Tamoxifen inhibits Na\(^+\) and K\(^+\) currents in rat ventricular myocytes

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Tamoxifen inhibits Na\(^+\) and K\(^+\) currents in rat ventricular myocytes. Am J Physiol Heart Circ Physiol 285: H661–H668, 2003. First published April 17, 2003; 10.1152/ajpheart.00686.2002.—Tamoxifen is an estrogen receptor antagonist used in the treatment of breast cancer. However, tamoxifen has been shown to induce QT prolongation of the electrocardiogram, thereby potentially causing life-threatening polymorphic ventricular arrhythmias. The purpose of the present study was to elucidate the electrophysiological mechanism(s) that underlie the arrhythmogenic effects of tamoxifen. We used standard ruptured whole cell and perforated patch-clamping techniques on rat ventricular myocytes to investigate the effects of tamoxifen on cardiac action potential (AP) waveforms and the underlying K\(^+\) currents. Tamoxifen (3 \(\mu\)mol/l) markedly prolonged AP duration, decreased maximal rate of depolarization, and decreased resting membrane potential. At this concentration, tamoxifen significantly depressed the Ca\(^2+\)-dependent transient outward K\(^+\) current (\(I_{\text{to}}\)), sustained outward delayed rectifier K\(^+\) current (\(I_{\text{so}}\)), inward rectifier K\(^+\) current (\(I_{\text{K1}}\)), and Na\(^+\) current (\(I_{\text{Na}}\) in the myocytes. Lower concentrations of tamoxifen (1 \(\mu\)mol/l) also decreased the resting membrane potential and significantly depressed \(I_{\text{K1}}\) to 79 ± 5% \((n = 5; \text{at } \sim 120 \text{ mV})\) of pretreatment values. The results of this study indicate that inhibition of \(I_{\text{to}}, I_{\text{so}},\) and \(I_{\text{K1}}\) by tamoxifen may underlie AP prolongation in cardiac myocytes and thereby contribute to prolonged QT interval observed in patients.

Electrophysiology; arrhythmia; cardiac; torsades de pointes; QT prolongation

The estrogen receptor antagonist tamoxifen has been used for >20 years to actively treat breast cancer and to help reduce the incidence of breast cancer in high-risk women (19). It has also been used to treat cancers of the liver, brain, and pancreas, which indicates that it may have more general application (3). However, increasing evidence suggests that tamoxifen may have untoward cardiovascular effects. Several cases of QT prolongation and ventricular arrhythmias, in particular torsades de pointes, have been reported with tamoxifen (13, 28). These reports prompted the warning of potentially serious adverse cardiac events.

It is likely that tamoxifen promotes QT-segment prolongation by selectively altering ionic currents; however, relatively little work has been done to address this possibility. Tamoxifen was shown to inhibit volume-activated Cl\(^-\) currents in many cell types and to inhibit a number of voltage- and ligand-gated cation channels primarily in noncardiac tissues (1, 16, 27, 43). As well, tamoxifen has been reported to block neuroblastoma cell proliferation that is mediated by voltage-dependent K\(^+\) channels (29). In rat vascular smooth muscle cells, tamoxifen inhibits both voltage-gated Ca\(^2+\) currents and contractility (35). Similar effects on Ca\(^2+\) currents are reported in a clonal pituitary cell line (32).

There are few published studies that describe the ionic mechanisms by which tamoxifen can initiate arrhythmias. In rabbit ventricular myocytes, Liu et al. (24) reported that tamoxifen inhibits the delayed rectifier K\(^+\) current (\(I_{\text{Kd}}\)) and the inward L-type calcium current (\(I_{\text{Ca,L}}\)). They did not, however, detect an effect on either the transient outward K\(^+\) current (\(I_{\text{to}}\)) or the inwardly rectifying K\(^+\) current (\(I_{\text{K1}}\)). They were also unable to demonstrate that tamoxifen increases action potential (AP) duration, a result that is inconsistent with \(I_{\text{K}}\) inhibition and is predicted based on the clinical observations of QT prolongation. Thus further studies are required to evaluate the ionic mechanisms of tamoxifen. Previously, we demonstrated that tamoxifen inhibits canine cardiac muscle sarcoplasmic reticulum (SR) Ca\(^2+\) uptake through a mechanism that is most consistent with the inhibition of an SR ion channel (21). We also showed that tamoxifen reduces the amplitude of electrically evoked Ca\(^2+\) transients and the amount of Ca\(^2+\) that can be released by caffeine in intact isolated rabbit cardiac myocytes. These results are consistent with an effect of tamoxifen on the SR but do not rule out the possibility that tamoxifen also affects plasma membrane ion channels. To test the latter possibility and to elucidate electrophysiological mechanism(s) that may underlie the arrhythmogenic effect of tamoxifen, we examined AP waveforms of rat ventricular myocytes. We determined the concentration-dependent effects of tamoxifen on AP waveforms and the K\(^+\) and Na\(^+\) currents present in these myocytes. Standard ruptured whole cell and perforated patch-clamping techniques were used for these studies.

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MATERIALS AND METHODS

Isolation of myocytes. Right ventricular myocytes were isolated according to methods previously described (42). Briefly, adult male Sprague-Dawley rats (200–225 g body wt) were killed by cervical dislocation. The heart was rapidly removed and mounted on a Langendorff apparatus by aortic cannulation. The heart was initially perfused with Tyrode solution that contained 1 mmol/l Ca²⁺ at 37°C for 5 min. Perfusion rate was 10 ml/min, and all perfusion solutions were bubbled with 100% O₂. The perfusate was then switched to Ca²⁺-free Tyrode solution for 5 min, followed by perfusion for 7 min with the same solution to which collagenase (0.02 mg/ml; Yakult) and protease (0.004 mg/ml, type XIV; Sigma Chemicals) had been added. After the perfusion, the right ventricular free wall was removed, minced in 10 ml of Tyrode solution that contained collagenase (0.5 mg/ml), protease (0.1 mg/ml), and bovine serum albumin (3 mg/ml; Sigma), and was gently shaken in a water bath (at 37°C) for 20–40 min. Aliquots of suspended cells were removed at 5-min intervals into tubes with Kraftbrühe solution (defined below), and the cells were stored at 4°C until use. Only single rod-shaped cells with clear cross striations and no spontaneous contractions were used for experiments. All the experiments were conducted at room temperature (21–23°C).

Solutions and chemicals. Tamoxifen (Sigma) was dissolved in ethanol to obtain 1 mmol/l stock solution, and aliquots of this stock solution were stored at −20°C until use. The standard Tyrode solution used for cell isolation and patch-clamp recordings contained (in mmol/l) 140 NaCl, 5.4 KCl, 1 Na₂HPO₄, 5 HEPES, 10 glucose, and 1 MgCl₂, and pH was adjusted to 7.4 with 1 mol/l NaOH. K⁺ currents [Iₖ, sustained outward delayed rectifier (Iₖsus), and Iₖ1] were recorded using a standard internal pipette solution that contained (in mmol/l) 20 KCl, 110 K-aspartate, 10 EGTA, 10 HEPES, 1 MgCl₂, 5 K₂ATP, 1 CaCl₂, and 10 NaCl, and pH was adjusted to 7.2 using 1 mol/l KOH. AP waveforms were recorded using solutions identical to those for K⁺ current recordings with the exception that amphotericin B (300 μg/ml) was added to the pipette solution to perforate the cell membrane. Unless stated otherwise, the standard 1 mM CaCl₂-containing Tyrode solution was used as the superfusion solution when K⁺ currents and APs are recorded. Na⁺ currents were recorded using a pipette solution that contained (in mmol/l) 120 CsF, 10 HEPES, 2 MgCl₂, 10 EGTA, 1 CaCl₂, and 10 NaCl, and pH was adjusted to 7.2 with 1 mol/l KOH. The superfusion solution for recording Na⁺ current (Iₙa) contained (in mmol/l) 10 NaCl, 10 HEPES, 5 KCl, 1 MgCl₂, 1 CaCl₂, 5 CsCl, 10 glucose, and 110 choline chloride, and pH was adjusted to 7.4 with 1 mol/l NaOH. Modified KB solution for cell storage contained (in mmol/l) 100 K-glutamate, 10 K-aspartate, 25 KCl, 20 glucose, 10 KH₂PO₄, 5 HEPES, 2 MgSO₄, 20 taurine, 5 creatine, 0.5 EGTA; plus 0.1% BSA with pH adjusted to 7.2 using 1 mol/l KOH.

Electrophysiological methods. Myocytes were transferred to a small chamber mounted on the stage of an inverted microscope (TE300, Nikon) and allowed to adhere onto the glass bottom of the chamber for 10–15 min. The flow rate through the chamber was 2 ml/min, and solution exchange of the bath was achieved within 30 s. Patch pipettes were pulled from borosilicate glass using a P-97 Puller (Sutter Instruments) and were polished on an MF-200 microforge (World Precision Instruments; Sarasota, FL). When filled with internal solution, the pipette resistance was 2–3 MΩ. Whole cell currents and APs were recorded with an Axopatch 1-D amplifier (Axon Instruments; Burlingame, CA) using the pCLAMP 8.0 Digidata 1200 data-acquisition system. We corrected for junction potentials (9–11 mV).

AP waveforms were recorded using the perforated patch-clamp technique. APs were elicited in current-clamp mode by 5-ms, 800-pA current injections at a frequency of 1 Hz. All APs and K⁺ currents were filtered at 2 kHz and sampled at 5 kHz.

The ruptured patch whole cell configuration was used to measure Iᵦ₀, Iₖᵦᵢᵰ, and Iₖᵢ₁. Whole cell K⁺ currents were evoked by 500-ms test pulses ranging between −120 and +50 mV in 10-mV increments from a holding potential of −80 mV. This voltage protocol was repeated with a 100-ms depolarizing step to −40 mV applied immediately before the 500-ms test pulses to voltage-inactive Iₖ₁. The currents derived by subtracting these two protocols are the isolated Iₖ₁ values. Iₖ₁ amplitude was measured as the peak current amplitude.

To measure Iₖᵦ₁, 500-ms test pulses ranging between −120 and +50 mV, in 10-mV increments, from a holding potential of −80 mV, were applied in the presence and absence of 200 μmol/l Ba²⁺. Ba²⁺ blocks Iₖᵦ₁, whereas it does not inhibit either Iₖ₁ or Iₖᵦᵢᵰ (25, 33). Subtraction of the current evoked in the presence and absence of Ba²⁺ yields the isolated Iₖᵦ₁ current. Values obtained at the end of the 500-ms pulse were used for analysis.

Iₖᵦᵢᵰ values were also recorded by 500-ms test pulses ranging between −120 and +50 mV, in 10-mV increments, from a holding potential of −80 mV. A 100-ms prepulse to −40 mV and 200 μmol/l BaCl₂ was applied to block Iₖ₁ and Iₖᵦ₁, respectively. Iₖᵦᵢᵰ amplitude was measured as the residual current at the end of the 500-ms pulses.

Whole cell Iₙa was elicited using 50-ms rectangular voltage steps from a holding potential of −90 mV to a command potential of −30 mV. These data were filtered at 2 kHz and recorded at 20 kHz.

For all experiments, tamoxifen was added to the superfusion solution 10 min before recording. Time-course studies (data not shown) indicated that the maximal effects of tamoxifen can be measured within 8 min of initial exposure.

Data analysis. Ionic currents were corrected for cell capacitance and are expressed in terms of current density (in pA/pF). All averaged and normalized results are presented as means ± SE. Statistical analysis was done using an ANOVA test followed by the Newman-Keuls multiple-comparison test. A P < 0.05 was taken to indicate a statistically significant difference.

RESULTS

Effects of tamoxifen on AP waveforms. Figure 1 illustrates the concentration-dependent effects of tamoxifen on the AP waveforms in rat ventricular myocytes. At all concentrations examined, tamoxifen altered AP waveforms. Typical effects of tamoxifen included reduced resting membrane potential, decreased upstroke velocity, reduced AP amplitude, and broadening of the entire waveform. The concentration-dependent effects of tamoxifen on AP parameters are summarized in Table 1. A complete AP characterization at 10 μmol/l tamoxifen was not possible, because this concentration completely suppressed our ability to stimulate APs. Vehicle control experiments (ethanol alone) did not significantly change the AP waveforms.

Effects of tamoxifen on Iₖ₁. Iₖ₁ was isolated using techniques outlined in MATERIALS AND METHODS; results are illustrated in Fig. 2D. The effects of tamoxifen at 1, 3, and 10 μmol/l on Iₖ₁ were assessed with cumulative
increasing-concentration applications. The concentration-dependent effects of tamoxifen on $I_{to}$ are summarized in Fig. 3. Consistent with the AP data, 1 μmol/l tamoxifen had little effect on $I_{to}$. In contrast, 3 and 10 μmol/l tamoxifen reduced peak outward current (at +50 mV) to 22.9 ± 1.8 (n = 4; P < 0.05) and 13.9 ± 1.4 pA/pF (n = 4; P < 0.05), respectively, from the pretreatment value of 29.2 ± 3.3 pA/pF. Examination of the current-voltage relationship for $I_{to}$ demonstrates that 3 and 10 μmol/l tamoxifen significantly attenuate this current at all command potentials positive to −20 mV (Fig. 3D).

Effects of tamoxifen on $I_{K1}$ current. Figure 2, A and C, shows representative current traces of $K^+$ currents before and after exposure to 200 μmol/l Ba$^{2+}$. The Ba$^{2+}$-sensitive current, $I_{K1}$, was obtained by subtracting the current in the presence and absence of Ba$^{2+}$ as shown by Fig. 2E. Superfusion of myocytes with 1, 3, and 10 μM tamoxifen for 10 min had significant concentration-dependent effects on $I_{K1}$ (Fig. 4). From a control current density of −13.8 ± 1.4 pA/pF, cumulative additions of tamoxifen decreased $I_{K1}$, at −120 mV, to −11.0 ± 1.2, −7.0 ± 0.6, and −2.0 ± 0.3 pA/pF (n = 5; P < 0.05 for all concentrations) for 1, 3, and 10 μmol/l, respectively (Fig. 4). The current-voltage rela-

Table 1. Concentration-dependent effects of tamoxifen on action potential parameters of isolated rat ventricular myocytes

<table>
<thead>
<tr>
<th>Tamoxifen Concentration, μmol/l</th>
<th>RMP, mV</th>
<th>AMP, mV</th>
<th>$V_{max}$, V/s</th>
<th>APD$D_{50}$, ms</th>
<th>APD$D_{90}$, ms</th>
</tr>
</thead>
<tbody>
<tr>
<td>0 (Control)</td>
<td>−80 ± 0.3</td>
<td>135 ± 3.7</td>
<td>145 ± 14</td>
<td>3.2 ± 0.4</td>
<td>21 ± 3.7</td>
</tr>
<tr>
<td>1</td>
<td>−78 ± 0.4</td>
<td>121 ± 3.0$^*$</td>
<td>92 ± 3.7$^*$</td>
<td>3.4 ± 0.2</td>
<td>20 ± 2.4</td>
</tr>
<tr>
<td>3</td>
<td>−74 ± 0.7$^*$</td>
<td>62 ± 0.6$^*$</td>
<td>54 ± 4.1$^*$</td>
<td>13 ± 1.4$^*$</td>
<td>59 ± 5.6$^*$</td>
</tr>
</tbody>
</table>

Values are means ± SE; n, 4 experiments. RMP, resting membrane potential in ventricular myocytes; AMP, action potential amplitude; $V_{max}$, maximum upstroke velocity; APD$D_{50}$ and APD$D_{90}$, action potential duration measured at 50 and 90% of repolarization, respectively. $^*$P < 0.05, data significantly different from pretreatment values.
Fig. 3. Concentration-dependent effects of tamoxifen on the Ca2+-independent \( I_{\text{Ca}} \). Representative traces of \( I_{\text{Ca}} \) families are shown with exposure to 10 \( \mu \text{mol/l} \) tamoxifen for command potentials of \(-50, -30, -10, +10, +30, \) and \(+50 \text{ mV} \). A: traces before exposure. B: traces after exposure. C: summary of changes of \( I_{\text{Ca}} \), following application of 1, 3, and 10 \( \mu \text{mol/l} \) tamoxifen as measured at \(+50 \text{ mV} \). D: current-voltage relationships for \( I_{\text{Ca}} \) before and after addition of 1 (\( \bullet \)), 3 (\( \circ \)), and 10 (\( \ast \)) \( \mu \text{mol/l} \) tamoxifen. All currents were normalized for cell capacitance and plotted as means \( \pm \) SE (\( n = 4 \)); \( +P < 0.05 \), data significantly different from pretreatment values; \( ^*P < 0.05 \), command potentials at which data for both 3 and 10 \( \mu \text{mol/l} \) groups were significantly different from pretreatment values (\( \ast \)).

Fig. 4. Concentration-dependent effects of tamoxifen on \( I_{\text{K1}} \). Representative traces of \( I_{\text{K1}} \) families are shown with exposure to 10 \( \mu \text{mol/l} \) tamoxifen for command potentials of \(-120, -100, -80, -60, -40, \) and \(-20 \text{ mV} \). A: before tamoxifen exposure. B: after tamoxifen exposure. C: summary of changes of \( I_{\text{K1}} \), following application of 1, 3, and 10 \( \mu \text{mol/l} \) tamoxifen as measured at \(-120 \text{ mV} \). D: current-voltage relationships for \( I_{\text{K1}} \) before and after addition of 1 (\( \bullet \)), 3 (\( \circ \)), and 10 (\( \ast \)) \( \mu \text{mol/l} \) tamoxifen. All currents were normalized for cell capacitance and plotted as means \( \pm \) SE (\( n = 5 \)); \( +P < 0.05 \), data significantly different from pretreatment values; \( ^*P < 0.05 \), command potentials at which data for both 3 and 10 \( \mu \text{mol/l} \) groups were significantly different from pretreatment values (\( \ast \)).

tionships for \( I_{\text{K1}} \) before and after exposure to tamoxifen are shown in Fig. 4D.

**Effects of tamoxifen on \( I_{\text{K1}} \).** Representative tracings of isolated \( I_{\text{K1}} \) are illustrated in Fig. 2F. Before tamoxifen exposure, peak \( I_{\text{K1}} \) at \(+50 \text{ mV} \) was determined to be \( 10.0 \pm 0.7 \mu \text{A/\mu F} \) (\( n = 5 \)). Tamoxifen attenuated \( I_{\text{K1}} \) to \( 8.5 \pm 0.8, 7.4 \pm 0.9, \) and \( 4.7 \pm 0.1 \mu \text{A/\mu F} \) (\( n = 5 \)) for 1, 3, and 10 \( \mu \text{mol/l} \) tamoxifen, respectively. These data are summarized in Fig. 5. Although there was a trend for 1 \( \mu \text{mol/l} \) tamoxifen to reduce this current, there were no significant differences over the entire voltage range examined (Fig. 5D). In contrast, 3 \( \mu \text{mol/l} \) tamoxifen significantly reduced \( I_{\text{K1}} \) at all command potentials \( >0 \text{ mV} \), whereas 10 \( \mu \text{mol/l} \) significantly reduced \( I_{\text{K1}} \) at all command potentials greater than \(-10 \text{ mV} \) (Fig. 5D).

For all \( \text{K}^+ \) current experiments, the effects of 1 and 3 \( \mu \text{mol/l} \) tamoxifen were completely reversible within 10-min washout of the drug. At 10 \( \mu \text{mol/l} \) tamoxifen, relatively longer washout periods were required (15–20 min), and occasionally, incomplete reversal was obtained.

**Effects of tamoxifen on \( I_{\text{Na}} \).** As noted above and shown in Table 1, tamoxifen at concentrations of 1 \( \mu \text{M} \) and greater significantly decreased the maximum upstroke velocity of the AP in rat ventricular myocytes. This suggests that the \( I_{\text{Na}} \) responsible for depolarization of the myocytes is also affected by tamoxifen.
test this possibility, $I_{Na}$ was measured in the presence and absence of tamoxifen (Fig. 6A). From a pretreatment value of $-37.4 \pm 3.5$ pA/pF ($n = 8$), tamoxifen decreased this current to $-34.4 \pm 3.6$, $-22.7 \pm 2.1$, and $-7.8 \pm 0.9$ pA/pF ($n = 8$) for 1, 3, and 10 $\mu$mol/l, respectively. Inhibition by both 3 and 10 $\mu$mol/l was statistically significant ($P < 0.05$). As observed with $K^+$ current recordings, $I_{Na}$ was not inhibited in vehicle control experiments (data not shown).

**DISCUSSION**

Tamoxifen is the leading antisteroidal substance available to treat breast cancer and acts as a weak estrogen by competing for estrogen receptors much like phytoestrogens. Currently, tamoxifen is prescribed to more women with breast cancer than any other drug. Nevertheless, evidence has accrued that tamoxifen may prolong cardiac repolarization and hence also prolong the QT interval of the surface ECG. Potentially life-threatening ventricular arrhythmias (e.g., torsades de pointes) may occur especially in cases of overdosage or pharmacokinetic interactions (13). The fact that a number of drugs that block cardiac $K^+$ channels and prolong AP duration can cause long-QT syndrome and induce ventricular arrhythmia (4) considered with the demonstrated ability of tamoxifen to block some types of ion channels motivated us to examine the effects of tamoxifen on cardiac $K^+$ channels. The roles that $K^+$ channels are known to play in the repolarization phase of the cardiac AP are consistent with the mechanisms thought to be involved in mediating acquired forms of long-QT syndrome (prolonged repolarization that leads to early afterdepolarizations and triggered arrhythmias; Ref. 36).

In the present study, we used standard whole cell and perforated patch-clamping techniques to investigate the effects of tamoxifen on cardiac AP waveforms and select $K^+$ currents ($I_{Na}$, $I_{sus}$, and $I_{K1}$) and $I_{Na}$ in isolated rat ventricular myocytes (2, 20, 34, 42). The rat model was chosen because the electrophysiological properties of these cells have been widely characterized and provide a better representation of human myocytes compared with those isolated from rabbits. Significant alterations of AP waveforms by tamoxifen were observed. All three $K^+$ currents and $I_{Na}$ were significantly and reversibly depressed by tamoxifen at 3 and 10 $\mu$mol/l concentrations. The marked reductions of these currents can explain in part the altered AP waveforms induced by tamoxifen in our experiments and also suggest ionic mechanisms that may contribute to the prolonged QT interval of the electrocardiogram that has been described in patients.

The contributions of individual $K^+$ currents to the overall AP waveform have been extensively characterized. The Ca$^{2+}$-independent $I_{Na}$ is present throughout the hearts of many species and is largely responsible for the early repolarization phase of the cardiac AP (6). Inhibition of this current would be expected to delay this repolarization phase and would contribute primarily to the increase in AP duration at 50% repolarization ($ADP_{50}$) that was seen in our experiments. $I_{K1}$ is a constitutively active, strong inward rectifier that determines the basic electrophysiological properties of both ventricular and atrial myocytes. It contributes to the acceleration of the final phase of phase III repolarization of the cardiac AP and stabilization of the resting membrane potential (5, 34). Inhibition of this current could therefore be responsible in part for the change in resting membrane potential and the increase in AP duration at 90% repolarization ($ADP_{90}$) seen in our experiments. The rapidly activating and nonactivating current, $I_{sus}$, also contributes to the overall...
is more sensitive to tamoxifen than either $I_{\text{to}}$ or $I_{\text{sus}}$. This finding is consistent with our AP data obtained in the presence of 1 $\mu$M tamoxifen in which we observe a depolarization of the resting membrane potential while other AP waveform parameters are not altered; this effect is characteristic of selective $I_{K1}$ inhibition. Inhibition of $I_{K1}$ is in direct contrast to the study of Liu et al. (24) and requires additional study. However, to fully elucidate the ability of tamoxifen to inhibit these currents in human tissues, further studies are required.

The distribution of tamoxifen into tissues and the availability of the drug and active metabolites to target cells are obvious determinants of tamoxifen effects. The apparent volume of distribution for tamoxifen is high (50–60 l/kg; Ref. 22), which suggests extensive distribution into peripheral tissues and the presence of only a minor portion of the drug in the serum. In humans, levels were 10- to 60-fold higher in tissues than in serum (23). Tamoxifen chronically administered for breast cancer at therapeutic doses (40 mg/day) reaches plasma concentrations of 0.75 $\mu$mol/l (30). Even higher doses such as 200 mg/day have been used for patients in clinical trials to test the efficacy of tamoxifen in the treatment of gliomas and in reverting multidrug resistance (8, 38). Therefore, the concentrations used in the present study could be clinically relevant. To more definitively explore this possibility requires additional information. Although the serum and tissue concentrations of tamoxifen can be quite high, the free concentration in extracellular fluid or in the blood is a more relevant parameter if tamoxifen acts directly on the ion channel proteins (see the discussion below). The solubility of tamoxifen also prevented us from determining its effects over a broader range of concentrations. As noted previously (21), tamoxifen at concentrations above ~15 $\mu$M precipitates in the buffers used in our experiments. This has prevented us from determining whether additional inhibition is observed at higher concentrations and, consequently, we have not been able to determine more complete dose-response curves for its action on the cardiac ion currents. Tamoxifen is extensively metabolized by cytochrome P-450 in humans and produces active estrogen receptor-binding metabolites. In addition to tamoxifen, N-desmethyltamoxifen, 4-hydroxytamoxifen, 4-hydroxy-N-desmethyltamoxifen, and N-desdimethyltamoxifen were abundant in most tissues (23). It is most likely that tamoxifen can modulate cardiac muscle $K^+$-channel function by at least two different pathways: directly by affecting ion channels and indirectly through conversion to a variety of metabolites. Additional studies to correlate ion-current blockade of metabolites of tamoxifen associated with arrhythmias would be beneficial.

Our results showing that tamoxifen inhibits $I_{\text{to}}$, $I_{K1}$, and $I_{\text{sus}}$ can explain some but not all of the alterations in the AP waveform that we observed in this study. On the basis of our data, we suggest that the observed decreases of $I_{\text{to}}$, $I_{K1}$, and $I_{\text{sus}}$ density by tamoxifen underlie the prolongation of the AP that we observed in rat ventricular myocytes. In the intact heart, this could
contribute to prolongation of the QT interval and arrhythmogenesis. From our AP data (see Table 1), however, we would also predict that \( I_{\text{Na}} \) is inhibited, because the AP upstroke velocity was significantly depressed in the presence of tamoxifen. Also consistent with strong \( I_{\text{Na}} \) inhibition is our inability to stimulate APs in the presence of 10 \( \mu \text{mol/l} \) tamoxifen. The effects of tamoxifen on \( I_{\text{Na}} \) were verified by direct measurement of peak inward \( I_{\text{Na}} \). Our results demonstrating that tamoxifen inhibits \( I_{\text{Na}} \) are consistent with our observed decreases in AP upstroke velocity and further demonstrate the nonselectivity of tamoxifen as an ionic channel inhibitor. As we have shown, at least four cardiac ion currents appear to be affected by tamoxifen. Tamoxifen is also known to inhibit volume-regulated Cl\(^-\) currents in cardiac muscle (15) and L-type Ca\(^{2+}\) channels (24) and to block cell-to-cell communication in neonatal cardiac myocytes (24). That tamoxifen appears to affect so many cardiac ion channels suggests that its actions are nonspecific. As discussed, there are, however, a number of ion channels that are not affected by tamoxifen (14, 24).

The mechanisms by which tamoxifen interacts with ion channels and other proteins have not been well determined. Tamoxifen and related compounds are highly lipophilic and readily insert into lipid bilayers (9–11, 18, 26). It has therefore been suggested that tamoxifen alters protein-lipid interactions and consequently exerts its effects on channels or other proteins indirectly (discussed in Refs. 10, 11, 35, 39, 40). It is also possible that tamoxifen interacts directly with proteins (see Refs. 12, 17, 35, 41). Sahebgharani et al. (31) showed recently that volume-activated Cl\(^-\) currents were inhibited by tamoxifen but not by a membrane-impermeant quaternary derivative of tamoxifen (ethylbromide tamoxifen). This indicates that tamoxifen must be in the lipid bilayer to affect these channels; however, whether the channel inhibition involves a direct interaction between tamoxifen and one of the transmembrane portions of the Cl\(^-\) channel protein or is mediated by tamoxifen-induced changes in lipid structure or lipid-protein interactions has not yet been established.

In summary, we have shown that tamoxifen has inhibitory effects on K\(^+\) and Na\(^+\) currents. These findings provide insight into the cellular mechanisms by which tamoxifen prolongs AP waveforms and potentially contributes to the induction of arrhythmias.

**DISCLOSURES**

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