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Sensitivity of the Slow Component of the Delayed Rectifier Potassium Current (I_{Ks}) to Potassium Channel Blockers: Implications for Clinical Reverse Use-Dependent Effects

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Key Words
Antiarrhythmic agents · Potassium channels · Voltage clamp

Abstract
The slow delayed rectifier potassium current (I_{Ks}) is unique in its slow activation and deactivation kinetics. It is important during cardiac repolarization, especially when the heart rate is fast. We compared the effects of quinidine, procainamide, sotalol, and amiodarone on I_{Ks} and correlated the findings with the clinical reverse use-dependent effects of potassium channel blockers. Human minK RNA was obtained by reverse transcription-polymerase chain reaction using explanted human heart. The RNA was injected into Xenopus oocytes for heterologous expression of I_{Ks}. A two-electrode voltage clamp technique was performed to investigate the I_{Ks}. We demonstrated that quinidine, sotalol and procainamide had no effects on I_{Ks} up to a concentration of 300 μM while amiodarone inhibited I_{Ks} in a concentration-dependent manner starting from 10 μM. The inhibition by amiodarone was state-dependent with gradual unblocking after depolarization. The degree of inhibition was 53% immediately after depolarization and 19% at the end of a 5-second depolarization. I_{Ks} is 30 times more sensitive to amiodarone than to quinidine, sotalol, and procainamide. Quinidine, sotalol and procainamide have reverse use-dependent effects while amiodarone does not. This is compatible with the hypothesis that no inhibition of I_{Ks} at clinical concentrations contributes to the clinical reverse use-dependent effects.

Introduction

The delayed rectifier potassium current (I_K) was first described by Noble and Tsien [16] in sheep cardiac Purkinje fibers. This current has since been identified in cardiac tissue from many of species including human beings [31]. Kinetic analysis studies on I_K have suggested that there may be more than one component of I_K. Sanguinetti and Jurkiewicz [21] have provided evidence showing that I_K in guinea pig cardiac cells can be separated into two kinetically distinct components, a more rapid component (I_{Kr}) and a slower component (I_{Ks}). These two components have also been observed in cardiac cells from dog and chick heart [7, 24]. The presence of I_{Kr} and I_{Ks} in human cardiac tissue has been debated. More recently, Wang et al. [32] and Li et al. [12] both reported that I_K in the human heart also has two components.

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Since I_K is one of the outward currents during the action potential, it contributes significantly to cardiac repolarization. Class Ia and class III antiarrhythmic drugs, by blocking I_K, delay the repolarization process, prolong the action potential duration and therefore increase cardiac refractoriness [5]. Most studies on the pharmacological properties of I_K were performed in single ventricular myocytes using the whole cell voltage clamp technique. Investigations on the pharmacological properties of human cardiac I_{Ks} are rare because the level of I_{Ks} is small compared to I_{to} in these cardiac myocytes and because it is not easy to obtain human cardiac tissue.

In the present study, we investigated the effects of quinidine, procainamide, *d,l*-sotalol, and amiodarone on I_{Ks} using an in vitro expression model in Xenopus oocytes. These drugs are widely used and have a QT prolonging effect in common. I_{Ks} is unique in its slow activation and deactivation kinetics and is important during cardiac repolarization especially when the heart rate is fast. Therefore, the pharmacological sensitivity of I_{Ks} to various drugs may have important clinical implications especially with regard to use-dependent properties of drugs.

Materials and Methods

Preparation of Human minK cDNA

Total cellular RNA was isolated from human left ventricular tissue from an explanted heart obtained during cardiac transplantation surgery with the consent of the patient. The patient was a 35-year-old man with advanced coronary artery disease, an old anterior wall myocardial infarction and left ventricular dysfunction. Cardiac transplantation was performed because the left ventricular ejection fraction was 11% and the patient had pulmonary edema and dyspnea on minimal exertion. The total cellular RNA underwent reverse transcription reaction using random hexanucleotide primers and avian myeloblastosis virus RNA-dependent DNA polymerase. Polymerase chain reaction was performed using the reverse transcription product as the template. The sense primer was 5′-GTGGGATCCTAATGCCCAGGATGATC and the antisense primer was 5′-GTGGTCGACTTCATGGGGAAGGCTTC. The temperature settings were 94°C for 30 s, 58°C for 2 min and 74°C for 1 min 30 s for 35 cycles. The polymerase chain reaction products were cloned into a TA cloning vector (Invitrogen, San Diego, Calif., USA) and then sequenced by a chain termination reaction with Sequenase Version 2 (United State Biochemical, Cleveland, Ohio, USA). The above-mentioned experiments were performed at National Taiwan University Hospital.

Expression of Human minK Protein in Xenopus Oocytes

The plasmid containing the minK cDNA was linearized by *Bam*H1. In vitro transcription was carried out with T7 RNA polymerase in the presence of a messenger RNA cap structure analogue. The minK messenger RNA was dissolved in sterile distilled water at 0.1 mg/ml.

Stage V and VI oocytes were isolated from *Xenopus laevis* (Nasco, Fort Atkinson, Wisc., USA). The isolated oocytes were digested with collagenase (1 mg/ml) in modified Barth's solution without calcium for 60–90 min to remove the follicular membrane. The oocytes were injected with 50 nl of the minK messenger RNA or sterile distilled water (as negative control).

Two-Electrode Voltage Clamp Study

After incubation in modified Barth's solution with calcium for 2–5 days, the oocytes were studied with a two-electrode voltage clamp technique. The microelectrodes were filled with 3 *M* KCl and had tip resistance of 0.5–1 MΩ. The experiment was performed at room temperature using an Axonclamp 2B amplifier (Axon Instruments, Foster City, Calif., USA). Command pulses and data acquisition were controlled via a 12-bit A/D converter (DigiData 1200, Axon Instruments, Foster City, Calif., USA) by an IBM PC/AT. A series of five 5-second pulses spaced 25 s apart, ranging from −40 to +40 mV at 20-mV increments were given from a holding potential of −60 mV. The tail currents were recorded after repolarization to −60 mV following the test pulses. We also performed voltage commands from a holding potential of −60 to +40 mV for different durations (envelop-tail studies). The pClamp 5.0 software was used for data acquisition and analysis. These recordings were low pass-filtered at 20 Hz and sampled at 1 kHz.

Drugs and Solutions

The stock solutions for drugs were 100 m*M* quinidine in dimethylsulfoxide, 30 m*M* *d,l*-sotalol in distilled water, 100 m*M* amiodarone in 100% ethanol, and 100 m*M* procainamide in distilled water. The modified Barth's solution without calcium contained 90 m*M* NaCl, 5 m*M* HEPES, 1.5 m*M* KCl, 1 m*M* $MgCl_2$, 1 m*M* $NaHCO_3$ at pH 7.6 while modified Barth's solution with calcium contained 82 m*M* NaCl, 7 m*M* HEPES, 1.5 m*M* KCl, 1 m*M* $MgCl_2$, 2 m*M* $NaHCO_3$, 1 m*M* $CaCl_2$ with antibiotic at pH 7.6. The external solution during the voltage clamp study was modified Barth's solution. For amiodarone study, bovine serum albumin was added to the external solution to a concentration of 1% to increase amiodarone solubility.

Data Analysis

Data are presented as mean ± standard deviation. Student's t test was used to test statistical significance, which was accepted at $p < 0.05$. Concentration-response relationship was examined by a linear regression test. Curve fitting of current tracings was performed using the exponential fitting algorithm in the pClamp 5.0 software. Adequacy of fit was assessed by visual inspection and an r value greater than 0.96.

Results

Effects of Drugs on the Peak and Tail Currents of I_{Ks}

Outward current characteristic of I_{Ks} was recorded during a depolarizing voltage command (from −60 mV to −20 mV or greater) in oocytes injected with minK mRNA, while no current was recorded in oocytes injected with distilled water. The dose-response curves of the I_{Ks} peak and tail currents to the drugs are shown in figure 1.

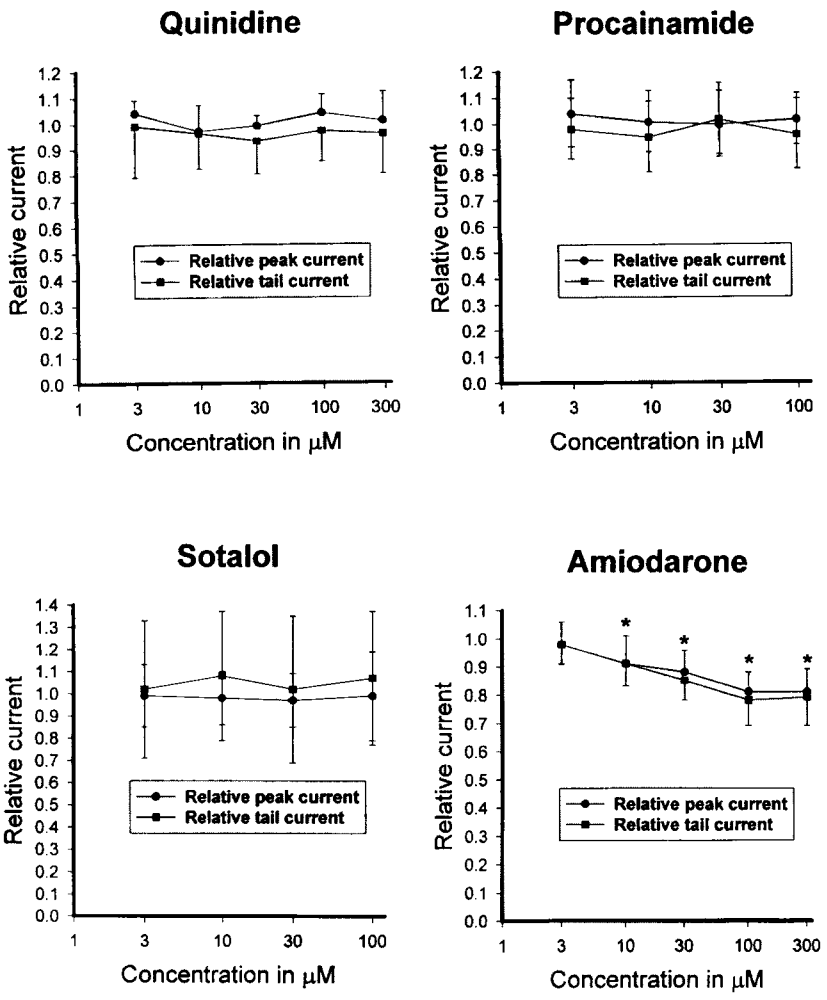


Fig. 1. Dose-response curves of quinidine (n = 8), procainamide (n = 8), *d,l*-sotalol (n = 9), and amiodarone (n = 9) on I_{Ks} peak and tail currents. The peak current was measured at the end of a 5-second depolarization pulse to +40 mV from a holding potential of −60 mV. The tail current was measured at the beginning of repolarization to −60 mV after depolarization to +40 mV. Amiodarone exerted significant inhibitory effects on both peak and tail currents while quinidine, sotalol and procainamide did not have inhibitory effects up to 300 μ*M*. * $p < 0.05$ when compared to baseline. When the relative current of amiodarone was correlated with the amiodarone concentration by linear regression, a negative correlation constant and a p value <0.05 were obtained for both peak and tail currents.

There was no inhibitory effect up to a concentration of 300 μ*M* for quinidine, procainamide and sotalol. However, amiodarone inhibited the I_{Ks} peak current in a concentration-dependent manner starting from 10 μ*M* (fig. 1). The inhibitory effect was saturated when the concentration was higher than 100 μ*M*. When the relative current of amiodarone was correlated with the amiodarone concentration by linear regression, a negative correlation constant and a p value <0.05 were obtained for both peak and tail currents. There were no changes of I_{Ks} current with 0.4% alcohol indicating that the inhibitory effects were due to drugs instead of the solvent. The inhibition of the peak current at the end of a 5-second voltage command to +40 mV was 19%. The tail current was suppressed to a degree similar to the peak current. The activation and deactivation time constants were calculated by fitting the activation and deactivation curves to a single exponential model. We found that the activation and deactivation time constants were not significantly changed by the drugs (table 1).

State-Dependent Inhibition of the I_{Ks}

We found that the degree of inhibition by amiodarone was different along the time course of the voltage command. Figure 2 shows the current tracing induced by depolarization to +40 mV before and after incubation with amiodarone 300 μ*M*. The peak and tail currents were both inhibited by amiodarone. When the inhibited current (Id, current with amiodarone) during depolarization was scaled by a factor of 1.3, it crossed over the current

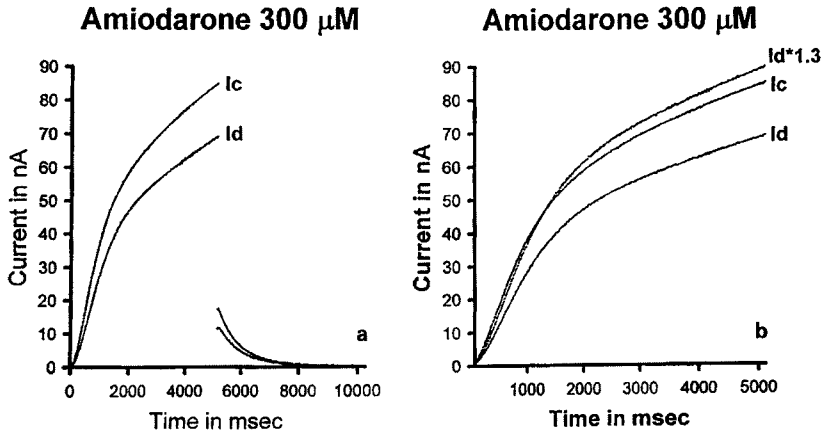


Fig. 2. Representative current tracing showing state-dependent inhibition of I_{Ks} by amiodarone. Similar results were observed in all oocytes. **a** I_{Ks} current during depolarization to +40 mV for 5 s from a holding potential of −60 mV, then repolarization to −60 mV. Ic is the current tracing without drug and Id is the tracing in the presence of 300 μ*M* amiodarone. The leak current has been subtracted. **b** When the Id was scaled up by a factor of 1.3, it crossed over the Ic. This phenomenon could be explained by unblocking of channels after depolarization.

Table 1. Effects of quinidine, *d,l*-sotalol, procainamide, and amiodarone (all at 300 μ*M*) on the activation and deactivation time constant

	Activation time constant, ms		Deactivation time constant, ms	
	baseline	drug	baseline	drug
Quinidine (n = 8)	1,717±175	1,943±413	597±215	677±153
Procainamide (n = 8)	1,630±890	1,730±910	603±154	622±142
Sotalol (n = 9)	1,810±770	1,650±590	637±180	597±108
Amiodarone (n = 9)	1,928±499	2,019±429	588±115	631±130

Data are expressed as mean ± standard deviation and n is the number of oocytes.

Table 2. Relationship between voltage and relative current after incubation with 300 μ*M* amiodarone

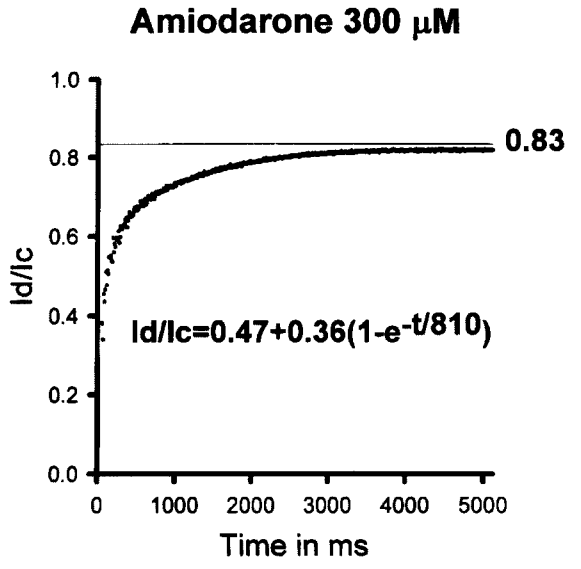
	Relative current	
	peak	tail
To 0 mV (n = 9)	0.82±0.10	0.83±0.11
To +20 mV (n = 9)	0.81±0.08	0.78±0.09
To +40 mV (n = 9)	0.81±0.07	0.79±0.08

Data are expressed as mean ± standard deviation and n is the number of oocytes.

before adding amiodarone (Ic). The 'crossover' was observed in all 9 oocytes. The probability of getting 'crossover' in all 9 oocytes would be 2^{-9} ($p < 0.01$). This phenomenon is best explained by unblocking of channels after depolarization. These unblocked channels contributed to the current and caused the 'crossover' phenomenon when the Id was scaled up. Figure 3 shows the plotting of relative current (Id/Ic) against time after the beginning of voltage command to +40 mV. The degree of inhibition was largest at the beginning of the voltage command and the I_{Ks} was unblocked during the voltage command. This curve could be fit to a single exponential model:

$$(Id/Ic)_t = (Id/Ic)_0 + B \cdot (1 - e^{-t/\tau})$$

where Id is the current after adding drugs; Ic is the current before adding drugs; t is the time after beginning of voltage command, $(Id/Ic)_t$ and $(Id/Ic)_0$ are the relative current (Id/Ic) at time t and 0; τ is the time constant of unblocking and B is the portion that is unblocked after depolarization. The calculated $(Id/Ic)_0$ and $(Id/Ic)_\infty$ [relative current at steady state: = $(Id/Ic)_0$+B] values at different voltages are shown in table 2. The dose-dependent inhibitory effects of amiodarone at different times after depolarization are shown in figure 4. Amiodarone had a



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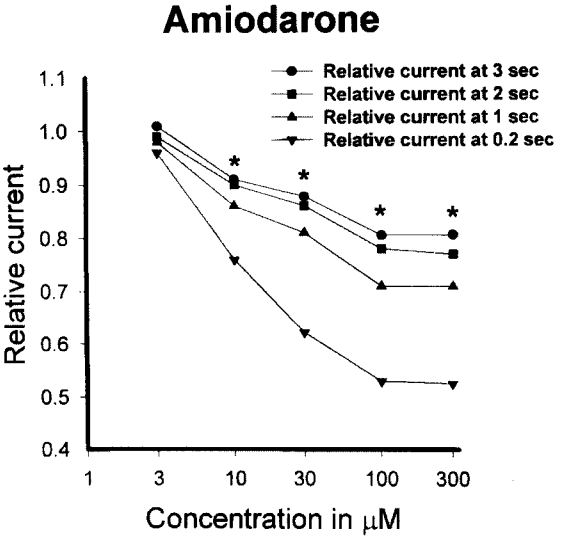

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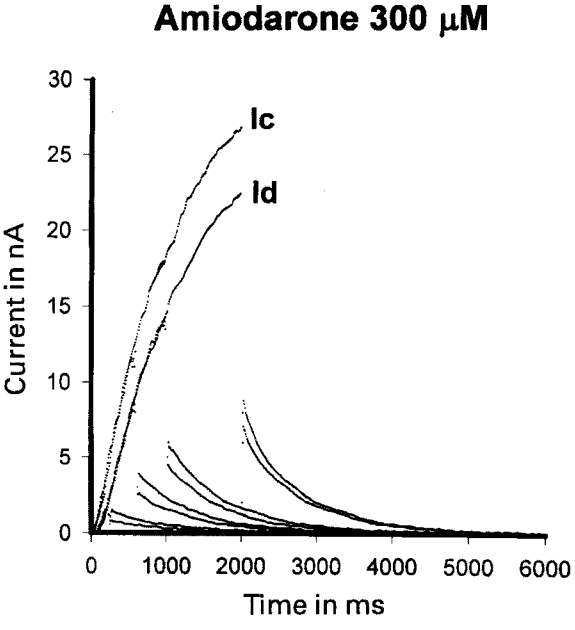
Fig. 3. Plot showing relative current (Id/Ic) against time after the beginning of depolarizing voltage command to +40 mV. The degree of inhibition was stronger initially and unblocking of channels occurred after depolarization to +40 mV. The kinetics of unblocking fit to an exponential curve. Ic = Current before adding drugs; Id = current after adding drugs; t = time after depolarization.

Fig. 4. Concentration-response curve of amiodarone on I_{Ks} at different times after depolarization. The effect of amiodarone was stronger with a shorter duration of depolarization. At 200 ms after depolarization, the inhibitory effect of amiodarone could reach 50–55%. This time interval (200 ms) is approximately equal to the duration of the human ventricular action potential. * $p < 0.05$ when compared to baseline.

Fig. 5. Effect of 300 μ*M* amiodarone on the envelop-tail studies of I_{Ks} current. The holding potential was −60 mV and the duration of the voltage commands to +40 mV were 200 ms, 600 ms, 1 s, and 2 s. The degree of inhibition was larger with shorter pulse duration. The relative amplitudes of peak and tail currents in the presence of 300 μ*M* amiodarone were 50 and 52%, respectively, with a pulse duration of 200 ms, and were 82 and 81% with a pulse duration of 2 s. Ic = Current before adding drugs; Id = current after adding drugs.



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stronger effect initially after depolarization at various concentrations. These findings indicate that amiodarone has a lower binding affinity with channels in the open state. At 200 ms after depolarization, the inhibitory effect of amiodarone reached 50–55%. This time interval (200 ms) is approximately equal to the duration of the human ventricular action potential. For further verification of the unblocking after depolarization, we used voltage commands from a holding potential of −60 mV to +40 mV with different pulse durations (envelop-tail studies). We showed that the degree of inhibition was larger with a shorter pulse duration. Figure 5 shows a representative experiment. The relative amplitudes of peak and tail current in the presence of 300 μ*M* amiodarone were

Table 3. Kinetics of unblocking of I_{Ks} by amiodarone at different voltage commands

	$(Id/Ic)_0$	$(Id/Ic)_\infty$	τ (ms)
To 0 mV (n = 9)	0.41 ± 0.02	0.81 ± 0.01	560 ± 42
To +20 mV (n = 9)	0.47 ± 0.01	0.83 ± 0.01	656 ± 36*
To +40 mV (n = 9)	0.47 ± 0.02	0.83 ± 0.01	810 ± 24*,**

The curve of unblocking was fit to a single exponential model: $(Id/Ic)_t = (Id/Ic)_0 + B \cdot (1 - e^{-t/\tau})$ where t is the time after beginning of voltage command; $(Id/Ic)_\infty$ and $(Id/Ic)_0$ are the relative current at time ∞ and 0; τ is the time constant of unblocking in ms. * $p < 0.05$ when compared to τ during clamp to 0 mV; ** $p < 0.05$ when compared to τ during clamp to +20 mV. Data are expressed as mean ± standard deviation and n is the number of oocytes.

50 and 52%, respectively, with a pulse duration of 200 ms, and were 82 and 81% with a pulse duration of 2 s (fig. 5).

Relationship between Voltage Command and I_{Ks} Inhibition

The inhibitory effect of amiodarone on the I_{Ks} was not significantly different among voltage commands to 0, +20 and +40 mV. With a more positive voltage command, the time constant of unblocking was longer (table 3). This indicates that unblocking is voltage-dependent and a more positive voltage may decelerate the unblocking process.

Discussion

Quinidine, sotalol and procainamide had no inhibitory effects on I_{Ks} channels expressed in oocytes up to a concentration of 300 μM. All these drugs are reverse use-dependent in prolonging action potential duration [3, 9, 23]. In contrast, amiodarone had concentration-dependent inhibitory effect on the I_{Ks} starting from 10 μM, and it is unique among potassium channel blockers which have minimal reverse use-dependent effects [18, 19]. We attribute clinical reverse use dependence to the failure to block I_{Ks}.

Production of I_{Ks} by Heterologous Expression of Human minK in Xenopus Oocytes

In our study, the I_{Ks} was obtained by heterologous expression of human minK protein in Xenopus oocytes. Since the amino acid sequence of minK protein hardly resembles any known ion channel, it has long been doubted that minK protein alone forms the potassium channel [14, 25]. Recent studies have shown that another protein KvLQT1, when coexpressed with minK protein, can induce I_{Ks} current in mammalian cells and this current cannot be produced by expression of either KvLQT1 or minK protein in these cells [2, 20]. Therefore, it is believed that KvLQT1 and minK protein together form the I_{Ks} channel. In the Xenopus oocytes, the KvLQT1 is endogenously present and the Xenopus KvLQT1 is 89–92% homologous to human KvLQT1 at the amino acid level [20]. The induced current in our study had an activation time constant of 1,000–2,000 ms and a deactivation time constant of 500–1,000 ms. The channels opened upon a voltage command to more than −40 mV and the reversal potential was close to potassium equilibrium potential. These kinetic, gating and ion selectivity properties are very similar to those of I_{Ks} in human cardiac tissue [12, 32].

Sensitivity of I_{Ks} to Potassium Channel Blockers

The I_{Ks} was at least 30 times more sensitive to amiodarone than to quinidine, procainamide, and sotalol. It has been reported that quinidine has a K_D value of 10–50 μM for I_{Ks} in guinea pig heart tissue [1, 17]. This concentration is much higher than the concentration needed for inhibition of I_{Na} and I_{to} [4, 8]. The effect on the prolongation of the action potential duration can be observed at a concentration much lower than that needed for inhibiting the I_{Ks} [4]. Therefore, quinidine does not block I_{Ks} at therapeutic concentrations. Our study corroborates previous studies which reported that sotalol is a selective I_{Kr} blocker without effects on I_{Ks} [22]. In our model, quinidine, procainamide and sotalol had no inhibitory effect on I_{Ks} at a concentration of 300 μM. In the same system, amiodarone inhibited I_{Ks} in a dose-dependent manner starting from 10 μM. This observation is in agreement with the observation of Yang and coworkers [33] that amiodarone inhibits KvLQT1-induced current in HEK293 cells. They reported that amiodarone at 10 μM reduced the peak current by 25–30% and reduced the tail current by 50%. Although KvLQT1 is the α-subunit of I_{Ks}, the current induced in that study is not typical of I_{Ks}. However, the concentration and degree of inhibition is comparable to our study. Therefore, it is likely that amiodarone inhibits I_{Ks} at clinical therapeutic concentrations while quinidine, procainamide and sotalol do not.

Significance of I_{Ks} in the Human Heart

The presence and importance of I_{Ks} in the human heart were controversial because I_{Ks} current in the human heart

is not prominent. Veldkamp et al. [28] reported that I_{Kr} was the only component of the delayed rectifier current in human ventricular myocytes. However, there has been growing evidence indicating that I_{Ks} is not only present but also contributes to the delayed rectifier current. Wang et al. [32] demonstrated that the I_K in human atrial tissue has kinetically distinguishable rapid and slow components. More recently, Li et al. [12] successfully dissected the delayed rectifier current in human ventricular myocytes into an E-4031-sensitive rapid component and an E-4031-insensitive slow component [12]. The I_{Ks} channels are formed by minK protein and KvLQT1. The genes of both proteins are expressed in human ventricle and therefore I_{Ks} channels should be present in the human ventricular tissue [20, 27]. Successful cloning of the I_{Ks} cDNA from cellular RNA extract from the human left ventricle in our study also proved that I_{Ks} gene is expressed in human ventricle. The shape and duration of the action potential is a delicate interplay of different ion currents. I_{Ks} can play an important role in repolarization even though it is not prominent in human heart tissue.

I_{Ks} and Clinical Reverse Frequency-Dependent Effects

After the disappointing Cardiac Arrhythmia Suppression Trial showed that potent sodium channel blockers caused excessive cardiac mortality, potassium channel blockers have become the focus of many recent investigations [26]. However, reverse use dependence is a major drawback of most potassium channel blockers [9, 29]. When the heart rate is slow, the action potential prolonging effect is strong leading to unwanted side effects such as early after-depolarization and torsades de pointes. When the heart rate is fast, such as during a tachyarrhythmia, the action potential prolonging effect is weak leading to the inability to terminate the tachyarrhythmia.

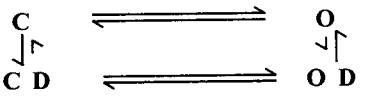
The mechanism of the reverse use-dependent effect has been debated. It has been shown in single ventricular myocytes that many potassium channel blockers are not selective inhibitors of channels in the closed state [4, 11, 13]. In the present study, we correlated clinical reverse use dependence to the failure to block I_{Ks}. I_{Ks} is unique in its slow activation and deactivation kinetics. There will be incomplete deactivation of the channels during tachycardia because of shortened diastolic periods. Therefore, they contribute more to repolarization during tachycardia than during a slow heart rate because open state channels tend to accumulate. Failure to block the I_{Ks} could result in less action potential duration prolongation effect during a faster heart rate. Jurkiewicz and Sanguinetti [10] have conducted a study on dofetilide using guinea pig ventricular myocytes. They showed that I_{Ks} contributed more to repolarization at a faster heart rate than at a slower heart rate. They also demonstrated that dofetilide, a class III agent with reverse use dependence, was a selective blocker of I_{Kr} without effects on I_{Ks}. In contrast, a study by Gintant [6] does not support the role of I_{Ks} in reverse use-dependent effects. In that report, the I_{Ks} in canine ventricular myocytes had rapid deactivating kinetics and channels in an open state would not accumulate at a fast heart rate. The discrepancy might be due to interspecies difference in the deactivating kinetics. In human beings, the I_{Ks} has slow deactivating kinetics similar to that of the guinea pig [12]. This favors the theory that I_{Ks} may play a role in clinical reverse use-dependent effects.

For the development of new antiarrhythmic agents with potassium channel-blocking properties, we suggest that I_{Ks}-blocking agents should be devoid of the undesirable reverse use-dependent effects and should be QT-prolonging agents. Many new antiarrhythmic agents, such as dofetilide and ibutilide, are specific I_{Kr} blockers and have strong reverse use-dependent effects. In contrast, ambasilide, a I_{Kr} and I_{Ks} blocker, has been reported to have less reverse use-dependent effects on refractoriness than sotalol, which is a pure I_{Kr} blocker [30]. Future clinical studies are needed to confirm the superiority of I_{Ks}-blocking agents.

Amiodarone is not a pure class III antiarrhythmic agent and has a sodium channel-blocking effect, β-adrenergic-blocking effect, and calcium channel-blocking effect in addition to the potassium channel-blocking effect. Is it possible that these additional properties contribute to its lack of reverse use-dependent effects? However, *d*, *l*-sotalol also has an additional β-adrenergic blocking effect, while quinidine and procainamide both have a sodium channel-blocking effect. These drugs have been proved to have reverse use-dependent effects. Therefore, it is unlikely that β-adrenergic blocking and sodium channel blocking effects contribute to the lack of reverse use-dependent effects of amiodarone. With regard to the calcium channel-blocking effect, the calcium current is an inward current during the action potential and calcium current blockade theoretically results in a shortening of the action potential duration. Since calcium channel-blockade is usually use-dependent [15], it contributes to the reverse use-dependent prolongation of the action potential duration by counteracting the potassium channel-blocking effects at fast heart rate. Therefore, it is unlikely that the calcium channel-blocking effect contributes to the lack of reverse use-dependent effects of amiodarone. We attribute the lack of reverse use-dependent effect to inhibition of I_{Ks}.

State-Dependent Inhibition of I_{Ks}

Amiodarone had stronger inhibitory effects during the initial portion of the voltage command. The steady state inhibition was 17–19% (I_∞) while the degree of inhibition was 53–59% (I_0) at the beginning of the voltage command. This observation can be explained by the following model:



Amiodarone has higher binding affinity with channels in the closed state and some of the I_{Ks} channels escape from blocking during the course of activation. In addition, we found that the process of unblocking was slower during a more positive voltage command as shown by a significantly larger unblocking time constant.

Limitations

The I_{Ks} channels were expressed in the Xenopus oocytes. The concentration for I_{Ks} inhibition by amiodarone was about one order higher than the plasma concentration in clinical use (typically 1–10 μM) and the degree of inhibition saturated at 100 μM. The lower sensitivity to drugs may be caused by the oocyte vitelline membrane which is a barrier between the drugs and the channels. Furthermore, the yolk in oocytes may form a reservoir for lipophilic drugs. The posttranslation modification and cofactors for channels may be absent in oocytes. All these factors may be responsible for the decreased drug sensitivity.

The I_{Ks} channel in the present study was formed by human minK protein and endogenous KvLQT1 in the Xenopus oocytes. There might be interspecies differences in the pharmacological properties of KvLQT1. However, the homology between human and Xenopus KvLQT1 is high (89–92%) and the induced current is very similar to I_{Ks} in human cardiac tissue with regard to its gating and kinetic properties.

The time-dependent inhibition of tail current could not be precisely calculated. The tail current was much smaller than the peak current. The crossover of tail current could not be demonstrated and the Id/Ic for the tail current suffered from large variation and data points showed serious scattering.

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