International Journal of Cardiology 207 (2016) 326–334

Contents lists available at ScienceDirect

International Journal of Cardiology

journal homepage: www.elsevier.com/locate/ijcard

Mechanisms underlying atrial-selective block of sodium channels by Wenxin Keli: Experimental and theoretical analysis☆

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ARTICLE INFO

Article history:
Received 30 September 2015
Received in revised form 29 December 2015
Accepted 1 January 2016
Available online 7 January 2016

Keywords:
Antiarrhythmic drugs
Arrhythmias
Electrophysiology
Pharmacology
Atria
Ventricles

ABSTRACT

Introduction: Atrial-selective inhibition of cardiac sodium channel current (I_{Na}) and I_{Na}-dependent parameters has been shown to contribute to the safe and effective management of atrial fibrillation. The present study was designed to examine the basis for the atrial-selective actions of Wenxin Keli.
Methods: Whole cell I_{Na} was recorded at room temperature in canine atrial and ventricular myocytes. Trains of 40 pulses were elicited over a range of pulse durations and interpulse intervals to determine tonic and use-dependent block. A Markovian model for I_{Na} that incorporates interaction of Wenxin Keli with different states of the channel was developed to examine the basis for atrial selectivity of the drug.
Results: Our data indicate that Wenxin Keli does not bind significantly to either closed or open states of the sodium channel, but binds very rapidly to the inactivated state of the channel and dissociates rapidly from the closed state. Action potentials recorded from atrial and ventricular preparations in the presence of 5 g/L Wenxin Keli were introduced into the computer model in current clamp mode to simulate the effects on maximum upstroke velocity (Vmax). The model predicted much greater inhibition of Vmax in atrial vs. ventricular cells at rapid stimulation rates.
Conclusion: Our findings suggest that atrial selectivity of Wenxin Keli to block I_{Na} is due to more negative steady-state inactivation, less negative resting membrane potential, and shorter diastolic intervals in atrial vs. ventricular cells at rapid activation rates. These actions of Wenxin Keli account for its relatively safe and effective suppression of atrial fibrillation.

1. Introduction

Experimental and clinical studies have demonstrated the value of sodium channel blockers in the management of atrial fibrillation (AF) [1–4]. A major limitation of some sodium channel blockers is their potential proarrhythmic actions in the ventricles. Atrial-selective sodium channel block has recently been advocated as a safe and effective mechanism for termination and suppression of inducibility of AF [1,5]. The anti-anginal agent, ranolazine, has been shown to be effective in suppressing atrial fibrillation with little effect on peak sodium channel current (I_{Na}) in the ventricle [1]. The atrial-selective effect of ranolazine is largely attributable to differences in the electrophysiological characteristics of sodium channels between atria and ventricle, namely a more negative steady-state inactivation relationship in atria [1].

Wenxin Keli (WK) is a Chinese herb extract that includes 5 main components: the *Nardostachys chinensis batal* extract, *codonopsis*, *notoginseng*, *amber*, and *Rhizoma polygonati*. Developed in various forms over the past 2000 years, it is used by millions today for the treatment of a variety of cardiac diseases. WK is reported to be of benefit in the treatment of cardiac arrhythmias [5–7], including atrial fibrillation, [8–10] and heart failure [11,12]. We previously reported that WK exerts atrial-selective inhibition of peak I_{Na} and thus effectively suppresses AF in experimental models of AF [13]. In the present study, we evaluate the nature and kinetics of interaction of WK with the cardiac sodium channel to gain insight into the basis for its atrial selectivity.

2. Methods

2.1. Isolation of adult canine cardiomyocytes

Myocytes were prepared from canine ventricles or atria as previously reported and described in the Online Supplement.

☆ All authors take responsibility for all aspects of the reliability and freedom from bias of the data presented and their discussed interpretation

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http://dx.doi.org/10.1016/j.ijcard.2016.01.016
0167-5273/

2.2. Voltage clamp recordings in cardiomyocytes

Cells were placed in a temperature-controlled chamber (PDMI-2, Harvard Apparatus, Holliston, MA) mounted on the stage of an inverted microscope (Nikon TE300) and recorded using the methods and protocols specified in the Online Supplement.

2.3. Cell culture and voltage clamp recordings in HEK293 Cells

HEK 293 cells stably expressing WT-*SCN5A* were transiently transfected with WT-*SCN1B* using Fugene 6. Sodium channel characteristics were studied using whole-cell patch clamp techniques 48 h after transfection, as described in the Online Supplement [13].

2.4. Experimental protocol and theoretical data analysis

Use-dependent inhibition of I_{Na} by WK was measured during trains of 40 pulses to −30 mV following a rest of 15 s at a holding potential of either −80 mV or −120 mV. To evaluate the effects of rate, the pulse duration was kept constant, and diastolic interval was either 150 ms, 50 ms, or 20 ms. Conversely, the influence of pulse duration was evaluated at constant diastolic interval and pulse durations of 5 ms, 20 ms, or 200 ms. Peak currents in the presence of WK were normalized to I_{Na} measured in the absence of drug. The goal of this experimental protocol was to calculate kinetic binding and unbinding rates of WK with closed, open, and inactivated states of the sodium channel using a guarded-receptor model [14]. Tonic block was determined as the block that develops with the first pulse of a train following a 15 s rest.

Steady-state inactivation was measured with a standard dual-pulse protocol. Cells were held at −120 mV before evoking a 1-sec conditioning pulse immediately followed by a 20-ms pulse to −30 mV to measure sodium current. Conditioning pulses ranged from −110 to −50 mV and were increased in 10-mV steps. Current was normalized to the peak current recorded after a conditioning step to −120 mV. The normalized current was plotted as a function of conditioning step voltage and fit to a standard Boltzmann function: 1 / (1 + exp. [(Vh − V) / Sh]).

The shifts (ΔVh) of the steady-state inactivation curves by different concentrations (D) of WK were analyzed using Bean's equation [15] to obtain dissociation constants for WK interaction with the inactivated (Ki) and closed (Kc) states of the sodium channel:

$$\Delta V_h = S_h \cdot \ln\left(\frac{1+\frac{D}{K_c}}{1+\frac{D}{K_i}}\right)$$

where Sh is the slope of the steady-state inactivation curve.

2.5. Statistical analysis of experimental data

Statistical analysis was performed using a paired Student's *t* test and one-way repeated-measures or multiple comparisons ANOVA followed by Bonferroni's test, as appropriate. All data are expressed as mean ± SD. Statistical significance was assumed at $p < 0.05$.

2.6. Mathematical modeling

We used a simplified Markovian model for I_{Na} that incorporates the interaction of WK with different states of the channel as previously described [16]. The main theoretical considerations for the development of the model and model equations are shown in the Online Supplement. The number of closed states was reduced to two: the fully closed state and the preopen non-conducting state (see Online Supplement Fig. 1). In addition, we assumed that the inactivation process is coupled with activation and requires at least one of four activation gates to open to proceed (Online Supplement Fig. 2). The absence of the closed-inactivated state in the Markovian scheme (Online Supplement Fig. 1) reflects this fact. In agreement with the experimental data, we shifted the voltage dependence for intrinsic steady-state inactivation in atrial cells to reproduce the observed approximately −10-mV difference in steady-state inactivation curves between ventricular and atrial cells. Due to the corresponding but smaller (−5 mV) difference in the steady-state activation curves, the inactivation time constant in atrial cells was smaller, in agreement with the experimental data [17].

3. Results

3.1. Use-dependent effects of WK during trains of pulses

We first examined whether WK interacts strongly with the inactivated state of the sodium channel. Opening of single sodium channels typically occurs in the initial 20 ms of a depolarizing pulse, after which channels enter an inactivated state [18]. If WK has significant interaction with the inactivated state, prolonging the test step during a train of pulses of constant diastolic interval should result in a greater steady-state inhibition of I_{Na}. Fig. 1 shows the results of an experiment in which I_{Na} was recorded from atrial and ventricular myocytes using 5 ms and 200 ms pulses to −30 mV separated by 150 ms holding at −80 mV. Note that the main effect of WK on peak I_{Na} appears to be tonic block, which was significantly larger in atrial cells than in ventricular cells (Table 1). Similar results were obtained using diastolic intervals of 50 and 20 ms (not shown). The use-dependent effect was much smaller and reached steady-state values after the first pulse. There was no statistically significant difference between the block recorded during the second pulse and the one recorded during the 40th pulse of the train. These results suggest that WK interacts with the inactivated state of the channel and that the rate of binding is very fast. Interestingly, with a holding potential of −120 mV, WK caused relative increases in peak I_{Na} (Supplement Figs. 4 and 5).

Next we tested the sensitivity of block to the diastolic interval during a train of pulses of fixed duration. If drug interaction with the closed state is weak, WK will primarily unbind during the "diastolic interval" between test pulses. Abbreviation of the time available for unbinding is expected to result in accumulation of block and greater steady-state inhibition as diastolic interval is reduced. The results of these experiments are presented in Fig. 2, which shows a decrease of peak I_{Na} during a train of 200 ms pulses delivered from −80 mV to −30 mV in the presence of 10 g/L WK and normalized to peak I_{Na} recorded during an identical train in the absence of drug (control). We observed very rapid unbinding of WK from the sodium channel at −80 mV, so that the steady-state block was only minimally dependent on the diastolic interval. The unbinding process reached steady state during the first interpulse interval. As a result, there was no statistically significant difference between the degree of block attained during the second pulse and the one attained during the 40th pulse. Similar results were obtained using other test pulse durations (5 ms and 20 ms, not shown). These results indicate that unbinding of WK from the inactivated sodium channels is very fast.

Finally, we explored the possibility that the tonic block of I_{Na} is due to WK interaction with the closed state of the sodium channel. For this purpose, we employed the same pulse protocols, but using a very negative diastolic (holding) potential of −120 mV. Under these conditions, WK did not produce any noticeable block of I_{Na} (see Supplementary Data) indicating that WK does not bind to the closed state of the sodium channel and that block is predominantly due to WK interaction with the inactivated state of the channel. If indeed this is the case, WK should shift the steady-state inactivation relationship toward more negative potentials [15], and this shift should be more pronounced in atrial cells when compared with ventricular cells.