

Original Article

Electrophysiological mechanisms of sophocarpine as a potential antiarrhythmic agent

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Aim: To examine the electrophysiological effects of sophocarpine on action potentials (AP) and ionic currents of cardiac myocytes and to compare some of these effects with those of amiodarone.

Methods: Langendorff perfusion set-up was used in isolated guinea pig heart, and responses to sophocarpine were monitored using electrocardiograph. Conventional microelectrode, voltage clamp technique and perforated patch were employed to record fast response AP (fAP), slow response AP (sAP) and ionic currents in guinea pig papillary muscle or rabbit sinus node cells.

Results: Tachyarrhythmia produced by isoprenaline (15 $\mu\text{mol/L}$) could be reversed by sophocarpine (300 $\mu\text{mol/L}$). Sophocarpine (10 $\mu\text{mol/L}$) decreased the amplitude by 4.0%, maximal depolarization velocity (V_{max}) of the fAP by 24.4%, and Na^+ current (I_{Na}) by 18.0%, while it prolonged the effective refractory period (ERP) by 21.1%. The same concentration of sophocarpine could also decrease the amplitude and V_{max} of the sAP, by 26.8% and 25.7%, respectively, and attenuated the Ca^{2+} current (I_{CaL}) and the K^+ tail current substantially. Comparison of sophocarpine with amiodarone demonstrated that both prolonged the duration and the ERP of fAP and sAP, both decreased the amplitude and V_{max} of the fAP and sAP, and both slowed the automatic heart rate.

Conclusion: Sophocarpine could reverse isoprenaline-induced arrhythmia and inhibit I_{Na} , I_{CaL} , and I_{Kr} currents. The electrophysiological effects of sophocarpine are similar to those of amiodarone, which might be regarded as a prospective antiarrhythmic agent.

Keywords: sophocarpine; action potential; anti-arrhythmia; voltage clamp; potassium current; sodium current; calcium current

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Introduction

Sophora flavescens is a traditional Chinese medicine that has been used for nearly 2000 years in China. It contains several types of alkaloids such as aloperin, matrine and sophocarpine. Some reports have found that *Sophora flavescens* has antiarrhythmic effects. Matrine, one extract from *Sophora flavescens*, was able to decrease abnormal spontaneous activities of the rat right atrium at 220 $\mu\text{mol/L}$ ^[1]. It was also reported that matrine protects against aconitine-induced arrhythmia^[2]. In 2008, Chen *et al* reported that sophocarpine could inhibit ouabain-induced arrhythmias in guinea pigs^[3].

Zhang *et al*^[4] found that sophocarpine, the extract from *Sophora flavescens*, was an effective drug in the treatment of viral myocarditis, not only for its antiviral effects, but also for its antiarrhythmic properties. According to Chen's data^[5], 220 patients were enrolled in the sophocarpine therapeutic

group, most of whom had shown different types of extra systoles. After treatment with sophocarpine (5–6 mg/kg in 500 mL of 5% glucose, iv for 4 h/d, 2–4 weeks as a course), the case analyses indicated that the antiarrhythmic efficiency of sophocarpine had reached 89.7%. Among these patients, complete data from 24 h Holter records were available for 49; the average number of extra systole cycles was decreased from 6120 ± 3966 to 112 ± 179 by sophocarpine in these patients. This report was very impressive, but the mechanism for its antiarrhythmic action needed to be further resolved. Chen *et al*^[6] reported that sophocarpine could prolong the effective refractory period and raise the ventricular fibrillation threshold. However, those results were not enough to explain the drug's efficacy in clinical use.

In spite of those experimental studies and case reports, very little work has been done regarding the electrophysiological effects of sophocarpine on the heart, cardiac myocytes and ion channels. The aim of this paper was to fully study the electrophysiological properties of sophocarpine and explore the mechanism of its antiarrhythmic function. We took 3

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steps to fulfill this purpose: first, we tried to confirm whether sophocarpine had antiarrhythmic effects in an arrhythmic model; second, we studied the effects of sophocarpine on action potentials (AP), whole cell and single channel currents to understand its electrophysiological properties; and third, we intended to compare the effects of sophocarpine with those of some known antiarrhythmic drugs.

Materials and methods

In the experiments, all of the procedures were approved by the Animal Care and Use Committee at Shanghai Jiaotong University. The investigation conforms to the Guide for the Care and Use of Laboratory Animals published by the US National Institutes of Health (NIH Publication No 85-23, revised 1996).

Solution preparation and materials

Tyrodé's solution for perfusion preparations consisted of the following (in mmol/L): NaCl, 138; KCl, 5.4; CaCl₂, 1.8; MgCl₂, 1; glucose, 10; and HEPES, 10; pH 7.4±0.05 saturated by 100% O₂.

For whole cell Na⁺ current recordings, the pipette solution contained (in mmol/L): KCl, 120; CaCl₂, 0.1; MgCl₂, 2; EGTA, 1.1; and HEPES, 10; pH 7.2. The bath solution was Tyrodé's solution.

For inside-out Na⁺ channel current recording, the bath solution was (in mmol/L): KCl, 120; MgCl₂, 2; CaCl₂, 0.1; HEPES, 10; and EGTA, 1.1; pH 7.4, while the pipette solution contained (in mmol/L): NaCl, 180; KCl, 1.3; MgCl₂, 0.5; CaCl₂, 1.5; glucose, 5; HEPES, 5; CoCl₂, 3; TEA, 10; 4-AP, 10; and CsCl, 10; pH 7.2.

The pipette solution for the perforated patch clamp contained (in mmol/L): K-aspartate, 130; MgCl₂, 2; CaCl₂, 5; EGTA, 11; Na-HEPES, 10; and Na₂-ATP, 2; pH 7.2. The concentration of amphotericin B was 240 μmol/L.

Sophocarpine (13,14-didehydromatridin-15-one, *M_w* 246.2, purity 99.7%, Figure 1A) was provided by Shanghai Institute of Pharmaceutical Industry (Shanghai, China). The injection solution of sophocarpine (10 g/L), produced by Shanghai Hejia Pharmaceutical Company (No 030827), was diluted to

the final concentrations for the experiments. Amiodarone was purchased from the Mingzhu Pharmaceutical Factory of Sanofi-aventis China.

Chromanol 293B was provided by Aventis Pharma (Frankfurt, Germany), dofetilide by EGIS Pharmaceuticals Ltd (Pfizer Global Research & Development, UK, Batch 3644-399). Chromanol 293 B and dofetilide were initially dissolved in 100% DMSO to make a stock solution (10 mmol/L).

Electrocardiogram (ECG) recording in isolated guinea pig hearts

Guinea pigs (weighing approximately 250–300 g) of either sex were anesthetized with an intraperitoneal injection of urethane (20%, 5 mL/kg). The hearts were quickly taken out of the chest and perfused using a Langendorff perfusion setup at the recorded pressure of 80 cm H₂O. The temperature of Tyrodé's solution was maintained at 37±0.5 °C. Three platinum electrodes, which had been placed on the cardiac apex, right atrium, and aortic root, were used to record the ECG. The signals were documented on a computer with the PowerLab system (PowerLab ML135, ML 785, ADInstruments, Australia)^[7].

Primary culture of neonatal rat ventricular myocytes

Neonatal Sprague-Dawley (SD) rats of either sex (postnatal 1 d) were anesthetized by intraperitoneal injection of urethane (20%, 5 mL/kg). After the heart had been taken out, the ventricles were cut into small pieces and processed by collagenase-containing solution for digestion. The myocytes were separated from the cell suspension by centrifuging twice. The isolated ventricular myocytes were planted and grown on glass coverslips in DMEM (Invitrogen, Burlington, OH, USA) with 10% fetal bovine serum for 5–6 d^[8,9].

Preparation of guinea pig papillary muscles and rabbit sinus node

Guinea pigs (250–300 g) or rabbits (1.5–2.5 kg) of either sex were sacrificed by venesection under deep anesthesia with an intravenous injection of sodium pentobarbital (30 mg/kg). The hearts were rapidly removed into the dissection chamber filled with Tyrodé's solution. The right ventricular papillary

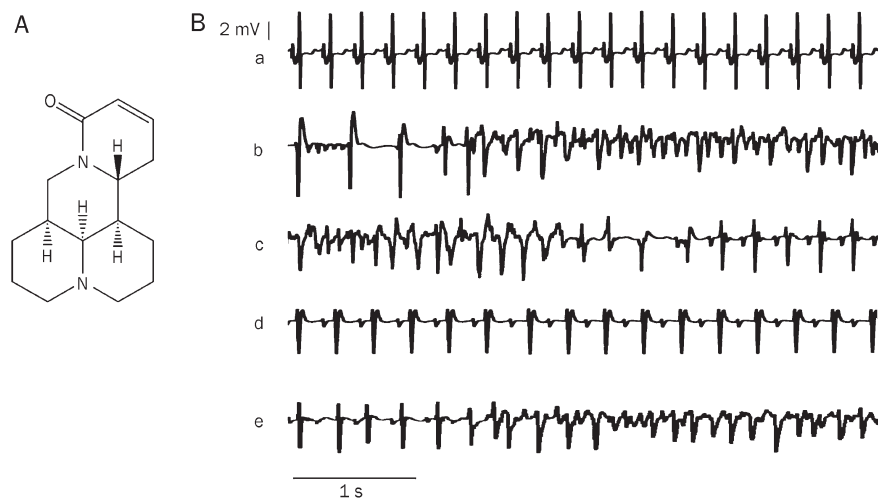


Figure 1. Sophocarpine (Soph) reversed the tachyarrhythmia of guinea pig.

A: Molecular structure of Soph.

B: Soph reversed the isoprenaline-induced tachyarrhythmia of guinea pig. (a) Control ECG. (b) The tachyarrhythmia was initiated after 6 min perfusion of the heart with isoprenaline 15 μmol/L. (c) The tachyarrhythmia began to be reversed after the heart had been exposed to Soph (300 μmol/L) plus isoprenaline solution for 13 min. (d) Two minutes later, the regular beating appeared on the ECG in the Soph-isoprenaline-contained solution. (e) The tachyarrhythmia recurred after Soph had been washed out for 7 min with the same solution as the one used in trace b.

muscles (from guinea pig) or the sinus nodes with partial right atrium (from rabbit) were excised and pinned to the bottom of a recording chamber. The chamber was perfused with Tyrode's solution at a constant rate of 3 mL/min and maintained at a temperature of $37\pm 0.5\text{ }^{\circ}\text{C}$ ^[7,10].

Recording of the fast-response and slow-response AP

Bipolar platinum electrodes were used to drive the preparations with rectangular current pulses at a stimulation frequency of 1 Hz for papillary muscle and 2.2 Hz for the sinus node. Each pulse lasted 0.1 ms with a strength of about 1.5 times the threshold value. After 30 min of stimulation, transmembrane AP was recorded by a conventional glass microelectrode that was filled with 3 mol/L KCl and had a tip resistance of 15–20 M Ω . The records were sampled and stored in the computer through the amplifier (MEZ8201, Nihon Kohden, Japan) and PowerLab interface (PowerLab ML785, ADInstruments, Australia)^[7,10]. The calculated parameters for the fast response AP included the resting potential (RP), the action potential amplitude (APA), the maximal depolarization velocity (V_{\max}), the action potential duration (APD₅₀ and APD₉₀), and the effective refractory period (ERP). To measure the ERP, the preparations of guinea pig were driven by a series of 8 stimuli pulses at a frequency of 1 Hz. Following the last pulse, an additional test pulse was added. By adjusting the time interval between the eighth pulse and the test stimulus, which was able to elicit an extra AP, the minimum interval was calculated as the ERP.

In our study of the sinus node, the parameters of the slow response AP were measured as APA, V_{\max} , APD₉₀, the maximum repolarization potential (MRP) and the spontaneous depolarization rate (SDR) in phase 4.

Voltage clamp experiment

The patch clamp technique was used to record the whole-cell and single channel currents. The cells were perfused with the bath solution mentioned above at a rate of 3 mL/min. The protocol for whole cell current recording is shown in the Results section.

Single Na⁺ channel current was recorded in an inside-out configuration. Since K⁺ and Ca²⁺ channels were blocked by TEA, 4-AP, CoCl₂, and CsCl in the pipette solution^[11], the only channel that allowed an inward current to pass through would be the Na⁺ channel.

Regarding the whole cell calcium current recording, the perforated patch clamp technique described in detail by our previous papers^[12,13] was used to prevent the run-down of calcium current. The perfusion solution contained 4-aminopyridine (4-AP, Sigma, USA), which was used to block the transient outward current (I_{to}) to reveal the Ca²⁺ current. An oscilloscope (DSS6521, Kikusui, Japan) and Axopatch-1D amplifier (Axon Instruments, Foster City, CA, USA) were used to monitor and record the clamped voltage and channel currents. The data were sampled with the Clampex 9.0 software suite (Axon Instruments) and stored in the computer through the interface (Digidata 1320, Axon Instruments). The sample

rate was 100 kHz with a filtering frequency of 10 kHz.

Statistical analysis

Chart 5 (ADInstruments, Australia) software, Clampex 9.0, and Origin 6 (Microcal Software, Inc, USA) were used for measurement and statistical analysis. All of the results were expressed as means \pm SEM. Statistical significance was determined using Student's *t*-test for paired data. $P < 0.05$ was considered statistically significant.

Results

Sophocarpine did exert an anti-arrhythmic effect

During tachyarrhythmia modeling, the heart was first perfused with isoprenaline (15 $\mu\text{mol/L}$)-containing Tyrode's solution. About 6–8 min later, the tachyarrhythmia or ventricular fibrillation was initiated and recorded. After that, sophocarpine was added to the isoprenaline-containing perfusion solution. About 11–17 min later, the tachyarrhythmia or the ventricular fibrillation was stopped, and the regular heartbeats appeared again on the ECG. Six guinea pig hearts were subjected to this protocol. All of them developed the tachyarrhythmia produced by isoprenaline, and all of the tachyarrhythmia was inhibited by sophocarpine (300 $\mu\text{mol/L}$). Isoprenaline increased the heart rate from 218 ± 26 to 284 ± 37 beats/min, leading to tachyarrhythmia; in contrast, sophocarpine returned the heart rate to 179 ± 24 beats/min and finally stopped the tachyarrhythmia. The washing out of sophocarpine and isoprenaline returned the heart rate to 207 ± 28 beats/min. Figure 1B-e shows that after the sophocarpine had been washed out with the same isoprenaline-containing solution, the tachyarrhythmia recurred.

The cardiac electrophysiological properties of sophocarpine

Four types of experiments were carried out to study the electrophysiological properties of sophocarpine: 1) recording of the fast response AP, 2) recording of the slow response AP, 3) measuring of Na⁺ current in whole cell and single channel configurations, and 4) observing the changes in Ca²⁺ and K⁺-tail currents.

The effects of sophocarpine on the fast response AP of ventricular cells

After the APs of the guinea pig papillary muscles had stabilized, the preparations were then perfused consecutively with different concentrations of sophocarpine-containing Tyrode's solution. Each perfusion lasted for about 8–10 min; meanwhile the RP, APA, V_{\max} , and APD were measured^[7].

The effective refractory period was obtained by an extra super-threshold stimulus, which was applied during different repolarizing phases of the AP. Figure 2A shows that the APA was decreased while the APD and the effective refractory periods of the AP were dose-dependently prolonged by sophocarpine. The data from 6 experiments are presented in Table 1.

For measurement of the dose relationship between APD and sophocarpine, the concentrations of sophocarpine used ranged

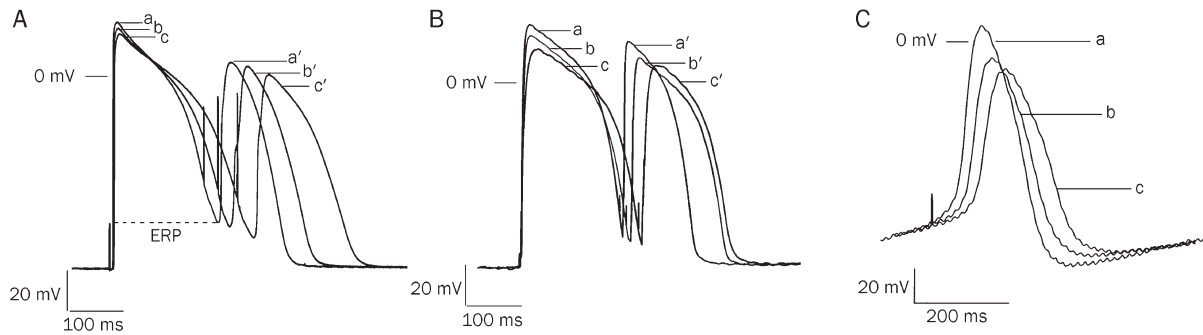


Figure 2. The effects of sophocarpine (Soph) and amiodarone (Amio) on the fast response AP of guinea pig papillary muscle and the slow response AP of rabbit sinus node cells.

A: The superimposed traces of the AP with the extra responses in different concentrations of Soph. The short upward straight lines before the AP and the each extra-response (a', b', and c') were stimulus artifacts. The figure showed that Soph not only decreased the APA (from trace a to c) but also prolonged the action potential durations and the effective refractory period (ERP) in a dose-dependent manner, and at the same time it did not change the resting membrane potential very much. (a) Control; (b) Soph 1 $\mu\text{mol/L}$. (c) Soph 10 $\mu\text{mol/L}$.

B: The effect of Amio on the AP. (a) Control; (b) Amio 1 $\mu\text{mol/L}$; (c) Amio 10 $\mu\text{mol/L}$.

C: The superimposed action potential traces of sinus node with different concentrations of Soph. The short vertical bars before APs were the stimulus artifacts. Soph dose-dependently decreased the APA and prolonged the APD (from trace a to c). (a) Control; (b) Soph 0.5 $\mu\text{mol/L}$; (c) Soph 1 $\mu\text{mol/L}$.

Table 1. The electrophysiological effects of sophocarpine (Soph) and amiodarone (Amio) on the AP of ventricular myocytes of guinea pig ($n=6$, mean \pm SEM). ^a $P>0.05$, ^b $P<0.05$, ^c $P<0.01$ vs control. ^d $P>0.05$, ^e $P<0.05$, ^f $P<0.01$ vs 1 $\mu\text{mol/L}$ group. ^g $P>0.05$, ^h $P<0.05$, ⁱ $P<0.01$ vs 5 $\mu\text{mol/L}$ group.

	$\mu\text{mol/L}$	RP (mV)	APA (mV)	V_{max} (V/s)	APD ₅₀ (ms)	APD ₉₀ (ms)	ERP (ms)
Soph	Control	-90.5 \pm 0.5	124.2 \pm 1.3	221.7 \pm 10.4	207.1 \pm 14.9	250.0 \pm 15.7	244.0 \pm 12.4
	1	-89.4 \pm 0.7 ^a	123.3 \pm 1.7 ^a	204.2 \pm 10.8 ^b	212.5 \pm 16.4 ^a	261.2 \pm 13.8 ^a	244.6 \pm 14.5 ^a
	5	-89.3 \pm 1.0 ^{ad}	121.6 \pm 1.5 ^{bd}	192.6 \pm 10.0 ^{cd}	225.5 \pm 9.5 ^{bd}	283.4 \pm 13.5 ^{ce}	270.2 \pm 17.9 ^{be}
	10	-89.9 \pm 1.1 ^{adg}	119.2 \pm 1.6 ^{efh}	167.5 \pm 11.5 ^{efi}	234.8 \pm 10.0 ^{ceg}	302.3 \pm 14.7 ^{cfg}	295.5 \pm 13.6 ^{cfh}
		(-0.7%)	(-4.0%)	(-24.4%)	(13.4%)	(20.9%)	(21.1%)
Washout	-90.1 \pm 0.8	123.5 \pm 2.0	202.9 \pm 13.1	210.5 \pm 15.7	264.3 \pm 16.1	247.3 \pm 16.4	
Amio	Control	-90.8 \pm 0.7	123.3 \pm 3.2	221.3 \pm 11.5	203.5 \pm 10.1	240.9 \pm 13.6	231.6 \pm 12.7
	1	-90.5 \pm 0.5 ^a	115.4 \pm 3.9 ^b	204.1 \pm 15.7 ^a	209.4 \pm 12.0 ^a	251.6 \pm 15.3 ^a	246.2 \pm 11.5 ^b
	10	-90.0 \pm 0.3 ^{ad}	109.6 \pm 4.5 ^{cd}	161.5 \pm 10.2 ^{cf}	226.9 \pm 12.3 ^{be}	267.6 \pm 16.0 ^{bd}	265.6 \pm 13.0 ^{ce}
		(-0.9%)	(-11.1%)	(-27.2%)	(11.5%)	(11.1%)	(14.7%)
	Washout	-89.9 \pm 0.6	118.9 \pm 4.7	187.8 \pm 13.4	205.6 \pm 9.8	243.7 \pm 14.8	228.9 \pm 13.1

The numbers in the brackets are the percentage changes vs controls.

from 1 to 100 $\mu\text{mol/L}$. EC₅₀ (median effective concentration) of the prolonged APD₉₀ was 5.93 $\mu\text{mol/L}$; the dose response curve is presented in Figure 3.

The effect of sophocarpine on the slow response AP of rabbit sinus node

In order to observe the effect of sophocarpine on the slow response AP of sinus node, the preparation of rabbit sinus node had to be driven by electric stimulus at the rate of 130 times/min (faster than its own spontaneous beating). In this way, we could eliminate the influence of the varying rhythm of spontaneous activity of the sinus node on the APD; otherwise, it might interfere with the calculation of the AP parameters, for it is known that the faster the heart beats, the shorter the APD will be. In those experiments, the stimulated APs

were recorded by glass microelectrode^[10]. The changes in AP parameters induced by sophocarpine are listed in Table 2. Figure 2C presents results from 5 experiments on the sinus node. In Figure 2C, the decreases in both the APA and the V_{max} were observed after the application of different concentrations of sophocarpine. Furthermore, the APD₉₀ was also prolonged by sophocarpine, which was in accordance with the results from the fast response AP recordings (Figure 2A).

The possible mechanism for the sophocarpine-mediated prolongation of the APD

Chromanol 293 B^[14] specifically blocks I_{Ksr} , while dofetilide^[15] blocks I_{Kr} . In this experiment, chromanol 293 B was first applied to block I_{Ksr} and then sophocarpine with different concentrations was consecutively added to the perfus-

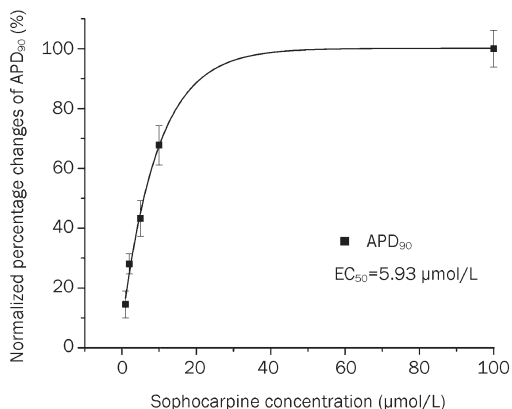


Figure 3. Dose-response of the APD₉₀ of the papillary muscle to sophocarpine (Soph). Abscissa, concentration of Soph. Ordinate, normalized percentage changes of APD₉₀ by Soph ($n=5$). Data was fitted with Exponential Decay Function. EC₅₀ was 5.93 µmol/L.

ing solution. Figure 4-IA shows that the prolongation of APD induced by chromanol 293 B (trace b) was further increased by sophocarpine (trace c and d), which suggests that sophocarpine might inhibit I_{Kr} , resulting in the increased APD. To confirm this, the following protocol was used (Figure 4-IB): Dofetilide was first used to inhibit the I_{Kr} , leading to the increase in APD (trace b); then sophocarpine at different concentrations was added to the perfusing solution. Figure 4-IB demonstrates that the APD was no longer affected by sophocarpine (traces c and d). The above results indicate that sophocarpine could block I_{Kr} rather than I_{Ksr} , leading to pro-

Table 2. The effects of sophocarpine (Soph) on the slow response AP of rabbit sinus node cells ($n=5$, mean±SEM). ^a $P>0.05$, ^b $P<0.05$, ^c $P<0.01$ vs control. ^d $P>0.05$, ^e $P<0.05$, ^f $P<0.01$ vs 0.1 µmol/L group. ^g $P>0.05$, ^h $P<0.05$, ⁱ $P<0.01$ vs 0.5 µmol/L group. MRP, the maximum repolarization potential.

Soph (µmol/L)	APA (mV)	V _{max} (V/s)	APD ₉₀ (ms)	MRP (mV)
Control	68.0±3.3	9.8±0.5	123.2±8.3	-65.8±3.1
0.1	64.7±2.2 ^a	9.1±0.4 ^b	124.8±7.1 ^a	-65.2±3.3 ^a
0.5	58.1±3.9 ^{ce}	8.8±0.5 ^{cd}	127.7±7.1 ^{ad}	-63.1±2.5 ^{ad}
1	53.4±3.0 ^{cf}	8.3±0.6 ^{cf}	140.7±7.0 ^{fh}	-61.6±2.7 ^{beg}
Washout	66.3±4.2	9.4±0.7	128.3±7.2	-65.5±2.9

longation of the APD. Figure 4-II presents the 5 experimental results with the washing-out data.

The effect of sophocarpine on the cardiac Na⁺ current

The action of sophocarpine on the cardiac Na⁺ current was carried out in 2 experimental levels: the whole cell I_{Na} recording and single Na⁺ channel current recording.

Sophocarpine decreased the whole cell I_{Na}

Whole cell current recording was carried out in enzyme-isolated ventricular cells of neonatal rat heart. The cell was depolarized from the holding potential (-110 mV) to the testing potential (-50 mV) for 300 ms before it was returned to the holding potential. The initial large inward current was invoked by depolarization clamping, as shown in Figure 5A.

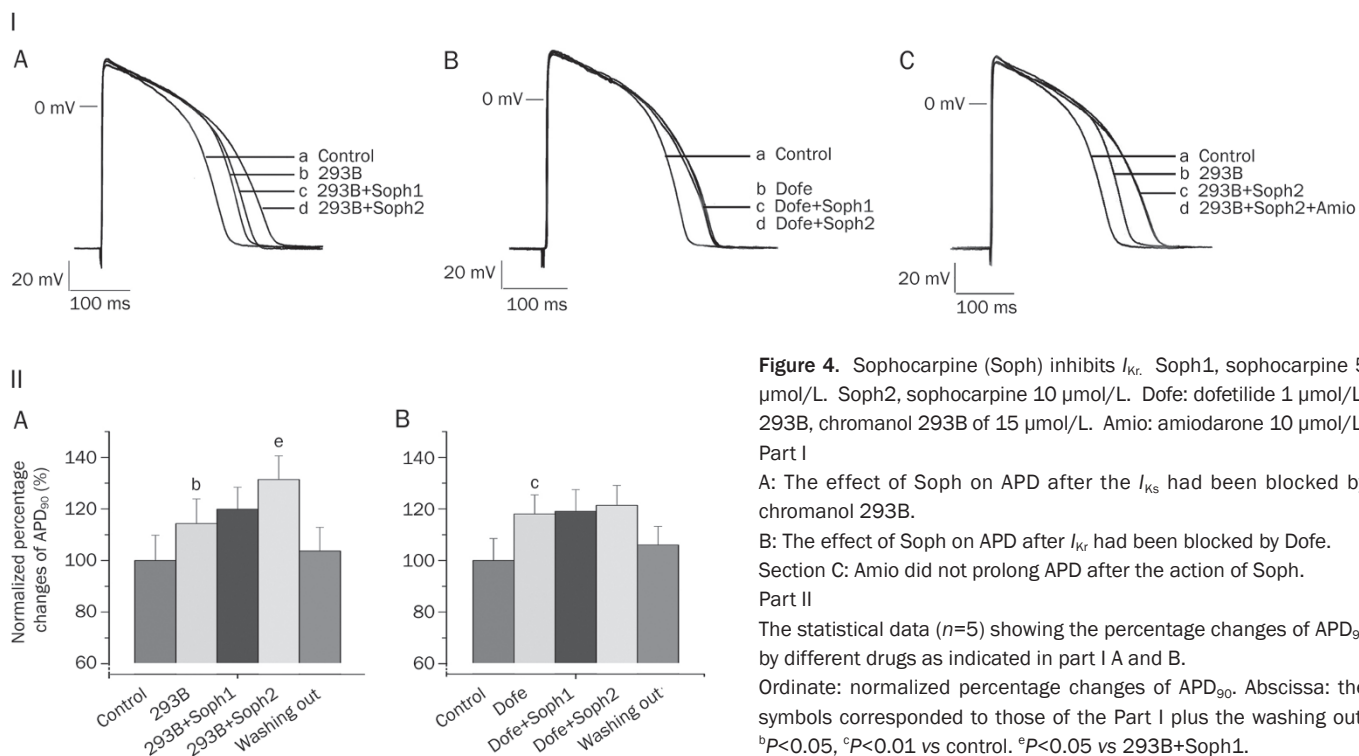


Figure 4. Sophocarpine (Soph) inhibits I_{Kr} . Soph1, sophocarpine 5 µmol/L. Soph2, sophocarpine 10 µmol/L. Dofe: dofetilide 1 µmol/L. 293B, chromanol 293B of 15 µmol/L. Amio: amiodarone 10 µmol/L. Part I

A: The effect of Soph on APD after the I_{Ks} had been blocked by chromanol 293B.

B: The effect of Soph on APD after I_{Kr} had been blocked by Dofe.

Section C: Amio did not prolong APD after the action of Soph.

Part II

The statistical data ($n=5$) showing the percentage changes of APD₉₀ by different drugs as indicated in part I A and B.

Ordinate: normalized percentage changes of APD₉₀. Abscissa: the symbols corresponded to those of the Part I plus the washing out. ^b $P<0.05$, ^c $P<0.01$ vs control. ^e $P<0.05$ vs 293B+Soph1.

As this inward current had been blocked by the special Na^+ channel blocker TTX $10 \mu\text{mol/L}$ (not shown), this current could be regarded as $I_{\text{Na}}^{[7]}$. After the cell was perfused with sophocarpine ($10 \mu\text{mol/L}$), the inward current was decreased substantially. From 3 experiments that involved whole cell recordings, the average decrease of the I_{Na} induced by sophocarpine ($10 \mu\text{mol/L}$) was about 18.0%.

Sophocarpine inhibited single Na^+ channel activity

Single Na^+ channel current recordings were carried out in an inside-out configuration on isolated ventricular myocytes from neonatal rats. The patch membrane was first held at -110 mV and then depolarized to and clamped at -50 mV for 500 ms before it was returned to the holding potential. As the pipette solution contained Ca^{2+} and K^+ channel blockers (see Material and methods section), the recorded channel current may have represented the Na^+ current.

Single channel openings were observed on the recording traces. Both the open occurrences and the averaged inward current were decreased after the cells had been perfused with a sophocarpine ($30 \mu\text{mol/L}$)-containing solution in 3 experiments. Figure 5B shows one of the results.

Sophocarpine inhibited the I_{CaL} of cardiac myocytes

The whole cell recording configuration and perforated patch technique were used in this experiment. The ventricular cells of neonatal rat were first held at -40 mV to inactivate the Na^+ channels and then depolarized to the command potential of 20 mV for 500 ms before they were returned to the holding potential (Figure 6). As 4-AP had been used to block the I_{to} , the inward current was clearly discernible at the command potential (Figure 6a). Sophocarpine ($10 \mu\text{mol/L}$) was then added to the perfusion solution. About 3 min later, not only had the inward current decreased, but also the tail current (outward K^+ current, a') was diminished. Compared with the controls in 3 experiments, average I_{CaL} decreased by $33.2\% \pm 11.7\%$ at the command potential of 20 mV .

Comparison of electrophysiological effects between sophocarpine and amiodarone

From the above results, we can see that sophocarpine induced effects similar to those of class III anti-arrhythmic drug amiodarone, *ie*, it was also able to prolong the APD. The following experiments were designed to compare these two drugs in their effects on both the fast and the slow response AP morphologies.

Effect of amiodarone on the fast response AP

The preparation and the experimental protocol were the same as sophocarpine section. Amiodarone at a concentration of $10 \mu\text{mol/L}$ not only prolonged the APD and ERP by 11.1% and 14.7%, respectively, but also decreased the APA and V_{max} . The results from 6 experiments are presented in Table 1, and one example is shown in Figure 2B.

Because our results confirmed that sophocarpine could inhibit I_{Kr} to prolong the APD and that amiodarone could

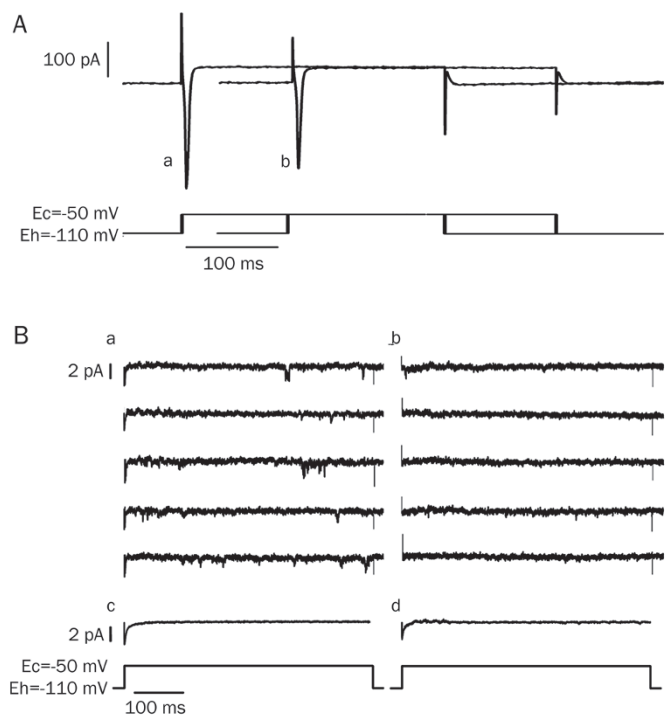


Figure 5. Sophocarpine (Soph) decreased the early Na^+ current.

A: Soph decreased I_{Na} in the whole cell recording configuration. The upper half showed the whole cell sodium current recordings; the lower one was the voltage clamp protocol. (a) Control inward current. (b) The decreased current by Soph $10 \mu\text{mol/L}$.

B: Soph decreased the Na^+ current in the single channel recording configuration. The upper parts (a, b, c, d) referred to the channel current recording; the lower was the voltage clamp protocol. (a) A group of control traces. (b) The traces after Soph $30 \mu\text{mol/L}$. (c and d) The averaged inward currents which were calculated before and after the Soph from 43 and 35 trails respectively.

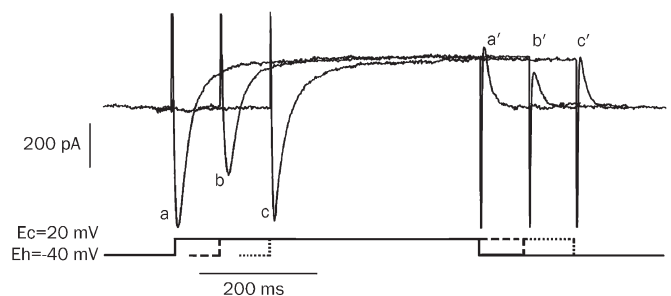


Figure 6. Sophocarpine (Soph) inhibited I_{CaL} and I_{K} tail current. The a, b, c, and a', b', c' represented I_{CaL} and I_{K} tail currents respectively. (a) Control. (b) Soph $10 \mu\text{mol/L}$. (c) Washing out.

block the I_{Kr} , the following experiment was designed to determine what would happen to amiodarone if sophocarpine were first used to inhibit I_{Kr} .

Figure 4-IC shows that after chromanol 293B had blocked the I_{Ks} (from trace a to trace b) leaving I_{Kr} , sophocarpine increased the APD (from b to c), and then amiodarone (at the

same concentration as sophocarpine) was added to the perfusing solution. The latter had no obvious effect on the APD (traces c and d were superimposed); neither were substantial changes to the APD observed when sophocarpine was administered before amiodarone (data not shown).

The effects of amiodarone and sophocarpine on the spontaneous AP of rabbit sinus node

The preparation of rabbit sinus node did not involve electric stimulus; spontaneous APs were recorded with a glass micro-electrode. After the control AP was recorded in Tyrode's solution, amiodarone (10 $\mu\text{mol/L}$) or sophocarpine (10 $\mu\text{mol/L}$) was added to the perfusion solution. About 10 min later, the changes in AP produced by the drugs were measured, and then the drugs were washed out with control Tyrode's solution. Sophocarpine-mediated inhibition of V_{max} and APA started at about 2 min and reached the maximum in another 1 min. The inhibitory effect on APA or V_{max} lasted stably for a considerable time. In our records, we only pre-treated the preparations for about 30–50 min and then washed them out to observe the recovery process. Full recovery could be obtained after 8 to 10 min of washing out. Figure 7 illustrates the comparison. The results from 5 experiments are shown in Table 3. In short, the data show that both drugs could decrease the APA, the V_{max} and the spontaneous heart rate and that both could prolong the APD to some extent.

Discussion

The electrophysiological effects of sophocarpine on the heart

Phase 0 of the fast response AP is initiated by the fast inward Na^+ current. The APA and V_{max} are related to the dynamics of this current. Our whole current experiments demonstrated that sophocarpine could substantially inhibit the I_{Na} , which would explain why sophocarpine decreased APA and V_{max} in the experiment on the fast response AP. In the inside-out recording experiments, K^+ and Ca^{2+} channels had been blocked by CoCl_2 , TEA, 4-AP, and CsCl in the pipette solution; hence, the inward current in Figure 5 must have been the Na^+ current. The decreased opening and reduced magnitude of the Na^+

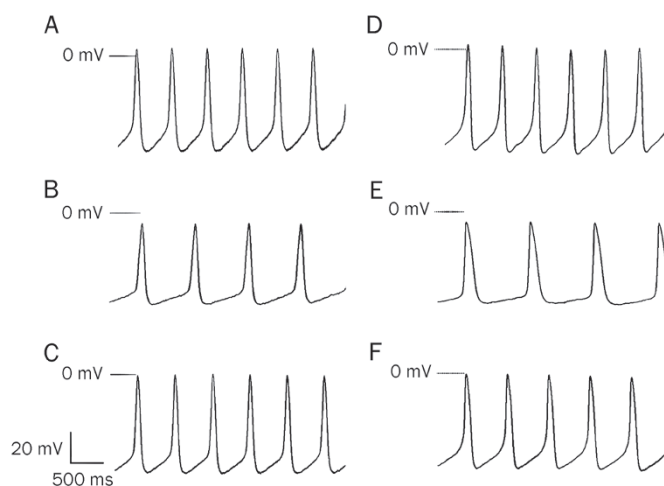


Figure 7. Sophocarpine (Soph) and amiodarone (Amio) had the similar effects on the spontaneous AP of rabbit sinus node. The traces of A, B, C, D, E, and F were spontaneous APs. A, B, C belonged to Soph experiment, and D, E, F to those of Amio's. A and D: Controls. B: Ten minutes after Soph 10 $\mu\text{mol/L}$. E: Ten minutes after Amio 10 $\mu\text{mol/L}$. C and F: Washout with the control Tyrode's solution.

channel current following sophocarpine treatment confirmed the inhibitory effect of sophocarpine on Na^+ channels.

During the whole cell recording of the slow inward current (I_{CaL}), sophocarpine substantially decreased the current. The slow response AP, such as the AP in sinus node, is initiated by the slow I_{CaL} . The APA and V_{max} of the slow AP are dependent on the dynamics or conditions of the Ca^{2+} channel. The decreases in APA and V_{max} induced by sophocarpine could result from the inhibitory effect of sophocarpine on the Ca^{2+} channel.

APD is mainly related to the balance between the inward I_{CaL} and the outward K^+ current. The observed prolongations of the APD in both the fast response AP (papillary muscle myocytes) and the slow response AP (sinus node cells) mediated by sophocarpine suggest that sophocarpine could also inhibit the outward K^+ current, which would result in retardation of

Table 3. The effects of sophocarpine (Soph) and amiodarone (Amio) on the slow response AP of rabbit sinus node ($n=5$, mean \pm SEM). ^a $P>0.05$, ^b $P<0.05$, ^c $P<0.01$ vs control.

	$\mu\text{mol/L}$	APA (mV)	V_{max} (V/s)	APD ₉₀ (ms)	MRP (mV)	SDR (mV/s)	HR (beat/min)
Soph	Control	70.2 \pm 1.2	10.1 \pm 0.7	110.5 \pm 7.2	-66.3 \pm 1.2	55.3 \pm 2.4	110.4 \pm 5.3
	10	51.4 \pm 3.0 ^c (-26.8%)	7.5 \pm 0.6 ^c (-25.7%)	122.7 \pm 8.1 ^b (11.0%)	-60.2 \pm 1.5 ^b (-9.2%)	29.2 \pm 2.8 ^c (-47.2%)	80.7 \pm 4.4 ^c (-26.9%)
	Washout	64.5 \pm 2.2	9.2 \pm 0.7	112.9 \pm 7.6	-64.2 \pm 1.0	43.6 \pm 4.5	102.5 \pm 6.8
Amio	Control	69.8 \pm 1.5	9.8 \pm 1.0	106.8 \pm 8.7	-67.2 \pm 1.7	56.0 \pm 2.6	112.7 \pm 6.1
	10	50.9 \pm 2.6 ^c (-27.1%)	4.7 \pm 0.8 ^c (52.0%)	135.6 \pm 10.2 ^c (27.0%)	-60.9 \pm 2.1 ^b (-9.4%)	18.8 \pm 3.2 ^c (-66.4%)	71.4 \pm 5.7 ^c (-36.6%)
	Washout	62.7 \pm 4.3	8.5 \pm 1.3	110.2 \pm 9.4	-63.1 \pm 3.8	40.1 \pm 4.7	100.9 \pm 7.5

SDR, spontaneous depolarization potential rate in phase 4; HR, heart rate.

the repolarization and prolongation of the APD. The direct evidence for sophocarpine-mediated inhibition of the K^+ current is presented in Figure 6, which presents the sophocarpine-mediated reduction of the K^+ tail current (Figure 6b'). Notably, after I_{Ks} had been blocked by chromanol 293B, sophocarpine was capable to dose-dependently prolong the APD; however, if I_{Kr} was first inhibited by the I_{Kr} -blocker dofetilide, sophocarpine did not demonstrate this effect. Although this design was unable to rule out whether I_{Kr} was involved in the process, our results coincided with those described by Yang *et al* in their study of the effect of sophocarpine on HERG channel stably expressed in human embryonic kidney-293 cells^[13]. The authors demonstrated that sophocarpine inhibited the transfected HERG channels by influencing the inactivation state^[16].

Sophocarpine could decrease the *L*-type calcium current, which might shorten the APD_{90} and interfere with its prolongation effect on APD ^[17] that there are two different repolarizing currents that are activated by a rise in cytosolic calcium concentration^[17]. One current, I_{KCa} , appears to be a member of the K_v family of potassium channels; the other, I_{to2} , is probably a calcium-activated chloride channel. Both respond to a rise in cytosolic calcium by shortening the action potential duration, limiting further calcium entry into the heart. According to this opinion, we suppose that the reduction of the calcium current by sophocarpine would therefore have less effect on I_{KCa} and be conducive to the prolongation of APD . Valenzuela *et al*^[18], in their experiments on guinea pig ventricular myocytes, found that amiodarone (16 $\mu\text{mol/L}$) induced dramatic tonic and phasic reductions of the Ca^{2+} current. Nonetheless, amiodarone still prolonged the APD .

The possible mechanism of the anti-arrhythmic function of sophocarpine

β -adrenergic agonists lead to the elevation of cytosolic cyclic AMP levels and the activation of protein kinase A. This kinase phosphorylates *L*-type Ca^{2+} channels and thereby increases the channel's open probability, resulting in large Ca^{2+} current into the cell. Normally, the inward Ca^{2+} current is balanced by the Ca^{2+} pumps located in the sarcoplasmic reticulum and cell membrane and in the Na^+/Ca^{2+} exchangers in the cell membrane. The tachyarrhythmia produced in our experiment may be due to the use of high concentrations of isoprenaline (15 $\mu\text{mol/L}$) in the perfused solution. Isoprenaline could induce the delayed after-depolarization (DAD) by increasing sarcoplasmic reticulum (SR) Ca^{2+} load, leading to spontaneous SR Ca^{2+} release and activation of transient inward current (I_{ti}), which underlies DADs^[19]. Ultimately, ECG revealed tachyarrhythmia or fibrillation of the ventricle.

Tosaki *et al*^[20] reported that in the isolated rat heart, reperfusion-induced arrhythmias were exacerbated by isoproterenol (0.01–1.0 $\mu\text{mol/L}$). The proarrhythmic action of isoproterenol was primarily the result of a β_1 -receptor-mediated tachycardia rather than a free radical mediated process^[21], since it was prevented competitively by the β_1 -receptor antagonist metoprolol, but not by the free radical scavengers. The authors

also found^[22] that drugs such as lidocaine, which prevent ion movements induced by some pathologic changes, protected against reperfusion-induced arrhythmias.

It has been said that 'Virtually all anti-arrhythmic drugs inhibit the opening or reactivation of voltage-gated ion channels'^[23]. Because sophocarpine inhibited Ca^{2+} channel current, it was expected to relieve the Ca^{2+} -overload produced by isoprenaline. Furthermore, sophocarpine, with its inhibitory effects on potassium and sodium currents, was not only able to prolong the effective refractory period but also able to decrease the conduction velocity via its inhibition of APA and V_{max} of the AP. Those two effects might be conducive to its antiarrhythmic effect because propagation of the AP can be interrupted by those two effects if reentry occurs during tachyarrhythmia. However, one reporter showed that the increase or decrease in heart rate was closely correlated with the development of arrhythmias^[24]. The investigation showed that increasing the heart rate in increments from 200 to 400 beats/min caused an increased incidence of ventricular fibrillation and ventricular tachycardia during reperfusion. Some researchers^[25] explained that a higher heart rate would cause the accumulation of extracellular potassium. Loss of cellular potassium was a critical factor in determining the vulnerability of the heart to arrhythmias. Furthermore, a lower heart rate also directly decreases the possibility of triggered activity^[26]. In our experiment with isoprenaline, the perfused heart rate increased from 218 ± 26 to 284 ± 37 beats/min and tachyarrhythmia developed. After sophocarpine, the heart rate decreased to 179 ± 24 beats/min and the tachyarrhythmia disappeared (Figure 1). In terms of Tosaki's point of view, the heart-rate-slowing effect of sophocarpine may explain one of its antiarrhythmic mechanisms. Although we have no direct evidence to support the above hypotheses or experiments to rule out whether sophocarpine could act as a β -receptor antagonist, the fact is that sophocarpine does reverse the tachyarrhythmia and fibrillation produced by isoprenaline. This antiarrhythmic function of sophocarpine has been proven by other experimental reports^[1–4] and clinical practice^[5, 27].

According to Chen's clinical observations^[5, 27], sophocarpine has no obvious harmful effects on the functions of the liver or kidney or on the blood system. A few patients have exhibited nausea or urticaria. After a decrease in the dosage, the symptoms disappeared in a few days. A clinical trial on sophocarpine conducted by researchers at Renji Hospital has progressed from stage II to stage III. The information gathered should be illuminating.

Comparison of sophocarpine with amiodarone with regard to electrophysiological characteristics

In our preliminary experiments, we found that sophocarpine had some inhibitory effect on potassium, sodium and calcium currents, similar to amiodarone^[28]. It has been reported that sotalol has no effects on sodium or calcium currents. That was the main reason we chose amiodarone instead of sotalol for comparison with sophocarpine. Furthermore, the clinical review maintains: Despite limited indication, amio-

darone is one of the most frequently prescribed specific antiarrhythmic drugs in the United States^[29-31]. Amiodarone is a class III antiarrhythmic drug. It inhibits repolarizing K⁺ currents and so prolongs the cardiac AP; it weakly inhibits depolarizing currents, such as those mediated by the Na⁺ and Ca²⁺ channels^[32-34]. Therefore, it was worth comparing the electrophysiological effects of these two drugs. Our results indicated that their effects were very similar: both can inhibit the APA and V_{max} of the fast and the slow response APs; both can prolong the APD and the ERP of ventricular myocytes; both can decrease the automatic heartbeat and spontaneous depolarization rate in phase 4 of the sinus node AP. However, in some respects, for example in the reduction of spontaneous heart beating, amiodarone seemed more powerful than sophocarpine: 10 μmol/L amiodarone decreased the heartbeat by 36.6%, while 10 μmol/L sophocarpine decreased the heartbeat by only 26.9%. In other respects, sophocarpine could increase the APD and prolong the ERP for a little longer than amiodarone. Actually, we do not know which is more effective in clinical use, for this kind of comparison must include many other factors, such as the side effects or the kinetics of the drugs, *etc.* However, Li *et al*, in their study on the antiarrhythmic properties of long-term treatment with matrine in rats with arrhythmia induced by coronary ligation, found that long term oral administration of Martine reduced arrhythmia and mortality more effectively than did amiodarone^[35].

Study limitations

One limitation of the present study is the lack of a comparison between sophocarpine and amiodarone and the short-lived duration of the effects of amiodarone on I_{Na}, I_{CaL} and I_{Kr} currents. Thus, further studies are needed.

Another limitation is that sophocarpine has obvious inhibitory effects on the isoprenaline-induced tachyarrhythmia, yet it is still unclear which of its electrophysiological properties plays the crucial role in this action. The mechanism by which isoprenaline induces tachyarrhythmia is complicated. Either the delayed after-depolarization or the re-entry mechanisms or the changes in heart rate may take part in the process. Further study will be necessary.

Conclusion

Sophocarpine could reverse isoprenaline-induced arrhythmia and inhibit I_{Na}, I_{CaL} and I_{Kr} currents. The electrophysiological effects of sophocarpine are similar to those induced by amiodarone, which might be regarded as a prospective antiarrhythmic drug in clinical practice.

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Author contribution

Ci-zhen LI and Yuan-mou LIU designed the research plan;

Zhi-fang YANG, Ci-zhen LI, Hong-wei WANG, Wei WANG, and Ying ZHANG performed the research; Zhi-fang YANG, Hong-wei WANG, Ci-zhen LI, and Ying-min CHEN analyzed the data; Hong-wei Wang and Yuan-mou LIU wrote the paper.

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