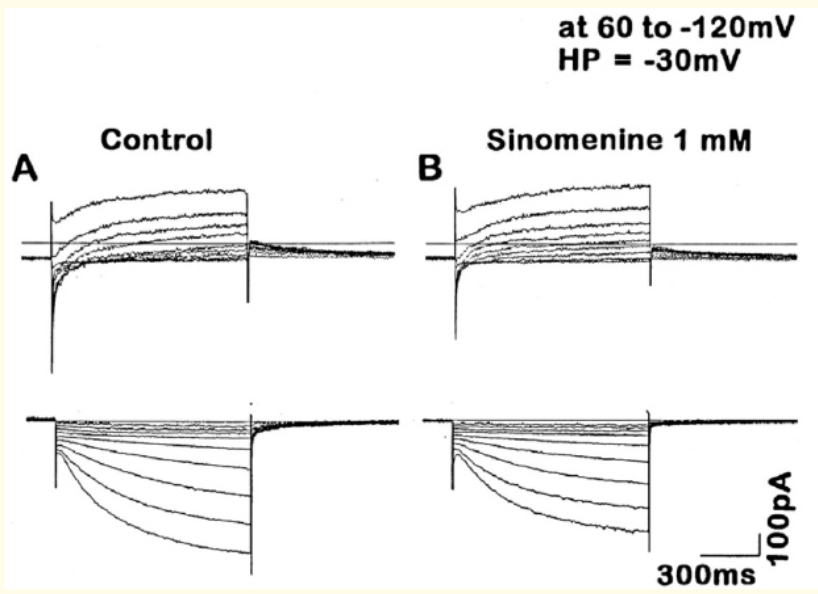



***Figure 3:*** *Modulation by magnoflorine of the spontaneously beating sino-atrial nodal cells. A: The action potentials and the maximum rate of depolarization (Vmax). B: Recordings at different time scale of spontaneous action potentials and Vmax at small letters (a-d) on panel A.*

In whole-cell patch-clamp experiments, the effects on the ionic currents of SA nodal cells were examined (Figure 4). Sinomenine at 300 μM inhibited the L-type $Ca^{2+}$ current ($I_{Ca}$) at 0 mV by 13.4 ± 2.6% (n = 6, P < 0.05), and at 1 mM by 38.1 ± 2.7% (n = 6, P < 0.05). And at 1 mM, the delayed rectifier $K^+$ current ($I_{Krec}$) at 60 mV was inhibited by 20.3 ± 3.6% (n = 6, P < 0.05), and the hyperpolarization-activated inward current ($I_f$) at –120 mV by 20.6 ± 3.6% (n = 6, P < 0.05). After washout for 15 to 20 minutes, the current was recovered to 50 - 60% of control value.



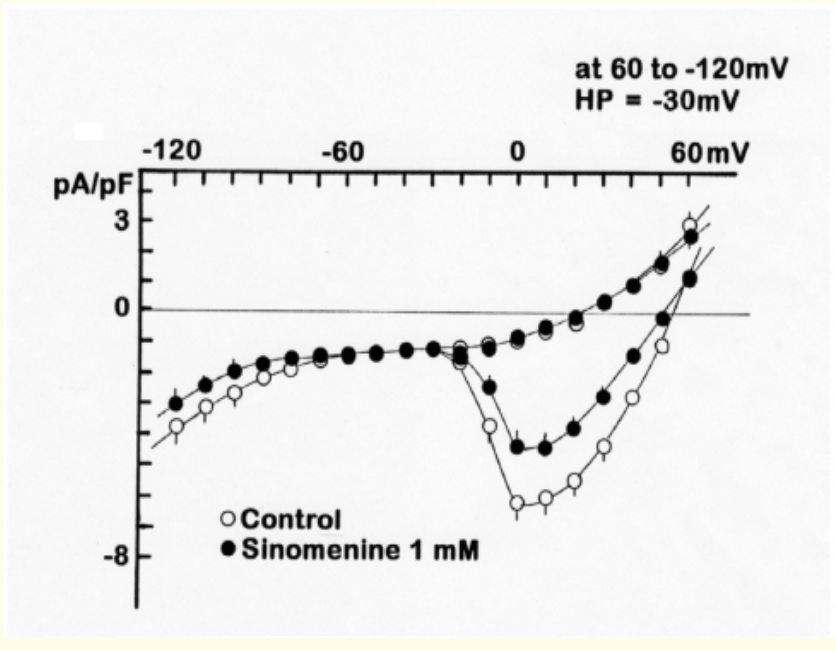




***Figure 4:*** *Modulation by sinomenine of the current channels in rat sino-atrial nodal cells. A: The superimposed ionic currents in control. Horizontal lines on the current recordings represent zero current level. B: The ionic currents by sinomenine. C: The current-voltage relationship. Symbols are in control (open circles) and in 1 mM sinomenine (closed circles). Vertical bars represent mean ± SEM.*

Tetrandrine (3 to 30 μM) also inhibited markedly the ionic currents concentration-dependently. Tetrandrine had the similar inhibitory effect on $I_{Ca}$ (at 0 mV); by 32.9 ± 3.7% (n = 6, P < 0.01) at 3 μM and by 62.2 ± 3.8% (n = 6, P < 0.001) at 10 μM, and blocked it at 30 μM. At 20 mV (as a peak of the action potential), the $I_{Krec}$ inhibition was 23.6 ± 3.7% (n = 6, P < 0.05) at 10 μM and 39.5 ± 3.5% (n = 6, P < 0.05) at 30 μM tetrandrine. The $I_f$ current at -120 mV markedly decreased; by 40.1 ± 3.5% (n = 6, P < 0.01) at 10 μM and by 88.3 ± 3.3% (n = 6, P < 0.001) at 30 μM. At -60 mV (as MDP), tetrandrine decreased the $I_f$, but not significantly. The responses were irreversible, and the recovery was 50 - 60% of control level.

Magnoflorine had the inhibitory actions on the ionic currents, but did not cause to significant extent. The $I_{Ca}$ inhibition (at 10 mV) was 5.2 ± 1.8% (n = 8) at 10 μM. Magnoflorine also tended to decrease the $I_{Krec}$ and the $I_f$ currents by 4.3 ± 1.3% (n = 7) and 4.1 ± 2.6% (n = 7) at 3 μM, and by 6.2 ± 1.3% (n = 8) and by 8.4 ± 2.0% (n = 8) at 10 μM, respectively.

## Discussion

The present study showed that (a) the phytochemicals such as sinomenine and tetrandrine depressed the spontaneous activity, resulting from the modulation of the action potential configurations, (b) simultaneously they inhibited the underlying ionic currents, (c) both sinomenine and tetrandrine often elicited dysrhythmias concentration-dependently, (d) magnoflorine had less or no effects, and (e) a washout incompletely recovered the spontaneous action potentials and the ionic currents to control values (approximately 50 - 60% of control).

### Effects on the spontaneous action potentials

The spontaneously beating (sinus rate) is regulated by (a) the rate of the slow diastolic potential (pacemaker potential), (b) the maximum diastolic potential (MDP), (c) the threshold potential, and (d) the action potential duration (APD). In the present experiments, sinomenine and tetrandrine caused the marked negative chronotropic effect, but magnoflorine caused slight effects. The phytochemicals decreased the APA, the $V_{max}$ and the MDP, and increased the APD of the SA nodal action potentials. Therefore, the phytochemicals affected the action potentials, and leaded to the strong prolongation of the CL.

The APD prolongation induced by sinomenine and tetrandrine is responsible for the negative chronotropic effect, although $Ca^{2+}$ channel plays a key role for the spontaneous rhythm in rat SA nodal cells. Because the APD prolongation would lead to an enhancement of cellular $Ca^{2+}$ level [15,18]. The faster repolarization induces to the faster inactivation of $Ca^{2+}$ channel and decreases the window current. Thus, the marked $I_{Ca}$ inhibition and the APD prolongation due to the $I_{Krec}$ channel inhibition cause strong negative chronotropic effect. Therefore, the negative chronotropic effect induced by the phytochemicals is mainly caused by the alteration of the rate of pacemaker depolarization via the modulation of the ionic currents. However, magnoflorine had less or no effect on the action potential parameters in this study, well consist with previous reports [19,20], although magnoflorine at the same concentrations has been reported to produce the effective responses on the other tissues [20,21].

### Effects on the pacemaking ionic currents

The modulation of the spontaneous action potentials is responsible for the underlying ionic channel currents across the SA nodal cell membrane [22,23]. In general, the ionic currents to regulate the spontaneously beating SA nodal cells are considered to be due to (a) $I_{Ca}$ current, (b) decline of $I_{Krec}$ conductance, and (c) $I_f$ current. Both sinomenine and tetrandrine decreased all the currents.

Major action of both phytochemicals was the marked inhibition of $I_{Ca}$ current, well consistent with our results of the vasodilation in rat aortic smooth muscles [5,6]. The great $I_{Ca}$ inhibition can strongly decline the intracellular $Ca^{2+}$ concentration $[Ca^{2+}]_i$, and produces the depression of the spontaneous activity of SA nodal cells. Under the conditions, a sinus arrest may occur. Liu., *et al.* [9] have shown that tetrandrine blocks the L-type $Ca^{2+}$ channel (IC = 0.3 - 8 μM) and the T-type $Ca^{2+}$ channel (IC = 2.5 - 20 μM). As well, the $Ca^{2+}$-activated $K^+$ channel (Kd = 0.2 μM) is blocked. Both sinomenine and tetrandrine also caused the $I_{Krec}$ inhibition, resulting in the APD prolongation

which leads to further suppressive pacemaker activity. The $I_f$ current is found mainly in the pacemaker nodal and Purkinje fiber cells, and both phytochemicals also decreased the $I_f$ current. Thus, the inhibitions in the ionic currents induced by the phytochemicals cause the negative chronotropic effect. The pacemaker potential is formed by the interaction with the contributing currents dependent on voltage and time [22,23]. Under the normal conditions, however, the $I_f$ current is considered unlikely to contribute the pacemaker activity. Because its activation needs the higher hyperpolarization (over -70 mV) and longer duration (over 1s) of stimulation pulse. Thus, the modulation by both phytochemicals of the $I_f$ current would cause minor changes in the spontaneous activity under the present (normal) conditions. Furthermore, no significant change in $I_f$ current at -60 mV of MDP occurred. Therefore, the modulation of $I_{Ca}$ and $I_{Krec}$ currents by both sinomenine and tetrandrine mainly contributes to the pacemaker activity. On the other hand, Mokuboito and SCR, as a mixture, enhanced $I_{Ca}$ and inhibited $I_{Krec}$ and inwardly rectifying $K^+$ current ($I_{K1}$) in guinea pig ventricular cardiomyocytes, different from the findings in the SA nodal cells [15,24].

### Occurrence and inhibition of the dysrhythmias

The higher concentrations of sinomenine and tetrandrine often elicited dysrhythmias, but magnoflorine never did it. The incidence was concentration-dependent. The dysrhythmias are responsible for the great inhibitions of the key currents for spontaneous activity.

Satoh [4] has already demonstrated that sinomenine effectively modulates the ionic channels in guinea pig ventricular cardiomyocytes. Sinomenine inhibited the $I_{Ca}$, and simultaneously caused the $I_{Krec}$ decrease which resulted in the APD prolongation. The $Ca^{2+}$ channel inhibition markedly dilates vascular smooth muscle cells [5,14,25]. Under the ischemia and heart failure, the resultant cellular $Ca^{2+}$ overload of heart muscles has been well known to elicit some arrhythmias and fall to dysfunctions [15,18]. In our previous studies, under the cellular $Ca^{2+}$ overload ($[Ca^{2+}]_o$ = 5.4 mM), abnormal action potentials occurred irregularly, in spite of the constant stimulation (1 Hz) [4]. Application of sinomenine suppressed and abolished the abnormal action potentials (dysrhythmias). The regulation of $Ca^{2+}$ influx may strongly regulate $Ca^{2+}$ overload in cardiomyocytes and produces protective actions for the myocardial cells, according with the vasodilating action [26]. Therefore, sinomenine may restrain the cell damages of heart muscles via the modulation of $[Ca^{2+}]_i$, and as a result, exert the cardioprotective actions.

In future, therefore, sinomenine as a cardioprotective drug would be expected to the respectable effectiveness for heart failure, mediated through the modulation of cardiac ion channels (including the inhibition of dysrhythmias) and blood vessels. These electro-physiological and -pharmacological actions contribute to regulate the pacemaker activity and simultaneously protect the cardiomyocytes.

### Clinical possibility of cardiovascular pharmacological effects

In general, the basic treatments of heart failure consist of (a) reducing workload of heart, (b) protection of cardiomyocytes, and (c) restriction and control of waters and sodium. In order to reduce both preload and afterload, the dilations of arterioles and veins are strongly required in the case of elevated filling pressures and reduced cardiac output.

Major action of both phytochemicals is the great inhibition of $I_{Ca}$ current, almost consistent with the results of the vasodilation in the experiments of rat aortic smooth muscles [5,6]. The vasodilating drug is one of the great useful tools for heart failure. Therefore, sinomenine-induced vasodilating actions under the heart failure may improve cardiac functions via the reductions of both preload and afterload, as well as the regulation of the $Ca^{2+}$ overload. The cardioprotective action of sinomenine on rat acute myocardial ischemia has already been demonstrated [27]. Reperfusion injury is induced by ligating the rat left coronary artery for 15 minutes and reopening. Sinomenine inhibits the incidence of arrhythmias and reduces the $[Ca^{2+}]_i$, well consistent with our results.

In addition, sinomenine inhibits bFGF-induced angiogenesis *in vitro* and *in vivo* [28]. Thus, sinomenine may be sufficiently expected as one of the therapeutic agents for heart failure [5,8,24]. Sinomenine reduces the production of prostaglandin (PG) $E_2$ and NO from macrophage [8]. Also, sinomenine depressed mRNA expression of tumor necrosis factor (TNF)-α and interleukin (IL)-β of peritoneal macrophages [29]. Thus, sinomenine may act as an anti-rheumatic drug through the anti-inflammatory effects on lymphocytes and cytokine.

In the studies for clinical pharmacology, oral administration of 80 mg sinomenine is performed for healthy volunteers [30]. In the pharmacokinetic parameters, $T_{1/2}\alpha$ is 1.04 ± 0.49 hr, $T_{1/2}\beta$ is 9.397 ± 2.433 hr, $T_{max}$ is 1.04 ± 0.27 hr, $C_{max}$ is 246.6 ± 71.17 ng/ml. Furthermore, AUC is 2651.158 ± 1039.050 ng.h/ml, and $C_L$ is 0.033 ± 0.010 ng/ml. As a cardiovascular protective drug, thus, sinomenine may contribute to clinical treatments via modulation of the ionic currents of cardiac cells, and via control of the tension of blood vessels.

For clinical treatment of Rheumatoid arthritis (RA), sinomenine have already been shown [31,32]. Sinomenine reduces joint stiffness and pain, attenuates proliferation of synovial fibroblasts in rat adjuvant arthritis models [33], and impairs signaling through NF-κB resulting in the anti-inflammatory and immunomodulative actions [31,34]. Furthermore, sinomenine possesses anti-proliferative effects on lymphocytes [35]. Sinomenine as well attenuates transmigration of granulocyte. The inhibition of leukocytes migration across the vessel wall and antiangiogenic effect may crucially contribute to the therapeutic effects for RA.

On the other hand, tetrandrine is similarly an alkaloid well known to inhibit $Ca^{2+}$ channel [9]. Tetrandrine is also used for the RA treatment to reduce joint damages of collagen-induced arthritis [36], and exerts immune-suppressive and anti-inflammatory activities [11]. As well, since tetrandrine causes the great inhibitions of cardiac ionic currents, tetrandrine possesses antiarrhythmic actions with quinidine-like effects. Furthermore, tetrandrine exerts the vasodilating effect resulting in antihypertensive actions, and protects concanavalin A-induced hepatitis via inhibiting NF-κB activation [11].

Although magnoflorine had less or no electrophysiological effect on the sino-atrial nodal cells similar to the ventricular cardiomyocytes, it has been shown to exert an inhibitory effect on $Cu^{2+}$-induced lipid peroxidation of high density lipoprotein (HDL). Magnoflorine exhibits the properties on protecting human HDL against lipid peroxidation [12]. Also, magnoflorine possesses antidiabetic activity by α-glucosidase inhibitor [13].

Mokuboito (Formula aristolochiae) consists of SCR, Cinnamomi Cortex (bark of Cinnamomum cassia Blume), Ginseng Radix (roots of Panax ginseng C. A. Meyer) and Gypsum Fibrosum. In SCR, the content of sinomenine is 0.3 - 1.3%, and the content of magnoflorine is 1.3 - 2.1%. On the other hand, tetrandrine has been reported to be almost less content in SCR of Kampo formulations (Japanese herbal medicine), but be much contained in Chinese herbal medicine [19].

## Conclusion

The phytochemicals (sinomenine and tetrandrine) cause the great inhibitory actions on the spontaneous action potentials and the underlying ionic currents, and thereby cause the negative chronotropic effect. The phytochemicals exert the direct actions on the ionic channels as the previous report [3,4,19]. In addition, sinomenine exhibits the antiarrhythmic action, and tetrandrine also cause the similar electrophysiological actions. Both phytochemicals exhibited the ameliorative cardioprotective actions. Especially sinomenine has been reported to possess the strong anti-inflammatory and immunomodulative actions. Further experiments need to clarify more in detail mechanisms.

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