

Original Article

Inhibitory effects of tetrandrine on the Na⁺ channel of human atrial fibrillation myocardium

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Aim: Tetrandrine (Tet) is a Ca²⁺ channel blocker and has antiarrhythmic effects. Less information exists with regard to the mechanisms underlying its antiarrhythmic action other than blocking Ca²⁺ channels. In this study, the effects of Tet on the Na⁺ current (I_{Na}) in the atrial myocardium of patients in atrial fibrillation (AF) and sinus rhythm (SR) were investigated, and the characteristics of the Na⁺ current were synchronously compared between the AF and SR patients.

Methods: Na⁺ currents were recorded using the whole-cell patch clamp technique in single atrial myocyte of the AF and the normal SR groups. The effects of Tet (40–120 μmol/L) on the Na⁺ current in the two groups were then observed.

Results: Tet (60–120 μmol/L) decreased I_{Na} density in a concentration-dependent manner and made the voltage-dependent activation curve shift to more positive voltages in the SR and AF groups. After exposure to Tet, the voltage-dependent inactivation curve of I_{Na} was shifted to more negative voltages in the two groups. Tet delayed the time-dependent recovery of I_{Na} in a concentration dependent manner in both AF and SR cells; however, there were no differences in the effects of Tet on I_{Na} density and properties in the two groups. The I_{Na} density of AF patients did not differ from that of the SR patients.

Conclusion: Tet can block sodium channels with slow recovery kinetics, which may explain the mechanisms underlying the antiarrhythmic action of Tet. The decreased conduction velocity (CV) in AF patients is not caused by the Na⁺ current.

Keywords: tetrandrine; electrophysiology; arrhythmia; sodium channels; heart

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Introduction

Atrial fibrillation is the most common arrhythmia in clinical practice and can produce electrical remodeling that contributes to the self-perpetuation of AF. Electrical remodeling of AF is characterized by a marked reduction in the atrial effective refractory period, a decrease or reversal of its physiologic adaptation to heart rate, and a decrease in atrial conduction velocity (CV)^[1–3]. Sodium current is one of the critical parameters affecting CV. In the canine rapid pacing model of AF, Gaspo *et al* have observed a decreased I_{Na} during AF that has been correlated with the slowing of atrial conduction^[4]. Only small amounts of data have been reported with regard to the differences in the characteristics of I_{Na} between AF and SR patients.

Tet, an alkaloid extracted from the Chinese medicinal

herb *Radix stephania tetrandrae*, possesses a wide spectrum of pharmacological activities. Tet is a nonspecific Ca²⁺ channel blocker and has been clinically used to treat patients with angina and hypertension in China^[5]. Tet has played an antiarrhythmic role in several experimental arrhythmic models^[6–9]. Tet reduces the incidence of arrhythmias induced by cesium chloride in rabbits^[7]. Tet has also been reported to produce equally potent ameliorating effects on arrhythmia and infarcts induced by ischemia and reperfusion without further inhibiting ischemia-reduced heart rate and coronary artery flow; the results indicate Tet may be a better choice for the treatment of arrhythmia than the classical Ca²⁺ channel blocker^[8,9]. Clinical reports have shown that Tet can terminate acute episodes of paroxysmal supraventricular tachycardia^[10]. Previous studies have also demonstrated that Tet can block Ca²⁺ channels in ventricular cells^[11], vascular smooth muscle cells^[12] and hepatocytes^[13]. Despite the antiarrhythmic action of Tet, little is known about its mechanism of action, other than that it includes blocking of Ca²⁺ chan-

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nels; no information is available about whether the antiarrhythmic actions of Tet are related to the sodium channels of humans.

The purpose of the present study was to investigate the following: 1) the effects of Tet on Na⁺ current density and kinetics in human atrial myocardium in sinus rhythm; 2) whether AF can affect the action of Tet on Na⁺ channels in humans; and 3) the differences in sodium current density and characteristics between AF and SR patients.

Materials and methods

Characteristics of patients

Right atrial appendages were obtained during the course of cardiac surgery using the following procedures approved by the Hospital Ethics Committee. Twenty-one patients were assigned to the SR group and 12 to the chronic AF group, according to the underlying cardiac rhythm. Chronic AF was defined as permanent AF of at least 7 days duration. Patient characteristics are listed in Table 1.

Table 1. Characteristics of patients with SR and AF. Mean±SD.

	SR	AF
<i>n</i> (man/feminine)	21 (12/9)	12 (8/4)
Age (year)	43±9	48±7
Underlying heart disease		
Rheumatic heart disease	9	8
Coronary artery disease	13	4
Heart function (NYHA)		
I	10	3
II	8	7
III	3	2
IV	0	0
Medication		
Digitalis	1	2
β-blockers	2	5
Ca ²⁺ -channel blockers	1	3
ACE-Inhibitors	5	6
Sotalol	0	2

SR: sinus rhythm. AF: atrial fibrillation.

Isolation of single myocytes

Single myocytes were isolated by enzymatic dissociation using the chunk method^[14]. The specimens were transported to the laboratory in a Ca²⁺-free cardioplegic solution (100% O₂, room temperature) containing (in mmol/L) 50 KH₂PO₄,

8 MgSO₄, 10 HEPES, 5 adenosine, 140 glucose, 100 mannitol, and 10 taurine, with pH adjusted to 7.4 using KOH. Chunks were washed in 25 mL EGTA solution containing (in mmol/L) 137 NaCl, 5 KH₂PO₄, 1 MgSO₄, 10 glucose, 5 HEPES, 10 taurine, and 0.1 EGTA, with pH adjusted to 7.4 using NaOH. After blood and calcium were washed out, the chunks were incubated for 35 min in 5 mL Ca²⁺-free Enzyme Incubation Medium (EIM) with the addition of 150 U/mL collagenase (TypeV), 12.6 U/mL protease (TypeXXIV) and 1 mg/mL albumin (100% O₂, 35 °C). The EIM solution contained the same composition of EGTA solution but without EGTA. This period of digestion was followed by a 1 min wash in EIM solution without any enzymes. Subsequently, the supernatant was removed and replaced by a fresh enzyme medium with the same composition but without protease and incubated until rod-shape cells were present. Cells were kept in a KB storage solution containing (in mmol/L) 30 KCl, 10 KH₂PO₄, 25 glucose, 5 mannitol, 0.1% albumin, 75 L-glutamic acid, 10 β-hydroxybutyric acid, 10 taurine, and 10 EGTA, with pH adjusted to 7.2 using KOH.

Patch clamp technique

Only quiescent, rod-shape cells with clear cross-striations were studied. These cells were transferred to the tissue chamber mounted on the stage of an inverted microscope. Myocytes were superfused (2–3 mL/min) with a solution containing (in mmol/L) the following: 132.5 CsCl, 5 NaCl, 1 MgCl₂, 1 CaCl₂, 20 HEPES, 11 glucose, and 2 CoCl₂, with pH adjusted to 7.2 using CsOH. The resistances of glass electrodes (outer diameter 1.5 mm) equaled 1–2 MΩ when filled with the following solution (in mmol/L): 135 CsF, 5 NaCl, 5 HEPES, 10 EGTA, 5 Mg-ATP, with pH adjusted to 7.35 using CsOH. K currents were suppressed with the use of this K-free solution, and I_{Ca} was blocked with 2 mmol/L Co²⁺. Na⁺ currents were recorded using the whole cell configuration of the voltage clamp technique, which was performed with an Axopatch 200B clamp amplifier (Axon Instruments). The membrane capacitance of each cell was measured using a 5 mV hyperpolarizing pulse from a holding potential of -120 mV, and these areas were divided by voltage step. The membrane capacitance was identical in the two groups: 69.17.2 pF (*n*=26) in SR and 73.68.8 pF (*n*=19) in AF; *P*=NS vs SR. To account for the differences in cell size, all mean current data are expressed as current densities. I_{Na} was recorded at room temperature.

Drugs

The main drugs were purchased from Sigma (St Louis, MO, USA), including adenosine, mannitol, taurine,

collagenase, protease, albumin, β -hydroxybutyric acid, Mg-ATP, CsOH and CsF. Tet was obtained from the Research Institute of Drug and Biologic Product, China (lot number: 000201).

Statistics

Group data are expressed as means \pm SEM. Comparisons between SR and AF groups were made using a Student's non-paired *t*-test. A *P* value <0.05 was considered statistically significant. Statistical comparisons of multiple group means were obtained by ANOVA. A nonlinear, least-square, curve-fitting program (CLAMPFIT in pCLAMP 6.0) was used to perform curve-fitting procedures.

Results

In order to avoid any contaminating effects of time-dependent changes in the I_{Na} *I*-*V* relationship, all studies began 20 min after membrane rupture, with protocols performed in the same sequence in all cells, beginning with the I_{Na} density-voltage relationship, followed immediately by analysis of voltage-dependent inactivation, and finally time-dependent recovery from inactivation.

Voltage- and concentration-dependent blockade of Na^+ current by Tet in SR cells

The data were obtained with a holding potential of -120 mV and a series of 5 mV depolarizing steps in voltage from -90 to 0 mV. Tet can decrease I_{Na} density in a concentration-dependent manner in the SR cells (Table 2). The maximal I_{Na} density was reduced by 28.1%, 46.5%, 67.6%, and 87.5% by Tet 60, 80, 100, and 120 μ mol/L, respectively. This inhibitory effect was readily reversed on the washout of Tet. Typical original current and *I*-*V* curves are shown in Figure 1A–1D. Tet did not change the threshold potential (around -70 to -60 mV), but it did change the shape of *I*-*V* curves of I_{Na} . The potential of maximum peak was shifted to a more positive potential, indicating that the block was voltage-

dependent.

We studied the voltage-dependent activation properties of the current (Figure 1E). The data were well fitted by a Boltzmann equation, with a half-maximum activation voltage averaging from -50.1 ± 0.5 mV in the control ($n=26$) to -45.9 ± 0.2 mV ($n=10$, $P<0.05$ vs control) and -41.3 ± 0.3 mV ($n=8$, $P<0.01$ vs control) by Tet 80 and 100 μ mol/L, respectively. The slope factors were not changed by Tet (Table 3). These results indicate that changes of I_{Na} density by Tet in SR cells are associated with changes in the voltage dependence of I_{Na} activation.

Voltage- and concentration-dependent blockade of Na^+ current by Tet in AF cells

The maximal I_{Na} density of AF was -61.8 ± 8.8 pA/pF ($n=19$) compared with SR cells; there was no difference between the two types of cells (Table 2). Tet also decreased I_{Na} density in a concentration-dependent manner in AF cells (Table 2). The maximal I_{Na} density was reduced by 29.7%, 44.4%, 70.9%, and 88.1% in AF cells after exposure to Tet 60, 80, 100, and 120 μ mol/L, respectively. This inhibitory effect was reversed on the washout of Tet. Figure 2 shows a typical original current and *I*-*V* curves. Tet caused the potential of maximum peak to shift to a more positive potential as well. Figure 2E shows the voltage-dependent activation properties of the current. The half-maximum activation voltage averaged from -51.5 ± 0.4 mV in the control ($n=19$) to -47.7 ± 0.4 mV ($n=8$) and -42.5 ± 0.3 mV ($n=7$) in the group exposed to Tet 80 and 100 μ mol/L, respectively, $P<0.05$ vs control). The slope factor was not changed by Tet (Table 3). The voltage-dependent activation curves of I_{Na} in SR and AF cells were all shifted to more positive voltages after exposure to Tet, which indicated that Tet could inhibit the activation of I_{Na} ; there was no difference in the inhibitory effects of Tet on SR and AF cells.

Figure 3 shows the relationships between the concentration of Tet and the percentage inhibition of Na^+ current density in the SR and AF cells. The half-maximal concentration

Table 2. The effects of Tet on I_{Na} density in SR and AF atrial myocytes. Mean \pm SEM. ^c $P<0.01$ vs control.

C_{Tet}	Maximal I_{Na} density (pA/pF)						
	0 μ mol/L	40 μ mol/L	60 μ mol/L	80 μ mol/L	100 μ mol/L	120 μ mol/L	Wash out
SR	-58.6 ± 7.8 ($n=26$)	-53.3 ± 8.5 ($n=10$)	-42.1 ± 6.9^c ($n=12$)	-31.3 ± 6.2^c ($n=10$)	-18.9 ± 5.7^c ($n=8$)	-7.3 ± 2.6^c ($n=8$)	-49.6 ± 10.8 ($n=5$)
AF	-61.8 ± 8.8 ($n=19$)	52.7 ± 9.5 ($n=8$)	-43.4 ± 7.1^c ($n=10$)	-34.4 ± 6.0^c ($n=8$)	-13.7 ± 4.3^c ($n=7$)	-5.2 ± 2.2^c ($n=7$)	-53.7 ± 11.8 ($n=4$)

SR: sinus rhythm. AF: atrial fibrillation. C_{Tet} : concentration of tetrandrine.

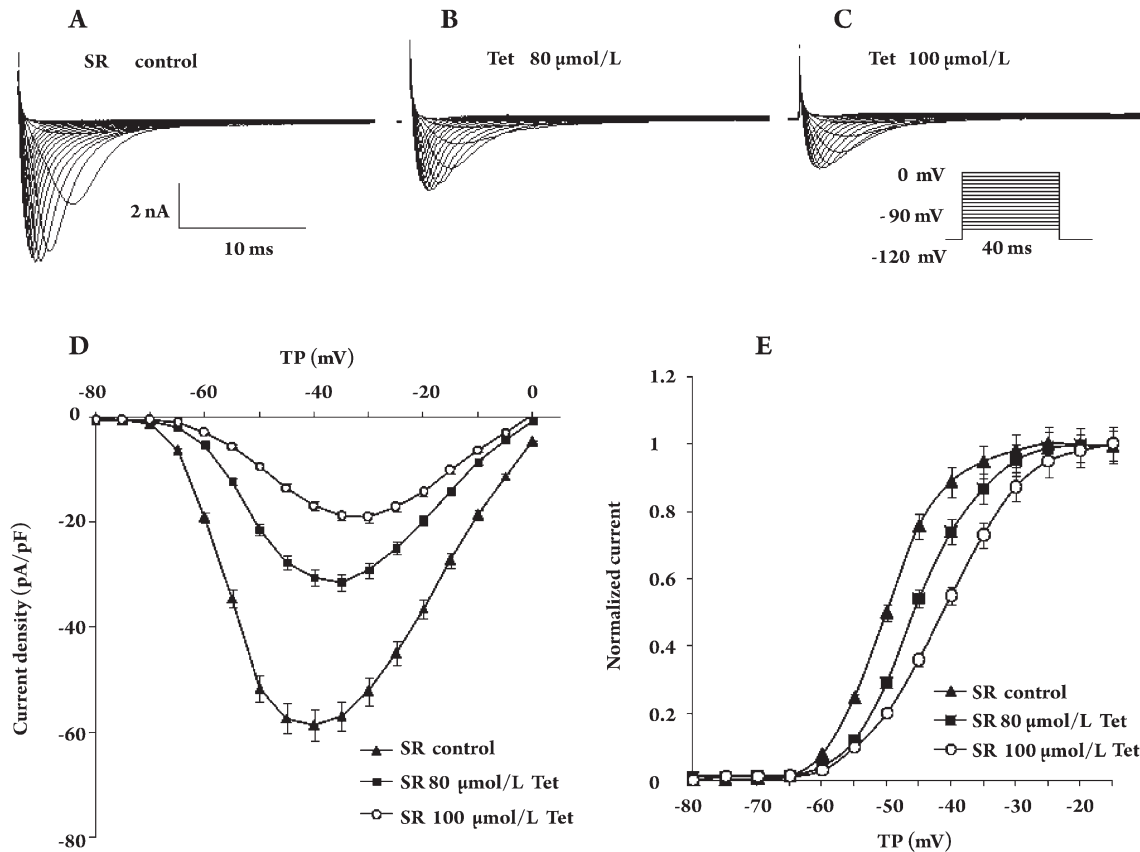


Figure 1. Effects of Tet on I_{Na} density in the SR myocytes of humans. (A) Current traces were obtained by a holding potential of -120 mV and a series of 5 mV depolarizing steps to voltages from -90 to 0 mV in the absence of Tet. (B) Original sodium current in the presence of 80 $\mu\text{mol/L}$ Tet. (C) Original sodium current in the presence of 100 $\mu\text{mol/L}$ Tet. (D) I - V relation curves of I_{Na} densities before and after the application of 80 $\mu\text{mol/L}$ ($n=10$, $P<0.01$) and 100 $\mu\text{mol/L}$ ($n=8$, $P<0.01$) Tet. (E) The effects of Tet on voltage-dependent activation curves of sodium current in SR cells; the data were fitted with a Boltzmann equation. The $V_{1/2}$ was as follows: control, -50.1 ± 0.5 mV, 80 $\mu\text{mol/L}$ Tet, -45.9 ± 0.2 mV ($n=10$, $P<0.05$ vs control), 100 $\mu\text{mol/L}$ Tet, -41.3 ± 0.3 mV ($n=8$, $P<0.01$ vs control). TP: test potential.

required for the inhibitory effect of Tet on the Na^+ current was 76.9 ± 4.1 $\mu\text{mol/L}$ and 74.4 ± 3.6 $\mu\text{mol/L}$ in the SR and AF cells, respectively ($n=7$, $P=\text{NS}$). There were no differences between the effects of Tet on I_{Na} in AF and SR cells. That is, Tet identically inhibited I_{Na} in AF and SR cells.

Effects of Tet on the voltage dependence of I_{Na} inactivation

To further characterize the inhibitory effects of Tet on I_{Na} , we examined whether Tet had an effect on voltage-dependent inactivation of I_{Na} . A double-pulse protocol was used to assess the voltage dependence of I_{Na} inactivation. A 1-s conditioning pulse of voltages between -140 and -60 mV was followed by a 30 ms test pulse of -40 mV. Figure 4A shows a representative original recording of the steady-state inactivation of the sodium channel. The peak current elicited by the test pulse was normalized to a current without a pre-

pulse. The results were well fitted by a Boltzmann relation. The voltage-dependent inactivation curve of I_{Na} (Figure 4B) was shifted to a more positive voltage in AF cells. The mean values for $V_{1/2}$ changed from -93.4 ± 3.3 mV ($n=15$) in SR to -83.0 ± 2.3 mV ($n=12$) in AF ($P<0.01$ vs SR). Exposure to Tet shifted the voltage-dependent inactivation curves of I_{Na} to more negative voltages in both AF and SR cells; $V_{1/2}$ was shifted from -93.4 ± 3.3 mV ($n=15$) to -98.7 ± 2.4 mV ($n=9$) ($P<0.05$ vs control) with application of 80 $\mu\text{mol/L}$ Tet in SR, from -83.0 ± 2.3 mV ($n=12$) to -90.5 ± 4.1 mV ($n=8$) ($P<0.05$ vs control) with application of 80 $\mu\text{mol/L}$ Tet in AF. The slope factor (K) was not altered by Tet (Table 3). These results indicate that Tet can inhibit the amplitude of I_{Na} in a voltage-dependent manner in AF and SR cells.

Effects of Tet on the time-dependent recovery of I_{Na}

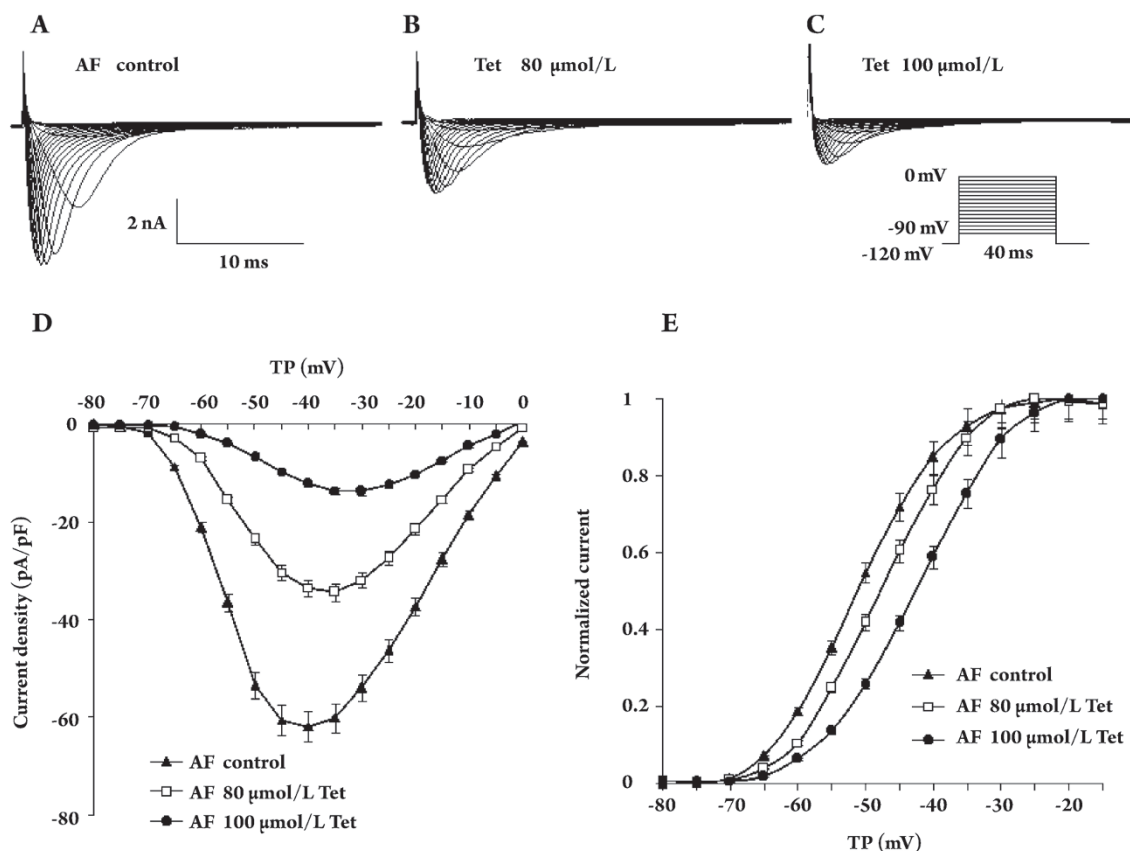
The time-dependent recovery of I_{Na} was studied with

Table 3. The effects of Tet on properties of INa in SR and AF atrial myocytes. Mean±SEM. ^b*P*<0.05, ^c*P*<0.01 vs control. ^e*P*<0.05, ^f*P*<0.01, AF vs SR.

C_{Tet}	SR			AF		
	0 $\mu\text{mol/L}$	80 $\mu\text{mol/L}$	100 $\mu\text{mol/L}$	0 $\mu\text{mol/L}$	80 $\mu\text{mol/L}$	100 $\mu\text{mol/L}$
Voltage-dependent activation						
<i>n</i>	26	10	8	19	8	7
$V_{1/2 \text{ act}}$ (mV)	-50.1±0.5	-45.9±0.2 ^b	-41.3±0.3 ^c	-51.5±0.4	-47.7±0.4 ^b	-42.5±0.3 ^c
K_{act}	6.0±0.2	5.9±0.2	6.4±0.3	6.2±0.2	6.2±0.3	6.4±0.2
Voltage-dependent inactivation						
<i>n</i>	15	9		12	8	
$V_{1/2 \text{ inact}}$ (mV)	-93.4±3.3	-98.7±2.4 ^b		-83.0±2.3 ^f	-90.5±4.1 ^b	
K_{inact}	6.2±0.2	6.3±0.2		4.6±0.2 ^e	4.6±0.1	
Recovery from inactivation						
<i>n</i>	10	8	7	8	6	5
Time constant (ms)	8.62±0.5	15.59±2.8 ^c	32.56±2.5 ^c	8.55±1.8	14.91±2.2 ^c	34.90±1.9 ^c

C_{Tet} : concentration of tetrandrine). $V_{1/2 \text{ act}}$: half-maximum activation voltage. K_{act} : slope factor for activation.

$V_{1/2 \text{ inact}}$: half-maximum inactivation voltage. K_{inact} : slope factor for inactivation.

**Figure 2.** Effects of Tet on I_{Na} density in human AF myocytes. (A–C) Representative sodium currents recorded from AF cells before and after incubation with 80 $\mu\text{mol/L}$ and 100 $\mu\text{mol/L}$ Tet. Currents were recorded at test potentials between -90 mV and 0 mV with 5 mV depolarizing steps. (D) I - V relation curves of I_{Na} densities in control, 80 $\mu\text{mol/L}$ ($n=8$, $P<0.01$) and 100 $\mu\text{mol/L}$ ($n=7$, $P<0.01$) Tet. (E) The voltage-dependent activation curves are shifted to more positive voltages by Tet in AF cells. The $V_{1/2}$ was as follows: control, -51.5±0.4 mV, 80 $\mu\text{mol/L}$ Tet, -47.7±0.4 mV ($n=8$, $P<0.05$ vs control), 100 $\mu\text{mol/L}$ Tet, -42.5±0.3 mV ($n=8$, $P<0.01$ vs control). TP: test potential.

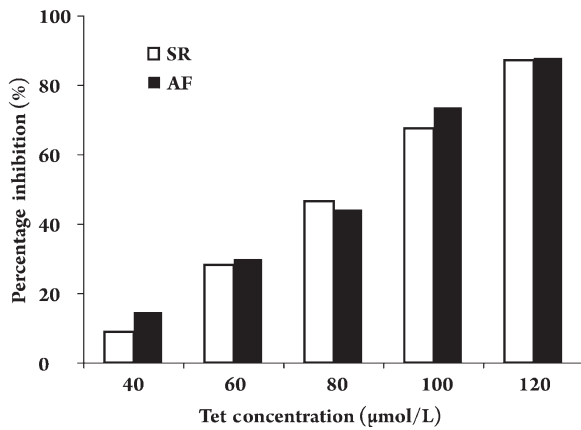


Figure 3. Relationship between the concentration of Tet and the percentage inhibition of Na⁺ current density in SR and AF cells. There were no differences between SR and AF with respect to the inhibition rate of Tet on I_{Na} .

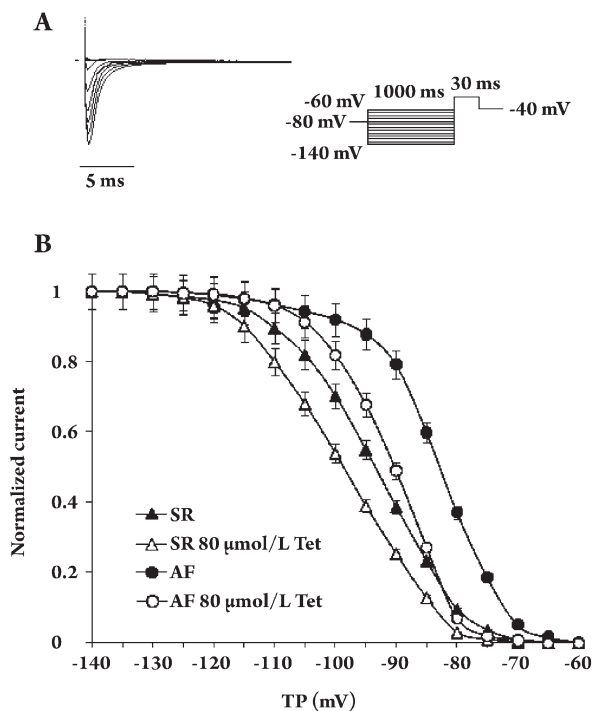


Figure 4. Effects of Tet on I_{Na} voltage-dependent inactivation in SR and AF cells. (A) Representative original recording of the steady-state inactivation of sodium channels. After a 1-s conditional pulse at voltages between -140 and -60 mV, the membrane potential was held to a test pulse (-40 mV, 30 ms). (B) Voltage-dependent inactivation curves of I_{Na} in both SR and AF cells. Peak current elicited by the test pulse was normalized to a current without a prepulse. The results were well fitted by a Boltzmann relation. Compared with the SR cells, the voltage-dependent inactivation of I_{Na} was shifted to a more positive voltage in AF cells. However, the voltage-dependent inactivation of I_{Na} by Tet was shifted to more negative voltages in both AF and SR cells. TP: test potential.

the use of a double pulse protocol with two identical 30-ms pulses (P_1 and P_2) at an increasing P_1 - P_2 interval. Figure 5A shows the original recording of time-dependent recovery of sodium channels. The P_2 current amplitude was normalized to the P_1 amplitude and then plotted with the P_1 - P_2 interval. Tet delayed the time-dependent recovery of I_{Na} in a concentration-dependent manner in both AF and SR cells (Figure 5B, 5C). Recovery kinetics was best fitted with a monoexponential function. The time constant of SR changed from

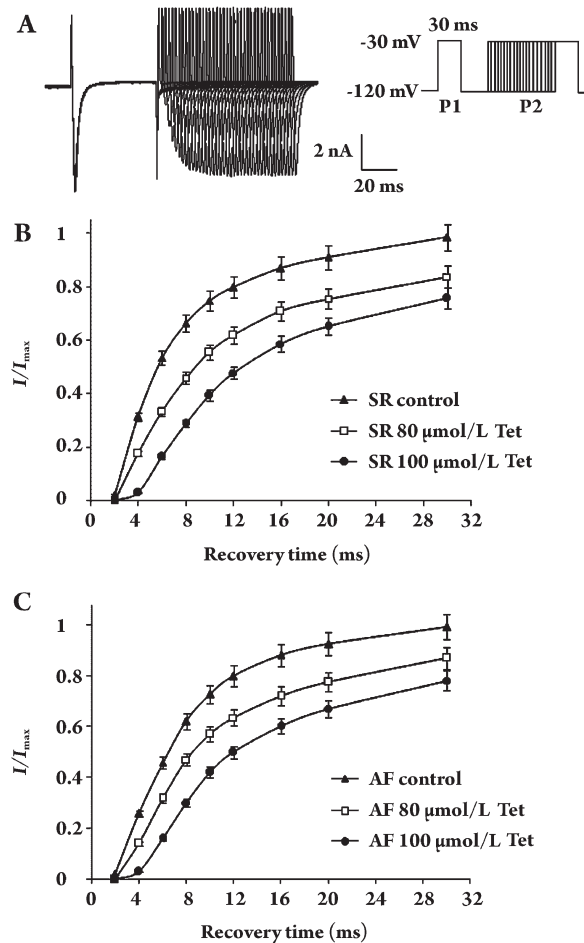


Figure 5. The effects of Tet on I_{Na} time-dependent recovery. (A) Representative original recording of the time-dependent recovery of sodium channels. A double pulse protocol with two identical 30 ms, -30 mV pulses (P_1 and P_2) at an increasing P_1 - P_2 interval was used to record the current. (B) The effect of Tet on the time-dependent recovery curve of I_{Na} in SR cells. The P_1 - P_2 interval is shown on the X-axis; the P_2 current amplitude was normalized to the P_1 , which is shown on the Y-axis. The recovery curve is best fitted with a monoexponential function; the time constant of SR was prolonged by Tet. (C) The effect of Tet on the time-dependent recovery curve of I_{Na} in an AF cell. Tet also prolonged the recovery process of inactive sodium channels in AF cells.

8.62±0.5 ms ($n=10$) to 15.59±2.8 ($n=8$, $P<0.01$ vs control) and 32.56±2.5 ms ($n=7$, $P<0.01$ vs control) by Tet (80 and 100 $\mu\text{mol/L}$, respectively). The time constant of AF changed from 8.55±1.8 ms ($n=10$) to 14.91±2.2 ($n=8$, $P<0.01$ vs control) and 34.90±1.9 ms ($n=7$, $P<0.01$ vs control), respectively, with exposure to Tet (80 and 100 $\mu\text{mol/L}$, respectively). There were no differences between the effects of Tet on I_{Na} recovery kinetics in AF and SR cells.

Discussion

Conduction velocity and sodium current of AF

AF can lead to electrical remodeling that contributes to the self-perpetuation of AF. Some data obtained from the animal model of AF by rapid pacing have shown that the slowing of CV is related to the decrease in I_{Na} ^[4, 15]. Our results found that neither current density nor time-dependent recovery of I_{Na} was altered by AF in humans; however, voltage-dependent inactivation of I_{Na} was shifted to more positive voltages in AF cells, which would have increased sodium channel availability at the resting potential and thus increased CV. This result is similar to research on human myocardium by Bosch^[14]. The reasons for these contrasting results might include differences in the type of AF (experimental vs clinical AF), the influence of the underlying cardiac disease, or antiarrhythmic drugs. Other mechanisms must be involved in the slowing of atrial conduction in AF. Atrial gap junctions are another major determinant in myocardial conduction. Several studies found that sustained AF led to downregulation of connexin40^[16, 17] and abnormal phosphorylation of connexin40^[16] in humans, which decreased the atrial CV and resulted in the initiation and/or perpetuation of AF.

Mechanisms of atrial fibrillation

Moe and coworkers postulated that AF was maintained by multiple wavelets circulating randomly through the myocardium^[18]. This hypothesis was confirmed by Allesie and colleagues, who observed atrial activation patterns during AF in isolated canine hearts and estimated that the critical number of wavelets for sustained fibrillation was between 4 and 6^[19]. The number of wavelets in the atrium is strongly affected by the wavelength, defined as the distance traveled by the activation wave during functional refractoriness, which is determined by the local effective refractory period and the local CV. Therefore, modification of these parameters is thought to affect the vulnerability and stability of AF. Other evidence shows that multiple-wavelet fibrillation is not the only mechanism of fibrillation. Mother rotor fibrillation,

which is fibrillation driven by a stable high-frequency source, may also be involved^[20, 21].

Sodium channel blockers characteristically slow the CV. According to the wavelength theory, there is a possibility that slowing CV could decrease the wavelength and increase the number of wavelets, thus compromising the antifibrillatory effect of sodium channel blockers. Yet sodium channel blockers can terminate atrial fibrillation effectively, as shown in clinical and experimental studies^[22–24]. Nattel^[23] and Kneller^[25] used computer simulations to show that Na^+ channel blockade destabilized the mother rotor, favoring AF termination.

Interaction between Tet and the Na^+ channel

According to the modulated receptor hypothesis^[26], the affinity of the Na^+ channel blocker for the binding site is determined by the electrophysiological state of the Na^+ channel (*ie*, resting, activated and inactivated states). Our results show that Tet not only reduced the maximal conductance of I_{Na} , but also produced a negative shift in the steady-state inactivation curve, indicating that the effect of Tet on I_{Na} is voltage-dependent and that the extent of current inhibition caused by Tet can be altered by the membrane potential. This negative-shift of the inactivation curve suggests that Tet has a preferential affinity for the inactivated states of Na^+ channels. Reactivation of I_{Na} is slowed down in the presence of Tet in our results, which indicates that the speed of the dissociation of Tet from inactivated Na^+ channels decreases.

Mechanisms of antiarrhythmic action of Tet

Our results indicate that Tet can delay the time-dependent recovery of I_{Na} from inactivation in a concentration-dependent manner. Tet is a type of sodium channel blocker with slow recovery kinetics in atrial myocardium. Previous reports have indicated that the efficacy of sodium channel blocking agents with slow recovery kinetics for terminating atrial fibrillation and maintaining sinus rhythm is relatively high^[27]. The reason for this is that, at any given time during the repolarization process, fewer sodium channels have recovered from inactivation in the presence of this kind of drug than in the drug-free state. It then follows that the degree of sodium channel recovery that will produce an action potential occurs at a more hyperpolarized potential, thereby prolonging refractoriness^[28]. Sodium channel blockers with slow recovery kinetics can prevent progressive encroachment by prolonging refractoriness near or beyond repolarization, an effect that has been called postrepolarization refractoriness (PRR)^[27, 29]. PRR can oppose premature excitations and induction of reentrant arrhythmias and

therefore protect the myocardium, which is the most important mechanism for the treatment of AF by sodium channel blockers^[27, 29, 30]. Moreover, sodium channel blockers with slow recovery kinetics are use-dependent, which can maintain the ability of PRR to terminate AF even in the case of high frequent stimulation^[27].

Indeed, sodium channel blockers slow CV, therefore increasing the number of wavelets. This is the main reason why sodium channel blockers are proarrhythmic. However, I_{Na} is not the only factor affecting CV; atrial gap junctions are another major determinant in myocardial conduction. Recent research has shown that Tet can inhibit the degradation of connexin 40 in an atrial rabbit model of AF^[31], which can increase the atrial CV of AF patients and therefore increase the wavelength and reduce the proarrhythmic action of Tet. Consequently, Tet may terminate AF effectively.

It should be noted that Tet is a non-specific calcium channel blocker. It produces a negative inotropic effect and vasodilation. Consequently, administration of Tet can reduce blood pressure. On the other hand, Tet can induce apoptosis in the hepatic cells and produce hepatotoxicity^[32]. If patients have AF combined with cardiac insufficiency, hypotension or abnormal function of the liver, we do not suggest using Tet for treatment. However, Tet is being used for the treatment of angina and hypertension in China^[5]. Tet can reduce the size of infarcts induced by myocardial ischemia^[33]. In addition, Tet can reverse cardiac and vascular remodeling^[34] and reduce arterial blood pressure. Tet is recommended for the treatment of AF patients who have complications of hypertension or angina or a myocardial infarct.

The results of this experimental study suggest that the reason of CV decrease in AF patients is not the change in sodium current. Tet can block sodium channels with slow recovery kinetics, which can explain the mechanism underlying the antiarrhythmic action of Tet. Tet could be a promising agent for the effective treatment of AF in humans.

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Author contributions

Xiao-rong ZENG, Li CHEN, and Yan YANG designed the research; Li CHEN, Zhong-wen LI, and Qi-yong LI

performed the research; Qi-yong LI and Li CHEN analyzed data; Li CHEN and Qi-yong LI wrote the paper.

References

- 1 Gaspo R, Bosch RF, Talajic M, Nattel S. Functional mechanisms underlying tachycardia-induced sustained atrial fibrillation in a chronic dog model. *Circulation* 1997; 96: 4027–35.
- 2 Shinagawa K, Shiroshita-Takeshita A, Schram G, Nattel S. Effects of antiarrhythmic drugs on fibrillation in the remodeled atrium: insights into the mechanism of the superior efficacy of amiodarone. *Circulation* 2003; 107: 1440–6.
- 3 Ashikaga K, Kobayashi T, Kimura M, Owada S, Sasaki S, Iwasa A, *et al*. Effects of amiodarone on electrical and structural remodeling induced in a canine rapid pacing-induced persistent atrial fibrillation model. *Eur J Pharmacol* 2006; 536: 148–53.
- 4 Gaspo R, Bosch RF, Elias BA, Nattel S. Tachycardia-induced changes in Na^+ current in a chronic dog model of atrial fibrillation. *Circ Res* 1997; 81: 1045–52.
- 5 Wong TM, Wu S, Yu XC, Li HY. Cardiovascular actions of Radix Stephaniae Tetrandrae: a comparison with its main component, tetrandrine. *Acta Pharmacol Sin* 2000; 21: 1083–8.
- 6 Cha L, Qian JQ, Lue FH. Antiarrhythmic action of tetrandrine and the total alkaloids of *sophora flavescens*. *Acta Pharmacol Sin* 1981; 2: 26–8.
- 7 Jiang JQ, Zeng QT, Cao LS. Effects of tetrandrine on early after depolarizations and arrhythmias induced by cesium chloride in rabbits. *J Cardiovasc Pulm Dis* 2002; 21: 239–41. Chinese.
- 8 Yu XC, Wu S, Wang GY, Shan J. Cardiac effects of the extract and active components of radix stephaniae tetrandrae. II. Myocardial infarct, arrhythmias, coronary arterial flow and heart rate in the isolated perfused rat heart. *Life Sci* 2001; 68: 2863–72.
- 9 Yu XC, Wu S, Chen CF, Pang KT, Wong TM. Antihypertensive and anti-arrhythmic effects of an extract of Radix Stephaniae Tetrandrae in the rat. *J Pharm Pharmacol* 2004; 56: 115–22.
- 10 Li WH. The clinical cardiac electrophysiologic effects of tetrandrine. *Zhonghua Xin Xue Guan Bing Za Zhi* 1993; 21: 225–54. Chinese.
- 11 Liu QY, Karpinski E, Pang PK. Tetrandrine inhibits both T and L calcium channel current in ventricular cells. *J Cardiovasc Pharmacol* 1992; 20: 513–9.
- 12 Wu SN, Hwang TL, Jan CR, Tseng CJ. Ionic mechanisms of tetrandrine in cultured rat aortic smooth muscle cells. *Eur J Pharmacol* 1997; 327: 233–8.
- 13 Zhou HY, Wang F, Cheng L, Fu LY, Zhou J, Yao WX. Effects of tetrandrine on calcium and potassium currents in isolated rat hepatocytes. *World J Gastroenterol* 2003; 9: 134–6.
- 14 Bosch RF, Zeng X, Grammer JB, Popovic K, Mewis C, Kühlkamp V. Ionic mechanisms of electrical remodeling in human atrial fibrillation. *Cardiovasc Res* 1999; 44: 121–31.
- 15 Yue L, Melnyk P, Gaspo R. Molecular mechanisms underlying ionic remodeling in a dog model of atrial fibrillation. *Cir Res* 1999; 84: 776–84.
- 16 Nao T, Ohkusa T, Hisamatsu Y, Inoue N, Matsumoto T, Yamada J, *et al*. Comparison of expression of connexin in right atrial myocardium in patients with chronic atrial fibrillation versus those in sinus rhythm. *Am J Cardiol* 2003; 91: 678–83.

- 17 Wilhelm M, Kirste W, Kuly S, Amann K, Neuhuber W, Weyand M, *et al*. Atrial distribution of connexin 40 and 43 in patients with intermittent, persistent, and postoperative atrial fibrillation. *Heart Lung Circ* 2006; 15: 30–7.
- 18 Moe GK, Rheinboldt WC, Abildskov JA. A computer model of atrial fibrillation. *Am Heart J* 1964; 67: 200–20.
- 19 Allesie M, Lammers WJ, Bonke FI, Hollen J. Experimental evaluation of Moe's multiple wavelet hypothesis of atrial fibrillation. In: Zipes DP, Jalife J, eds. *Cardiac electrophysiology and arrhythmias*. New York: Grune & Stratton, 1985: 265–75.
- 20 Mandapati R, Skanes A, Chen J, Berenfeld O, Jalife J. Stable microreentrant sources as a mechanism of atrial fibrillation in the isolated sheep heart. *Circulation* 2000; 101: 194–9.
- 21 Nash MP, Mourad A, Clayton RH, Sutton PM, Bradley CP, Hayward M, *et al*. Evidence for multiple mechanisms in human ventricular fibrillation. *Circulation* 2006; 114: 536–42.
- 22 Chou CC, Zhou S, Miyauchi Y, Pak HN, Okuyama Y, Fishbein MC, *et al*. Effects of procainamide on electrical activity in thoracic veins and atria in canine model of sustained atrial fibrillation. *Am J Physiol Heart Circ Physiol* 2004; 286: H1936–45.
- 23 Nattel S, Kneller J, Zou R, Leon LJ. Mechanisms of termination of atrial fibrillation by class I antiarrhythmic drugs: evidence from clinical, experimental, and mathematical modeling studies. *J Cardiovasc Electrophysiol* 2003; 14: S133–9.
- 24 Kawase A, Ikeda T, Nakazawa K, Ashihara T, Namba T, Kubota T, *et al*. Widening of the excitable gap and enlargement of the core of reentry during atrial fibrillation with a pure sodium channel blocker in canine atria. *Circulation* 2003; 107: 905–10.
- 25 Kneller J, Kalifa J, Zou R, Zaitsev AV, Warren M, Berenfeld O, *et al*. Mechanisms of AF termination by pure sodium channel blockade in an ionically-realistic mathematical model. *Circ Res* 2005; 96: e35–47.
- 26 Hondeghem LM, Katzung BG. Time-and voltage-dependent interaction of antiarrhythmic drugs with cardiac sodium channels. *Biochim Biophys Acta* 1977; 472: 373–98.
- 27 Kanki H, Mitamura H, Takatsuki S, Sueyoshi K, Shinagawa K, Sato T, *et al*. Postrepolarization refractoriness as a potential anti-atrial fibrillation mechanism of pilsicainide, a pure sodium channel blocker with slow recovery kinetics. *Cardiovasc Drugs Ther* 1998; 12: 475–82.
- 28 Roden DM. Treatment of cardiovascular disorders: arrhythmias. In: Melmon KL, Morrelli HF, Hoffman BB, Nierenberg DW, editors. *Clinical pharmacology: basic principles in therapeutics*. New York: McGraw-Hill; 1992. p151–185.
- 29 Kirchhof PF, Fabritz CL, Franz MR. Postrepolarization refractoriness versus conduction slowing caused by class I antiarrhythmic drugs: antiarrhythmic and proarrhythmic effects. *Circulation* 1998; 97: 2567–74.
- 30 Koller BS, Karasik PE, Soloman AJ, Franz MR. The relationship between repolarization and refractoriness during programmed electrical stimulation in the human right ventricle: implications for ventricular tachycardia induction. *Circulation* 1995; 91: 2378–84.
- 31 Li DQ, Feng Y, Zhang JM, Jang JM, Zhang HQ, Hu HZ. Tetrandrine prevent connexin 40 degradation in the rabbit atria by rapid pacing. *J Fourth Milit Med Univ* 2004; 25: 150–2.
- 32 Cai Y, Qi XM, Gong LK, Liu LL, Chen FP, Xiao Y, *et al*. Tetrandrine-induced apoptosis in rat primary hepatocytes is initiated from mitochondria: caspases and endonuclease G (Endo G) pathway. *Toxicology* 2006; 218: 1–12.
- 33 Shen YC, Chen CF, Sung YJ. Tetrandrine ameliorates ischemia-reperfusion injury of rat myocardium through inhibition of neutrophil priming and activation. *Br J Pharmacol* 1999; 128: 1593–601.
- 34 Rao MR. Effects of tetrandrine on cardiac and vascular remodeling. *Acta Pharmacol Sin* 2002; 23: 1075–85.