Short- and Long-Term Effects of Amiodarone on the Two Components of Cardiac Delayed Rectifier $K^+$ Current

Kaichiro Kamiya, MD; Atsushi Nishiyama, MD; Kenji Yasui, MD; Mayumi Hojo, BS; Michael C. Sanguinetti, PhD; Itsuo Kodama, MD

Background—Amiodarone is the most promising drug for the treatment of life-threatening tachyarrhythmias in patients with structural heart disease. The pharmacological effects of amiodarone on cardiac ion channels are complex and may differ for short-term and long-term administration.

Methods and Results—The delayed rectifier $K^+$ current ($I_K$) of ventricular myocytes isolated from rabbit hearts was recorded with the whole-cell voltage-clamp technique. $I_K$ was separated into 2 components by use of specific blockers for either $I_{Kr}$ (chromanol 293B, 30 μmol/L) or $I_{Ks}$ (E-4031, 10 μmol/L). Short-term application of amiodarone caused a concentration-dependent decrease in $I_{Kr}$ with an $IC_{50}$ of 2.8 μmol/L (n=8) but only a minimal reduction in $I_{Ks}$. The short-term effects of amiodarone were also determined in Xenopus oocytes expressing the cloned human channels that conduct $I_{Kr}$ and $I_{Ks}$ (HERG and KvLQT1/minK). HERG current in oocytes was reduced by amiodarone ($IC_{50}$=38 μmol/L), whereas KvLQT1/minK current was unaffected by 300 μmol/L amiodarone. To study the effects of long-term drug administration, rabbits were treated for 4 weeks with oral amiodarone (100 mg · kg$^{-1}$ · d$^{-1}$) before cell isolation. Long-term administration of amiodarone decreased $I_K$ to 55% (n=10) in control rabbits and altered the relative density of $I_{Kr}$ and $I_{Ks}$. The majority (92%) of current was $I_{Kr}$. mRNAs of rabbit $ERG$, $KVLQT1$, and $minK$ in left ventricular myocardium did not differ between control and long-term amiodarone.

Conclusions—Amiodarone has differential effects on the 2 components of $I_K$, depending on the application period; short-term treatment inhibits primarily $I_{Kr}$, whereas long-term treatment reduces $I_{Ks}$. (Circulation. 2001;103:1317-1324.)

Key Words: amiodarone • potassium • mRNA

Amiodarone is an effective drug for the treatment of life-threatening ventricular tachyarrhythmias. Amiodarone has been referred to as a class III antiarrhythmic agent, but its pharmacological action is in fact very complex. It prolongs both action potential duration and refractory period when administered long term but blocks Na$^+$ and Ca$^{2+}$ channels after short-term administration. Amiodarone also has noncompetitive antisympathetic effects and modulates thyroid function, phospholipid metabolism, and production of certain cytokines. What specific action or combination of actions is fundamental and salutar for potent antiarrrhythmic activity is not known. As recently reviewed, the short-term and long-term effects of amiodarone on action potentials and ionic currents of cardiac cells are quite different.

Delayed rectifier $K^+$ currents ($I_K$) are important determinants of cardiac repolarization. $I_K$ is composed of 2 distinct components: a rapidly activating component showing inward rectification ($I_{kr}$) and a slowly activating component ($I_{ks}$) with a linear current-voltage (I-V) relationship. $K^+$ channel genes that encode $I_{kr}$ and $I_{ks}$ are $HERG^2$ and $KVLQT1$ plus $minK^8,9$ respectively. The effects of amiodarone on $I_{kr}$ are still limited and controversial. Therefore, in the present study, we examined the short- and long-term effects of amiodarone on $I_{kr}$ and $I_{ks}$ in rabbit ventricular myocytes. In addition, we studied the short-term effects of amiodarone on human $ERG$ and $KVLQT1/minK$ channels expressed heterologously in Xenopus oocytes. The effects of long-term amiodarone on rabbit $ERG$, $KVLQT1$, and $minK$ mRNA were also examined.

Methods

Rabbit Ventricular Myocytes

Japanese white rabbits of either sex weighing 1.5 to 2.2 kg were killed under anesthesia with thiramal sodium (30 mg/kg IV), and the hearts were removed. Single myocytes were isolated enzymatically from the apical region of the left ventricle (approximately one third in the apex/base axis) as described previously. To observe the long-term effects of amiodarone, the rabbits were administered oral amiodarone (100 mg · kg$^{-1}$ · d$^{-1}$) for 4 weeks before cell isolation.

Electrophysiological Recording

The single-pipette, whole-cell, voltage-clamp technique was used for recording membrane currents. Cell capacitance was measured by

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integrating the capacitive transient evoked by applying a hyperpolarizing pulse to −5 mV from a holding potential of −50 mV. The cell capacitance and series resistance were electrically compensated by 60% to 90%. Command potential generation and data acquisition were controlled by pCLAMP software (version 6.0.3, Axon Instruments) and an IBM-compatible computer. Current signals were filtered at 1 kHz and digitized at a sampling frequency of 2 kHz. Current measurement was started after 40-minute superfusion with normal Tyrode’s solution to avoid possible retention of amiodarone and its active metabolite (desethylamiodarone) on the cell surface in rabbits with oral treatment.

cRNA Injection and Voltage-Clamp of Oocytes
Isolation and maintenance of Xenopus oocytes and injection with HERG cRNA were performed as described previously.7 Stage V and VI oocytes were injected with 10 ng HERG cRNA. KVLQT1 cRNA (5 ng) and minK cRNA (1 ng) were coinjected to induce I Ks. Currents were recorded at room temperature (22°C to 24°C) by standard 2-microelectrode voltage-clamp techniques15 2 to 4 days after cRNA injection.

Ribonuclease Protection Assay
For the ribonuclease protection assay, rabbit ERG, KVLQT1, and minK subunit cDNA fragments were amplified by reverse-transcriptase polymerase chain reaction. The nucleotide sequences of the primers and the amplified regions are listed in Table 1. Gene bank accession numbers are U75212 (nucleotides 187 to 456) for ERG, U70068 (nucleotides 502 to 1133) for KVLQT1, and L41659 (nucleotides 239 to 477) for minK. A HindIII site (AAGCTT) was introduced into the 5’ end of the sense primers of the rabbit ERG, KVLQT1, and minK. The amplified cDNA was cloned into pGEM-T vector with the TA cloning system (Promega). The ribonuclease protection assay was performed as described previously.16

Solution and Drugs
The Tyrode’s solution used for cell isolation and the single myocyte experiments was composed of (mmol/L) NaCl 143, KCl 5.4, CaCl2 1.8, MgCl2 0.5, NaH2PO4 0.25, HEPES 5.0, and glucose 5.6; pH was adjusted to 7.4 with NaOH. The glass pipette had a resistance of 3 to 5 MΩ after filling with the internal pipette solution containing (mmol/L) KOH 60, KCl 80, aspartate 40, HEPES 5, MgATP 5, sodium creatinine phosphate 5, and CaCl2 0.65 (P Ca 8.0) at pH 7.2. The external solution used to measure K 1 currents was maintained at 34°C and was composed of the following (mmol/L):
N-methyl-D-glucamine 149, MgCl₂ 5, HEPES 5, and nisoldipine 0.003. 

Iₖₛ and Iₖᵣ were separated by applying 2 specific blockers, chromanol 293B (30 μmol/L) and E-4031 (10 μmol/L), respectively.

Oocytes were bathed in a modified ND96 solution containing (mmol/L) NaCl 94, KCl 4, MgCl₂ 2, CaCl₂ 0.1, and HEPES 5 (pH 7.6). CaCl₂ was reduced to 0.1 mmol/L to suppress endogenous Ca²⁺-activated Cl⁻ current.

Amiodarone hydrochloride (Sigma Chemical Co) was dissolved in dimethyl sulfoxide to prepare a stock solution of 300 mmol/L. On the day of experiments, aliquots of the stock solution were diluted with the bath solution. Dimethyl sulfoxide at 0.1% had no significant effect on outward currents in rabbit ventricular myocytes or Xenopus oocytes. E-4031 was kindly provided by Eisai Pharmaceuticals (Tokyo, Japan); chromanol 293B, by Aventis Pharma (Frankfurt, Germany).

Statistical Analysis
Data are presented as mean±SEM unless otherwise specified. When relative densities of Iₖᵣ and Iₖₛ and effects of long-term amiodarone administration on the ratio were determined, current amplitudes from 2 to 3 myocytes per each rabbit heart were averaged and served as 1 datum point. Statistical comparisons between the different experimental groups were obtained by ANOVA. Comparisons between multiple group means were performed with a Bonferroni-corrected t test for all group comparisons. Differences were considered significant at P<0.05. Concentration-response relationships were fit to the Hill equation to determine the concentration of drug required for 50% inhibition (IC₅₀). A nonlinear least-squares curve-fitting program (Calmpfit 6.0) was used to analyze deactivation kinetics.

Results
Iₖᵣ and Iₖₛ of Rabbit Ventricular Myocytes
The relative contributions of chromanol 293B–resistant and –sensitive components of Iₖ in rabbit isolated ventricular

<table>
<thead>
<tr>
<th>Activation Time Constants, τ, ms</th>
<th>Deactivation Time Constants, ms</th>
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<tbody>
<tr>
<td>Control (n=7)</td>
<td></td>
</tr>
<tr>
<td>293B-resistant current (Iₖᵣ)</td>
<td>482±41</td>
</tr>
<tr>
<td>293B-sensitive current (Iₖₛ)</td>
<td>585±34</td>
</tr>
<tr>
<td>Chronic amiodarone (n=8)</td>
<td></td>
</tr>
<tr>
<td>293B-resistant current (Iₖᵣ)</td>
<td>465±43</td>
</tr>
</tbody>
</table>

Figure 2. Short-term effects of amiodarone on Iₖᵣ. Iₖᵣ was elicited by same voltage-clamp pulse protocol as in Figure 1. A, Representative current traces in ventricular myocyte pretreated with 30 μmol/L chromanol 293B. Bath application of 10 μmol/L amiodarone caused marked inhibition of 293B-resistant component of Iₖᵣ (Iₖᵣ). Additional application of 10 μmol/L E-4031 resulted in complete elimination of time-dependent currents. B, Iₖᵣ-V relationship for Iₖᵣ before and after application of 1 and 10 μmol/L amiodarone. Density of peak tail current (mean±SE, n=8) was plotted vs test potentials (*P<0.05 vs control). C, Concentration-response relationship for block of tail current after pulse to 50 mV by amiodarone. IC₅₀ and Hill coefficient were 2.8 μmol/L and 0.91, respectively (n=8).
myocytes are illustrated in Figure 1. Depolarizing pulses for 2 seconds from −40 to 70 mV were applied from the holding potential at −75 mV at 0.03 Hz. The tail current (\(I_{K, \text{tail}}\)) was measured on repolarization back to 250 mV (Figure 1A).

Application of 30 \(\mu\text{mol/L}\) chromanol 293B decreased the time-dependent outward current during depolarization and the tail current on repolarization. Additional application of 10 \(\mu\text{mol/L}\) E-4031 eliminated most time-dependent current. Figure 1B illustrates the chromanol 293B–sensitive component obtained by subtraction of the current traces. The chromanol 293B–resistant and –sensitive components of \(I_K\) were used in this study to define \(I_{Kr}\) and \(I_{Ks}\), respectively.

Figure 1C shows a tail current-voltage (I-t) relationship for total \(I_K\), \(I_{Kr}\), and \(I_{Ks}\) in control rabbit ventricular myocytes (n=10, 22 cells). Based on sensitivity to chromanol 293B, 71% of the tail current amplitude after depolarization to 50 mV was attributable to \(I_{Kr}\) and 29% to \(I_{Ks}\). Tail current amplitudes were normalized to peak values, and the resulting I-V relation was fitted to a Boltzmann equation to construct isochronal activation curves (Figure 1D).

The time courses of activation at 50 mV and deactivation at −50 mV were determined by exponential fitting. The time constants of \(I_{Kr}\) and \(I_{Ks}\) in control myocytes are summarized in Table 2. There were no significant differences between the 2 components of \(I_K\) at these potentials.

**Short-Term Effects of Amiodarone on \(I_{Kr}\) and \(I_{Ks}\)**

The short-term effects of amiodarone on \(I_K\) were examined in ventricular myocytes pretreated with 10 \(\mu\text{mol/L}\) E-4031. Application of 10 \(\mu\text{mol/L}\) amiodarone caused minimal inhibition of E-4031–resistant component of \(I_K\) (a, b). Additional application of 30 \(\mu\text{mol/L}\) 293B resulted in complete elimination of the time-dependent currents (c). Changes in 293B-sensitive component of \(I_K\) after application of 10 \(\mu\text{mol/L}\) amiodarone are shown by digital subtraction (a-c, b-c). B, I-t-V relationship for \(I_{Ks}\) before and after 10 \(\mu\text{mol/L}\) amiodarone (n=10; *P<0.05 vs control).

**Effects of Amiodarone on HERG and KvLQT1/minK Currents**

HERG currents were measured with a 2-step pulse protocol at a frequency of 0.03 Hz. From a holding potential at −90 mV,
a 2-second depolarization (−80 to 50 mV) was applied to activate outward currents, followed by return of the membrane potential to −70 mV to evoke tail currents (Figure 4A). The amplitude of outward tail currents exceeded the amplitude of the activating currents, characteristic of HERG channel behavior.\textsuperscript{11,18} Bath application of amiodarone (1 to 100 \(\mu\)mol/L) for 30 minutes resulted in a concentration-dependent decrease in outward currents during depolarization and tail currents. Figure 4B and 4C shows the I-V relationship for currents at the end of the depolarization step and at the peak of the tail after repolarization, respectively (\(n=5\)). The \(IC_{50}\) for amiodarone block of the HERG channel tail current was 37.9 \(\mu\)mol/L (Hill coefficient=0.61; \(n=5\)).

Injection of oocytes with cRNA encoding KVLQT1 and minK subunits induced \(I_{Ks}\), characterized by a linear I-V relationship (Figure 4D).\textsuperscript{8,9} Currents were measured during a 7.5-second depolarizing pulse to potentials ranging from −80 to 40 mV, with tail currents measured at −80 mV. Pulses were applied at a frequency of 0.03 Hz. Bath application of amiodarone did not affect the time-dependent currents even at the highest concentration tested (300 \(\mu\)mol/L). Figure 4E and 4F shows the I-V relationships for currents during the test pulse (step) and for tail currents. Unlike what was observed for \(I_{Ks}\) in rabbit myocytes, the averaged I-V curve was not shifted by 300 \(\mu\)mol/L amiodarone.

**Long-Term Effects of Amiodarone on \(I_{Kr}\) and \(I_{Ks}\)**

Figure 5 shows \(I_{Kr}\) and \(I_{Ks}\) in ventricular myocytes isolated from rabbits treated with oral amiodarone (100 mg·kg\(^{-1}\)·d\(^{-1}\)) for 4 weeks. Averaged current densities of total \(I_{K}\) in these myocytes were significantly less than total \(I_{K}\) measured in cells isolated from control rabbits. The tail current density after depolarization to 50 mV was 0.81 ± 0.13 pA/pF in controls (\(n=10\); 22 cells) and 0.45 ± 0.04 pA/pF in cells isolated from rabbits treated long term with amiodarone (\(n=9\); 26 cells). Application of 30 \(\mu\)mol/L 293B caused no substantial change in both time-dependent outward currents and tail currents (Figure 5A). Additional application of 10

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**Figure 4.** Short-term effects of amiodarone on HERG and KVLQT1/minK currents in *Xenopus* oocytes. A, Representative HERG current traces recorded from oocyte before and after application of 30 \(\mu\)mol/L amiodarone. Voltage-clamp pulse protocol is shown above current traces. B, I-V relationship for currents measured at end of 2-second depolarization step (\(n=5\)). Data were obtained before (•) and 30 minutes after amiodarone at 3 \(\mu\)mol/L (○), 30 \(\mu\)mol/L (△), and 100 \(\mu\)mol/L (●). C, I-V relationship for peak tail currents. D, Representative current traces recorded from oocyte expressing KVLQT1 and minK subunits before and after application of 300 \(\mu\)mol/L amiodarone. E, I-V relationship for currents measured at end of 7.5-second depolarization step (\(n=5\)). Data were obtained before (○) and 30 minutes after (●) 300 \(\mu\)mol/L amiodarone. F, I-V relationship for peak tail currents (\(n=5\)).

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mol/L E-4031 resulted in complete elimination of the time-dependent currents. These findings indicate that IKs was very small in cells isolated from rabbits treated long term with amiodarone.

Figure 5B shows I-t-V relationships for total IK, IKr, and IKs in myocytes treated with long-term amiodarone (n=9; 26 cells). After depolarization to 50 mV, 92% of the tail current was IKr resistant (IKr), and only 8% was chromanol 293B sensitive (IKs). The tail current density of IKr in the amiodarone-treated myocytes (0.41±0.04 pA/pF at 50 mV) was reduced by 29% (P<0.05) compared with control myocytes (0.58±0.11 pA/pF at 50 mV). In contrast, the tail current density of IKs in the amiodarone-treated myocytes (0.04±0.02 pA/pF at 50 mV) was reduced by 83% (P<0.05) compared with control (0.23±0.02 pA/pF at 50 mV). Figure 5C shows isochronal activation curves for total IK and its 2 components. V1/2 of total IK and IKr did not differ significantly from the corresponding values in control myocytes. The amplitude of IKs was too small to analyze precisely, but its activation curve (Figure 5C, dotted line) was apparently shifted toward more positive potentials compared with those of IKr and IKs. There were no significant differences between control and amiodarone-treated myocytes in the activation and deactivation time constants of IKr (Table 2).

Channel Subunit Expression

The effects of long-term treatment of amiodarone on mRNA encoding ERG, KVLQT1, and minK subunits were measured with the ribonuclease protection assay using hearts obtained from control and amiodarone-treated rabbits killed after 28 days. Cyclophilin mRNA expression levels were used for the internal control. As shown in Figure 6, the levels of rabbit ERG, KVLQT1, and minK mRNAs did not exhibit a significant difference between control and amiodarone-treated rabbits.

Discussion

Short-Term Effects of Amiodarone on IKr and IKs

We used myocytes isolated from the apical region of the left ventricle to minimize cell-to-cell variation of IKr and IKs.
resulting from their regionally different distribution. The activation-deactivation kinetics of \( I_{Kr} \) (chromanol 293B–resistant component) and \( I_{Ks} \) (chromanol 293B–sensitive component) in control rabbits were similar to those in our previous reports, in which \( I_{Kr} \) and \( I_{Ks} \) were estimated as E-4031–sensitive and –insensitive components, respectively. Unlike other animal species, activation-deactivation kinetics of \( I_{Kr} \) and \( I_{Ks} \) in rabbits are similar (Table 2). Therefore, it was difficult to discriminate between the 2 components on the basis of kinetics, necessitating a pharmacological approach to distinguishing between \( I_{Kr} \) and \( I_{Ks} \).

Short-term application of amiodarone to rabbit isolated ventricular myocytes resulted in a concentration-dependent decrease in the 293B-resistant component of \( I_{K} \) with minimal change in the 293B-sensitive component (\( I_{Ks} \)). This confirms our previous findings, also in rabbit ventricular myocytes, that short-term amiodarone (1 to 10 \( \mu \)mol/L) inhibited the E-4031 (10 \( \mu \)mol/L)–sensitive component of \( I_{K} \) (\( I_{Kr} \)) without affecting the E-4031–resistant component (\( I_{Ks} \)). However, these results are at odds with a previous report on guinea pig ventricular myocytes by Balser et al. Differences in animal species and experimental conditions might explain the discrepancy.

The study of heterologously expressed human channels in Xenopus oocytes confirmed the short-term effects of amiodarone in isolated rabbit cardiac myocytes. We confirmed the findings of a recent study by Kiethn et al. that HERG channels can be blocked by short-term amiodarone (IC\(_{50}\), 9.8 \( \mu \)mol/L) and demonstrated a lack of effect of the drug on KvLQT1/minK current.

**Long-Term Effects of Amiodarone on \( I_{Kr} \) and \( I_{Ks} \)**

The most prominent effect of long-term amiodarone on cardiac muscles is a moderate and frequency-independent prolongation of action potential duration. Information available to explain the mechanism underlying the action potential duration prolongation is limited. It was shown in our previous study in ventricular myocytes isolated from rabbits treated with oral amiodarone (100 mg \( \cdot \) kg\(^{-1}\) \( \cdot \) day\(^{-1}\) for 4 weeks) that the current densities of \( I_{Kr} \) and \( I_{Ks} \) (transient outward current) were decreased significantly compared with control rabbits (by \( \approx 50\% \) and \( \approx 30\% \), respectively), without any appreciable changes in their voltage dependence. Qualitatively similar findings have been reported by Varró et al. In these reports, \( I_{Ks} \) was not separated into the 2 components \( I_{Kr} \) and \( I_{Ks} \). In the present study, the tail current density of total \( I_{K} \) in amiodarone-treated rabbits was decreased by 29\%, whereas the tail current density of \( I_{Ks} \) was reduced by 83\% from controls. This indicates that \( I_{Ks} \) inhibition by long-term amiodarone is predominantly due to a reduction in \( I_{Kr} \).

Recently, Bosch et al. reported that long-term treatment of guinea pigs with intraperitoneal amiodarone (80 mg \( \cdot \) kg\(^{-1}\) \( \cdot \) day\(^{-1}\) for 7 days) caused a substantial reduction in the current density of \( I_{Kr} \) and \( I_{Ks} \) of ventricular myocytes to a similar extent (\( \approx 60\% \) reduction) without affecting their voltage-dependence or kinetics. Different periods of amiodarone administration (4 versus 1 week), different experimental protocols, and different animal species (rabbits versus guinea pigs) may underlie the discrepancy between our data and their observations.

**Study Limitations**

We focused our investigation on the short- and long-term effects of amiodarone on \( I_{Kr} \) and \( I_{Ks} \) because block of these currents is an obvious candidate mechanism for the class III properties of this drug. However, the antiarrhythmic action of amiodarone likely results from inhibition of multiple channels and receptors. In addition, long-term effects of amiodarone on the heart are modulated by plasma and tissue accumulation of the parent drug and its active metabolite desethylamiodarone, which would impose their direct effects.

mRNA levels of \( I_{Kr} \), \( I_{Ks} \), and \( I_{Ks} \) potassium channel subunits were not affected by long-term amiodarone, indicating that the reduction in \( I_{Ks} \) density cannot be ascribed to reduced transcription of mRNA. The mechanism of reduced \( I_{Kr} \) is unknown but may be due to an effect on the posttranscriptional processes of channel protein synthesis.

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**References**


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