The Novel Mechanism of Oxymatrine Affecting hERG Currents at Different Temperatures

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Key Words

HEK293 cell • hERG channel • Patch clamp • Oxymatrine • Temperature

Abstract

Background/Aims: *Human ether-à-go-go-related gene* (hERG) has an important role in the repolarization of the cardiac action potential. Our studies were to investigate the effects of oxymatrine (one of the natural constituents extracted from Chinese herb *Sophora flavescens* Ait) on hERG-encoded K⁺ channels at different temperatures and its underlying mechanism. **Methods:** The effects of oxymatrine were examined on hERG channels stably expressed in HEK293 cells using a whole-cell patch clamp technique. **Results:** At the temperature 30°C, oxymatrine inhibited hERG current in a concentration-dependent manner and the IC₅₀ was ~665 μM. However at the temperature of 20°C, low concentration oxymatrine C ≤ 100 μM increased hERG current density. However, high concentration oxymatrine C > 100 μM inhibited the hERG current density significantly. Oxymatrine only affected the activation kinetic of hERG channels at all temperatures and had a high binding affinity for open

state of hERG channels except the 300 μM-20°C group which had a high binding affinity for inactive state of hERG channels. **Conclusion:** Oxymatrine is a low potency blocker of hERG K⁺ channels at 30°C, low concentration oxymatrine affect the hERG activation gating with accelerating hERG tail current at 20°C, oxymatrine is a potential hERG activator at low temperatures.

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Introduction

The human ether-a-go-go related gene (hERG) potassium channel plays a central role in regulating cardiac excitability and maintenance of normal cardiac rhythm [1]. HERG has been regarded as an important target for the pharmacological treatment of cardiac arrhythmias by class III anti-arrhythmias [2]. In fact, if hERG current is accelerated or blocked too much short QT interval or long QT interval will be induced, even cause arrhythmia. HERG locus in the seventh chromosome is the responsible gene for LQT2. Currently, we have discovered 12 Long QT syndrome (LQTS) loci called LQT1–LQT12 in all

[3]. Excessive prolonging of the QT interval can cause a potentially fatal ventricular cardiac arrhythmia, termed Torsade de Pointes (TdP), which occurs when cardiac repolarization is delayed [1].

I_{Kr}/HERG represents an important target for the pharmacological management of arrhythmias [4]. A surprisingly diverse group of drugs from a wide variety of classes, including class β anti-arrhythmia drugs [5], antihistamines [6], antipsychotics [7], antibiotics, antimalarials [8] and gastrointestinal prokinetic agents [9], can induce fatal ventricular cardiac arrhythmia by blockage of hERG channels. For this reason some of these drugs have to be withdrawn from market. This side effect is also a common reason for the failure of new drug development in preclinical safety trials.

Compared to western medicines which easily cause arrhythmia, Chinese herbs have superiority because of their fewer side effects on inducing arrhythmia. Oxymatrine is one of the active constituents extracted from Ku Shen, which is the dried root of *Sophora flavescens* Ait. The herb used in different herbal formulations is commonly known with specific pharmacological properties for treatment of liver disorders and other diseases such as arrhythmia, eczema and skin disorders, leukopenia and bronchitis [10]. It is also reported that administration of oxymatrine relieved myocardial injuries during ischemia by protecting cardiomyocytes and brains from apoptotic death [11, 12]. However, little information is available about the effect of oxymatrine on HERG K⁺ channel and the underlying mechanisms.

Therefore, in this study, we used the HERG channel expressed in human embryonic kidney (HEK293) cells to observe the interaction of oxymatrine with HERG channels. We set out to establish: the effects of oxymatrine on the amplitude, concentration dependence, and kinetics of currents conducted by HERG channels stably expressed in HEK293 cells at different temperatures. This was done using a whole-cell patch clamp method.

Materials and Methods

Stably hERG-expressing cell lines and Drugs

Experiments were performed on HEK293 cells that stably expressed the wild-type hERG gene (provided by Professor Z-G Wang, Montreal Heart Institute, Montreal, Canada). Cells were maintained in Dulbecco's Modified Eagle's Medium (DMEM, Hyclone) supplemented with 10% (v/v) fetal bovine serum (FBS, Gibco) and 400 μ g/ml geneticin (G-418, Gibco) at 37°C in a humidified atmosphere of 5% CO₂. For electrophysiological study, the cells were harvested from the

culture dish by trypsinization, washed twice with standard DMEM medium, and stored in this medium at room temperature for later use. Cells were studied within 8 h of harvest. Oxymatrine standard substance was purchased from National Institute for the Control of Pharmaceutical and Biological Products, Beijing, China.

Patch-clamp recording techniques

hERG currents were measured using the whole-cell configuration of the patch clamp technique. Heat-polished patch pipettes had final resistances of 2–4 M Ω when filled with a pipette solution containing (in mM): 130 KCl, 1 MgCl₂·6H₂O, 10 HEPES, 5 Mg-ATP, 5 EGTA and 0.1 GTP (pH 7.3 with KOH). The extracellular solution contained (in mM): 136 NaCl, 5.4 KCl, 5 HEPES, 1 MgCl₂·6H₂O, 1 CaCl₂ and 10 glucose (pH 7.4 with NaOH). The drug solutions were freshly prepared daily with extracellular solution.

For electrophysiological recordings, submaximally confluent cells were used after 1–3 days of culture at a seeding density of 1–4×10⁵ cells per flask (Corning). The cells were harvested from the flask by treatment with 0.25% trypsin and 0.02% EDTA, and transferred to a small cell bath mounted on the stage of an inverted microscope (IX-70, Olympus), where they were allowed to attach to the glass bottom for about 10 min. The cells were then superfused continuously at a rate of 1.5 ml/min. Recordings were taken at physiological temperature of 20°C and 30°C, which were maintained with a TC-324B temperature controller (Warner Instruments Inc). An Axopatch-200B patch-clamp amplifier was used to record membrane current. Computer software (Clampex 9.2, Axon Instruments) was used to generate voltage clamp protocols and acquire data. Capacitance and series resistance compensation were optimized. Data were recorded on a computer via Digidata 1322A, and analyzed using Clampfit 9.2 (Axon Instruments) and Prism (Graphpad Inc.) software. Graphical fits of the data were made using previously described standard equations [13, 14].

Data were presented as mean $\pm$ standard error of the mean (S.E.M.), unless otherwise specified. Statistical comparisons were made using a two-tailed Student's t-test. P-values < 0.05 were interpreted as being statistically significant.

Results

Concentration dependence of hERG channel binding

The hERG current was elicited from the holding potential of -80 mV by test pulses ranging from -60 mV to +40 mV in 10 mV steps. The test pulses each had a 4 s duration and were applied every 8 s. Each test pulse was followed by a repolarization step to -50 mV, which evoked large, slowly decaying outward tail-currents (Fig. 1A). Currents were recorded first under control conditions, and then oxymatrine was washed into the bath for 10 min at 20°C or 30°C, with the cell kept at the holding

Fig. 1. Electrophysiological properties of hERG current (n=10). (A) Voltage clamp protocol and currents recorded from a stably expressed hERG cell before (upper set of current traces) and after exposure to 100 μ M Oxymatrine at 20°C (middle set of current traces) and 300 μ M (lower set of current traces). (B) Normalized I-V relationships for the tail current in the absence and presence of oxymatrine at temperature 20°C. At drug concentrations of 1, 10, 100 and 300 μ M, the peak tail-current amplitude were measured, normalized for each cell to the control value and then averaged. (C) Normalized I-V relationships for the tail current in the absence and presence of oxymatrine at temperature 30°C. (D) Voltage-dependent activation curves for the control and following exposure to 100 μ M oxymatrine at 20°C and 30°C (E), smooth curves were best fits of the data to a Boltzmann function. Voltage protocol was the same as used in Fig. 1A. (F) The half-inhibition concentration (IC_{50}) of Oxymatrine on hERG tail current at 30°C. (G) The ratio of voltage dependence peak tail current between drug group (I_{drug}) and control group ($I_{control}$). Compared to control group, * $P<0.05$, ** $P<0.01$, *** $P<0.001$ vs. control.

potential, before current recording commenced in the presence of drug. At drug concentrations of 1, 10, 100 and 300 μ M, the peak tail-current amplitude were measured, normalized for each cell to the control value and then averaged (n=10). Fig. 1B shows the effects of oxymatrine at 20°C on tail current of hERG channels. Oxymatrine at temperature 20°C accelerated hERG tail current in a concentration-dependent manner at drug concentrations 1, 10 and 100 μ M but inhibited hERG tail current at 300 μ M; Fig. 1C shows the I-V plot of hERG current amplitude at the peak tail current recorded from before and after exposure to oxymatrine at 30°C. The result was that oxymatrine at temperature 30°C inhibited hERG tail current in a concentration-dependent manner at drug concentrations 1, 10, 100 and 300 μ M. Percental

accelerated rate of oxymatrine at temperature 20°C were $29.5 \pm 1.75\%$ in the 1 μ M group ($P<0.05$), $40.0 \pm 0.63\%$ in the 10 μ M group ($P<0.01$) and $50.1 \pm 0.85\%$ in the 100 μ M group ($P<0.05$); Percental block rate of oxymatrine in the 300 μ M group at temperature 20°C was $36.5 \pm 0.35\%$ ($P<0.01$). In addition, the percental block rate of oxymatrine at temperature 30°C were $6.89 \pm 0.19\%$ in the 1 μ M group ($P>0.05$), $18.96 \pm 0.19\%$ in the 10 μ M group ($P>0.05$), $31.61 \pm 0.53\%$ in the 100 μ M group ($P<0.01$) and $43.21 \pm 0.28\%$ in the 300 μ M group ($P<0.01$). Fig. 1F shows the half-inhibition concentration (IC_{50}) of oxymatrine on hERG tail current at 30°C is $665.0 \pm 1.25 \mu$ M.

Thus, compared with the control, at 20°C, 1, 10, 100 μ M oxymatrine had significantly accelerated effects on

Fig. 2. Effect on steady-state inactivation of the hERG current (n=10). (A) Representative current tracing for steady-state inactivation using a double-pulse protocol with varying interpulse repolarization levels (from -120 mV to +20 mV). (B) Normalized steady-state inactivation curves before and after exposure to 100 μ M oxymatrine at 20°C. (C) Normalized steady-state inactivation curves before and after exposure to 100 μ M oxymatrine at 30°C. (D) The voltage dependent ratio of peak current for the third section of protocol in Fig. 2A, between drug group and control group.

tail current; 100, 300 μ M oxymatrine at 30°C and 300 μ M oxymatrine at 20°C had significantly inhibited effects on hERG tail current.

Voltage dependence of hERG channel binding

Fig. 1G and Fig. 2D show that the voltage dependence of oxymatrine binding hERG channels. The Y axon represents the ratio of hERG current between control and drug group. The ratios calculated from activated current were 1.27, 1.17, 1.03 at -60 mV (1.53, 0.64, 0.69 at 10 mV) in 100 μ M/20°C group, 300 μ M/20°C group, and 100 μ M/30°C group, respectively (see Fig. 1G). At -60 mV, hERG channels nearly all stayed at the close state and the number of hERG channels for open state became more and more with the membrane potential increasing. From the inactivated current (see Fig. 2D), the ratios of all four groups kept relatively stably, except 300 μ M/20°C group (1.06 at -120 mV and 0.61 at 10 mV). In this protocol, at -120 mV, nine tenth hERG channels stayed at open state (recover from inactivation), and the open state channels were shrank by the depolarized membrane potential.

Effects on hERG channel kinetics

The activation curves shown in Fig. 1D and Fig. 1E were obtained by normalizing the maximum tail currents amplitude, recorded with the protocol used in Fig. 1A. The normalized data were plotted against the test pulse potentials and fitted to the Boltzmann function. The

activation curve showed that the threshold voltage for hERG current activation is close to -50 mV and that it was fully activated with voltage steps to +10 mV.

The results were (a) 100 μ M oxymatrine at 20°C shifted activation curve in a negative direction, half-maximum activation voltage ($V_{1/2}$) values were -13.46 ± 0.13 mV before exposure to oxymatrine and -19.11 ± 0.11 mV after exposure to it ($p < 0.001$, $n = 10$); the corresponding slope factor (k) values were 8.3 ± 0.11 and 7.9 ± 0.10 , respectively ($p < 0.05$, $n = 10$). However, (b) 100 μ M oxymatrine at 30°C also altered the activation properties, but shift activation curve in a positive direction: the $V_{1/2}$ and k values were -21.06 ± 0.11 mV and 6.23 ± 0.10 before and -17.42 ± 0.44 mV ($p < 0.001$, $n = 10$) and 5.58 ± 0.38 ($p > 0.05$, $n = 10$) after using oxymatrine, respectively.

To measure inactivation, a special protocol was used that inactivated the channels at a holding potential of +40 mV, recovered the channels from inactivation at various potentials from -120 to +20 mV (12 ms) in 10 mV steps, and measured the resulting peak outward current at constant +20 mV as a measure of steady-state inactivation (Fig. 2A). In Fig. 2B and Fig. 2C, the inactivating outward current amplitude (measured at potential +20 mV) was normalized and plotted against the test pulse potential, giving the steady-state inactivation curve. Neither oxymatrine at 20°C nor at 30°C shifted the inactivation curve significantly. This curve could be fitted with a Boltzmann distribution, yielding half-maximum inactivation

Fig. 3. The effect of Oxymatrine on onset of inactivation and recovery from inactivation of hERG current at different temperature. (A) Three-pulse protocol used to study onset of inactivation properties of hERG current. (B) Representative current tracing for recovery from inactivation using a two-pulse protocol. (C, D) Time constant for onset of inactivation obtained by fitting the exponential function to the decaying current during the third pulse of the protocol, was plotted against membrane potential; Voltage dependence of the time constant for recovery from inactivation obtained by fitting a single exponential function to the initial increase amplitude, was plotted against membrane potential, before and after exposure to 100 μ M Oxymatrine at different temperatures.

voltage ($V_{1/2}$) and Slope factor (k). $V_{1/2}$ shifted mildly after exposure to 100 μ M oxymatrine at 20°C, from -92.0 ± 2.34 mV in the control to -88.16 ± 1.43 mV ($p > 0.05$, $n = 10$). After treatment with 100 μ M oxymatrine at 30°C, $V_{1/2}$ shifted slightly from -51.40 ± 0.18 mV to -54.24 ± 0.96 mV ($p > 0.05$, $n = 10$). The corresponding k values were from -26.18 ± 1.30 to -24.47 ± 0.87 at 20°C, and from -17.04 ± 1.20 to 18.19 ± 1.00 at 30°C, respectively.

The time courses of hERG current onset of inactivation and recovery from inactivation are shown in Fig. 3. To measure the effect on the onset of inactivation of the hERG current, we used a three-pulse protocol (Fig. 3A). The channels were first inactivated by clamping the membrane at +40 mV for 2.5s, followed by a prepulse to -100 mV for 10 ms. This prepulse was sufficiently long to allow rapid recovery of channels from inactivation but short enough to prevent significant channel deactivation. Following the recovery prepulse, a series of test pulses were delivered to potentials ranging from -120 to +30 mV, resulting in large, outwards inactivating currents. The onset of inactivation curves in the absence and presence of 100 μ M oxymatrine at 20°C or 30°C were shown in Fig. 3C and Fig. 3D. The time constant for the onset of inactivation was obtained by fitting a single exponential function to the decaying current traces during the third pulse of the protocol. Fig. 3C and Fig. 3D showed that the onset of inactivation was similar before and after exposure to 100 μ M oxymatrine either at 20°C or 30°C

at test potentials between -40 and +30 mV (at -30 mV, $\tau = 22.08 \pm 1.69$ ms in control group and 21.41 ± 1.67 ms at 20°C group, $p > 0.05$, $n = 10$; 7.53 ± 0.46 ms in control group and 7.47 ± 0.44 ms at 30°C group, $p > 0.05$, $n = 10$). To measure the time constant of recovery from inactivation directly, we used a two-pulse protocol (Fig. 3B), in which the cells were held a 2.5 s depolarizing pulse to +40 mV to inactivate the hERG channels, and then were repolarized to voltages between -120 mV and +30 mV to give tail current. The prepulse potential at +40 mV was positive enough to induce full conductance of the hERG channels but also rendered a large number of the channels in the inactivated state. The time constant for recovery from inactivation was determined by fitting a single exponential function to the initial increase in tail-current amplitude at potentials between -80 mV and -20 mV. Neither 100 μ M oxymatrine at 20°C nor at 30°C changed constant for recovery from inactivation significantly between -80 mV and -20 mV (at -60 mV, $\tau = 3.26 \pm 0.56$ ms in control and 3.13 ± 0.39 ms at 20°C, $p > 0.05$, $n = 10$; 2.02 ± 0.22 ms in control and 2.15 ± 0.23 ms at 30°C, $p > 0.05$, $n = 10$).

To determine activation and deactivation kinetic of hERG current, As whole cell current recordings in hERG channels are complicated by the concomitant inactivation of the channels. We separate the channel activation and inactivation processes using an envelope of tails protocol and thereby obtain an estimate of the time constant for

Fig. 4. The effect of oxymatrine on hERG activation kinetics at 20°C (A) The typical example of currents recorded in control group and 100 μ M oxymatrine group at 20°C. (B) Plots of the peak tail current amplitude vs. the duration of the test pulse interval from the traces shown in A. Cells were depolarized to 10 mV for variable durations before stepping the voltage to -50 mV where tail currents were recorded.

Fig. 5. The effect of 300 μ M Oxymatrine on hERG channels at 20°C and their kinetics. (A) The tail I-V plot of hERG before and after exposure to 300 μ M drug. (B) The activation curves were best fits of the data to a Boltzmann function. Voltage protocol was the same as used in Fig 1A. (C) Normalized steady-state inactivation curve before and after exposure to 300 μ M Oxymatrine. (D) The time constants of onset of inactivation obtained fitting the exponential function to the decaying current during the third pulse of the protocol (shown in Fig. 3A) and the time constants of recovery from inactivation of 300 μ M, the fitting methods were the same as in Fig. 3. Compared to control group, * P <0.05, ** P <0.01, *** P <0.001 vs. control.

the peak values of tail currents. Fig. 4A shows typical currents recorded from the same cell in control group and 100 μ M oxymatrine group. The peak amplitudes of the tail currents, indicated in Fig. 4A, which plotted against test pulse duration in Fig. 4B. The time constants of activation was obtain by fitting a single exponential function to the plots and we gained the time constants of activation in 100 μ M oxymatrine group at 20°C which were decreased significantly from 854.5 $\pm$ 210.5 ms to 358.6 $\pm$ 25.24 ms (n =6, p <0.05), and the time constants of activation at 30°C were decreased significantly from 349.5 $\pm$ 59.80 ms to 179.2 $\pm$ 34.99 ms (n =6, p <0.05) before and after exposure to 100 μ M oxymatrine. So we could suggest that the 100 μ M oxymatrine can speed up the hERG channel activation course at all temperatures. The

time constant of deactivation was ascertained by fitting a single exponential function to the decay of the tail current (see Fig. 1A). The time constant of deactivation was similar before and after exposure to 100 μ M oxymatrine either at 20°C or at 30°C at test potentials between -30 and +30 mV (at 0 mV, τ = 540.53 $\pm$ 30.64 ms in control and 547.14 $\pm$ 44.88 ms at 20°C, p >0.05, n =10; 676.99 $\pm$ 46.37 ms in control and 608.69 $\pm$ 17.50 ms at 30°C, p >0.05, n =10).

High concentration of oxymatrine blocked hERG current at low temperature

Fig. 5 shows the effects of 300 μ M oxymatrine on hERG current at 20°C. Fig. 5A shows the tail I-V plots before and after exposure to 300 μ M oxymatrine,

obviously, 300 μM oxymatrine inhibited hERG tail current significantly. Fig. 5B shows the normalized activation curve of hERG tail current, 300 μM oxymatrine shifted the activation curve negatively ($V_{1/2}$ from -15.20 ± 0.16 mV to -16.21 ± 0.37 mV, $p < 0.05$, $n = 10$), with no changes in the slope factor (k from 6.76 ± 0.14 to 6.75 ± 0.33 , $p > 0.05$, $n = 10$). Fig. 5C shows normalized steady-state inactivation curve by fitting the plots which obtained by normalizing the peak outward current amplitude (see Fig. 2) and plotted against the test pulse potential. 300 μM oxymatrine at 20°C shifted steady-state inactivation curve in a negative direction significantly ($V_{1/2}$ from -63.94 ± 1.30 mV to -72.84 ± 1.03 mV, $p < 0.001$, $n = 10$ and k from -19.14 ± 1.28 to -18.20 ± 0.90 , $p > 0.05$, $n = 10$). Fig. 5D shows time constant of onset of inactivation and recovery from inactivation of hERG channels. The fitting method was the same as in Fig. 3. The time constant of onset of inactivation at -30 mV changed slightly from 17.43 ± 2.50 ms to 18.92 ± 1.56 ms ($p > 0.05$, $n = 8$), and the time constant of recovery from inactivation at -60 mV also changed slightly from 2.96 ± 0.73 ms to 4.07 ± 0.69 ms ($p > 0.05$, $n = 10$).

Effect of temperature on hERG current kinetics

We also analyzed if the temperature could affect the hERG current kinetics. In cells studied at both temperatures, the time constants of inactivation at -30 mV was decreased significantly from 22.08 ± 1.69 ms to 7.53 ± 0.46 ms ($p < 0.001$, $n = 10$), and the time constants of recovery from inactivation at -50 mV was decreased significantly from 4.89 ± 0.61 ms to 2.39 ± 0.24 ms ($p < 0.01$, $n = 10$). The deactivation of hERG current was fitted by a single exponential function at both temperatures. The time constants of deactivation at -10 mV altered from 893.74 ± 55.45 ms to 691.29 ± 38.20 ms with significantly change ($p < 0.05$, $n = 7$). Finally, increasing temperature from 20°C to 30°C shifted the activation curve negatively ($V_{1/2}$ from -13.46 ± 0.13 mV to -21.06 ± 0.11 mV, $p < 0.001$, k from 8.30 ± 0.12 to 6.23 ± 0.10 , $p < 0.001$) and inactivation curve positively ($V_{1/2}$ from -92.00 ± 2.34 mV to -51.40 ± 1.78 mV, $p < 0.001$, k from -26.18 ± 1.30 to -17.04 ± 1.20 , $p < 0.001$).

Discussion

Oxymatrine, as previously described, could protect mice from fulminant hepatitis induced by GalN/LPS and might block hepatocyte apoptosis [15], and had anti-HBV effect *in vivo* through reducing the contents of HBsAg and HBeAg in transgenic mice liver [16], beyond that,

oxymatrine also has the inhibitory effect on hepatitis C virus *in vitro* [17] and against respiratory syncytial virus [18]. Except the antiviral effects on different viruses, oxymatrine also has anti-tumor effect on human colon cancer cell line SW1116 [19], human hepatoma SMMC-7721 cells [20]. Our previous studies show that oxymatrine could have anti-arrhythmic activity through affecting the hERG channel expression [21], and have found that low concentration matrine and oxymatrine increase hERG channels protein levels significantly. Based on these results, we had examined the effects of oxymatrine on hERG K^+ channels at different temperatures. The experiments were performed in HEK293 cells that were stably transfected with hERG cDNA. Our present data indicate that oxymatrine has variable effects on hERG channel gating accompany with bath temperature changed, which is supposed to be one of mechanisms of oxymatrine for reducing the effects of inducing arrhythmias or LQTS, and perhaps anti-arrhythmic.

In present studies, oxymatrine could increase hERG current density when concentration ≤ 100 μM at temperature 20°C , however when the concentration > 100 μM , the drug inhibited the hERG current density. But at temperature 30°C , oxymatrine blocked hERG current in a concentration-dependent manner and IC_{50} was about 665 μM , which indicated that oxymatrine was a low potency blocker of hERG channels at 30°C . As the result, oxymatrine was safer than those agents strongly affecting I_{Kr} current and inducing prolongation of QT interval, which could result in lethal arrhythmias such as torsade de pointes and had to be recalled from market.

HERG channels can exist in one of three main conformational states; closed, open or inactive. Transitions between these three states are voltage-dependent. Activation involves opening of an intracellular gate (intracellular half of the S6 helix), whereas inactivation involves opening of a gate at the extracellular end of the permeation pathway (between the pore helix and S6) [22]. Altered gating characteristics can lead to reducing hERG current due to one of two main mechanisms: reduced activation (i.e. slower activation kinetics/accelerated deactivation kinetics, or shift in the voltage-dependence of activation to more depolarized voltages) [23-25] or enhanced inactivation [26, 27].

The state dependent binding to hERG channel is shown in Fig. 1G and Fig. 2D, oxymatrine not a bit affected the hERG current at -60 mV (Fig. 1G) and when the membrane potential voltage increased, the current ratio between control and drug become bigger (accelerating)

Concentration(μ M)		1	10	100	300
20°C activation	CG	-9.57 $\pm$ 0.26	-15.42 $\pm$ 0.30	-13.46 $\pm$ 0.13	-15.20 $\pm$ 0.16
	DG	-13.84 $\pm$ 0.14 ***	-16.55 $\pm$ 0.09 **	-19.11 $\pm$ 0.11 ***	-16.21 $\pm$ 0.37 *
20°C inactivation	CG	-67.35 $\pm$ 1.55	-78.12 $\pm$ 1.63	-92.00 $\pm$ 2.34	-63.94 $\pm$ 1.30
	DG	-66.38 $\pm$ 1.22	-75.99 $\pm$ 1.39	-88.16 $\pm$ 1.43	-72.84 $\pm$ 1.03 ***
30°C activation	CG	-24.88 $\pm$ 0.20	-31.86 $\pm$ 0.64	-21.66 $\pm$ 0.11	-19.91 $\pm$ 0.20
	DG	-24.31 $\pm$ 0.18 *	-29.15 $\pm$ 0.16 ***	-17.42 $\pm$ 0.44 ***	-25.59 $\pm$ 0.33 ***
30°C inactivation	CG	-50.44 $\pm$ 4.19	-58.06 $\pm$ 1.09	-51.40 $\pm$ 1.18	-53.61 $\pm$ 2.18
	DG	-48.69 $\pm$ 4.51	-59.60 $\pm$ 1.595	-54.24 $\pm$ 0.96	-61.29 $\pm$ 1.19 **

Table 1. The $V_{1/2}$ of normalized (in)activation curves for oxymatrine at different temperatures. CG: control group, DG: drug group. * $p < 0.05$, ** $p < 0.01$, *** $p < 0.001$ vs. control group.

or smaller (inhibiting). These findings suggest that oxymatrine have a high binding affinity for the open state or inactivated state. The steady-state inactivation peak current ratio between control and drug maintained stably except 300 μ M group at 20°C (Fig. 2D). These results suggest that oxymatrine preferentially bind to activated channels (open state) with little or no effect on inactivated channels. However, the situation of high concentration at 20°C was exception, which had a high binding affinity for the inactivated state (‘ $\square$ ’ in Fig. 2D). The result that the 300 μ M oxymatrine speed the inactivation course in Fig. 5D also supported this conclusion.

In Fig. 1D and Fig. 5B, we found that when the bath temperature was 20°C, all concentrations of oxymatrine shifted the normalized activation curve negatively (Table 1), furthermore, low concentrations including 1, 10, 100 μ M oxymatrine couldn’t shift normalized inactivation curve, but 300 μ M oxymatrine shifted inactivation curve in a negative direction (Fig. 2B and Fig. 5C). According these results, we inferred that at 20°C (a) oxymatrine both impacted the activation and inactivation gating, and with high affinity to activation gating. (b) By affecting the activation gating oxymatrine improved hERG currents and impacting inactivation gating resulted in hERG channels being blocked. The results that oxymatrine sped up the activation of hERG channels also supported these inferences (Fig. 4). Are these inferences equally applicable to hERG current at 30°C? We calculated the inactivation curve parameters on concentrations 1, 10, 100 and 300 μ M at 30°C, the results (Fig. 2C and Table 1) were similar to that at 20°C, that oxymatrine indeed have low affinity to inactivation gating and only high concentration (> 100 μ M) could affect the inactivation gating of hERG channels, at both temperatures.

At both 20°C and 30°C, the time constants of onset of inactivation and the time constants of recovery from inactivation (shown in Fig. 3) did not have significant difference before and after exposure to 100 μ M oxymatrine. Furthermore, when increased the concentration of oxymatrine to 300 μ M at 20°C, the time constants of inactivation still changed slightly (Fig. 5D). These findings suggested that oxymatrine indeed have low influence on inactivation gating of hERG channel gating which were in accordance with our above results of inactivation curves.

With recent years, a new class compounds classified as hERG agonist with potential anti-arrhythmias properties has been introduced. Neuro Search compounds NS1643 and NS 3623 increase hERG current primarily inducing a right-ward shift of inactivation curve [28, 29], and NS 1643 also affects activation properties at the outer vestibule of the pore entrance (activation gate relative) [30, 31]. It is reported that Sanofi-Aventis compound RPR260243 affect the deactivation properties of the hERG channel [32] and also affect inactivation property though a right-ward shift of inactivation curve similar to the Neuro Search compounds [33]. It should also be mentioned that the naturally occurring hERG1 channel agonist mallotoxin has been shown to activate hERG1 channels by a left-ward shift of the activation curve and a slowing of the deactivation process [31, 34]. So the mechanisms of hERG agonist on hERG channel kinetic are ever-changing. As a hERG agonist oxymatrine affects the activation properties by a left-ward shift of activation curve and decreased activation time constants without any changes of other checked time constants.

In the previous study showed, the rates of activation, deactivation, inactivation and recovery from inactivation

all had different temperature sensitivities [35]. In our experiment, we also analyzed the temperature sensitivities of hERG gating. Our findings show that activation/inactivation curves and the time constants of inactivation/recovery from inactivation/deactivation all altered with temperatures. The activation curve shifted negatively, and inactivation curve shifted positively; the time constants of inactivation/recovery from inactivation/deactivation decreased with the temperature increasing. When the temperature increased, the rate of open state channels accordingly increase; and we have concluded above that oxymatrine have high affinity to open state (at 30°C) of hERG channels, so oxymatrine ought to bind more open hERG channels at high temperature and appear as inhibited effect on hERG channels. Our results suggested oxymatrine may trap into the hERG inner pore (open state); leading to hERG channels lose their normal function. Low concentration oxymatrine speeding up the activation course of hERG channel also support those inferences (Although oxymatrine boosting the activation at 30°C, the functional hERG channels lack in number). There are probably why oxymatrine inhibit the hERG currents at high concentrations (more drugs) or high temperatures (more open state channels). But the mechanisms that how oxymatrine trapping into the hERG inner pore need furthermore investigating.

Some class III anti-arrhythmic drugs are reported to bind near the pore helix [36], and the inhibitory actions of sophoridine and sophocarpine on hERG K⁺ channels have recently been reported [37], which have the similar structure with oxymatrine. They also have shown that the binding site for sophoridine and sophocarpine on the channel are around the pore helix that controls channel inactivation. Oxymatrine displayed similar properties about binding the inactivation relative structures (inner pore) of hERG channels resulting in hERG channels inhibition.

Conclusions

The differences in the temperature sensitivities of each of the kinetic processes that govern hERG channel activity mean that it is not possible to use data obtained at room temperature and extrapolate the results to physiological temperatures using a single temperature scaling factor [35]. In summary, oxymatrine is a low potency blocker of hERG K⁺ channels, and oxymatrine has high binding affinity for open state. Low concentration oxymatrine affect the hERG activation gating with accelerating hERG tail current at 20°C. From our previous study, low concentration oxymatrine can improved the protein level of hERG channel on cellular membrane, and in present study oxymatrine block the hERG channel current at physically temperature (~30°C). The both effects are of antagonism. For these reasons oxymatrine is a safer chemical using for anti-tumor and anti-virus even for long time application. The effect of oxymatrine on anti-arrhythmia appears in low concentration groups at temperature 20°C, because of their synergism including effects of protein levels and gating altering. Although this temperature isn't close to physical temperature, our finding can give a clue about discovering the similar structure Chinese herbs which can really use for anti-arrhythmia.

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