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

Jianying He, Margaret E. Kargacin, Gary J. Kargacin, and Christopher A. Ward

Department of Physiology, Queen’s University, Kingston, Ontario K7L 3N6; and Department of Physiology and Biophysics, University of Calgary, Calgary, Alberta T2N 4N1, Canada

Submitted 2 August 2002; accepted in final form 11 April 2003

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\(^{2+}\)-independent transient outward K\(^+\) current (\(I_{\text{Ks}}\)), sustained outward delayed rectifier K\(^+\) current (\(I_{\text{Kd}}\)), 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 with minimal effects upon \(I_{\text{K1}}\) to 79 \(\pm\) 5\% (\(n = 5\); at \(-120\) mV) of pretreatment values. The results of this study indicate that inhibition of \(I_{\text{Kd}}\), \(I_{\text{Na}}\), and \(I_{\text{K1}}\) by tamoxifen may underlie AP prolongation in cardiac myocytes and thereby contribute to prolonged QT interval observed in patients.

The costs of publication of this article were defrayed in part by the payment of page charges. The article must therefore be hereby marked “advertisement” in accordance with 18 U.S.C. Section 1734 solely to indicate this fact.

http://www.ajpheart.org 0363-6135/03 $5.00 Copyright © 2003 the American Physiological Society
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 CaCl\(_2\) at 37°C for 5 min. Perfusion rate was 10 ml/min, and all perfusion solutions were bubbled with 100% O\(_2\). The perfusate was then switched to Ca\(^{2+}\)-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 Na\(_2\)HPO\(_4\), 5 HEPES, 10 glucose, and 1 MgCl\(_2\), and pH was adjusted to 7.4 with 1 mol/l NaOH. K\(^+\) currents \([I_{Na}], \text{sustained outward delayed rectifier} (I_{NaS}, \text{and} I_{K1})\) were recorded using a standard internal pipette solution that contained (in mmol/l) 20 KCl, 110 K-aspartate, 10 EGTA, 10 HEPES, 1 MgCl\(_2\), 5 K\(_2\)ATP, 1 CaCl\(_2\), 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 \(\mu\)g/ml) was added to the pipette solution to perfurate the cell membrane. Unless stated, the standard 1 mM CaCl\(_2\)-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 MgCl\(_2\), 10 EGTA, 1 CaCl\(_2\), and 10 NaCl, and pH was adjusted to 7.2 with 1 mol/l KOH. The superfusion solution for recording Na\(^+\) current \([I_{NaS}]\) contained (in mmol/l) 10 NaCl, 10 HEPES, 5 KCl, 1 MgCl\(_2\), 1 CaCl\(_2\), 5 CoCl\(_2\), 5 CsCl, 10 glucose, and 110 choline chloride, and pH was adjusted to 7.4 with 1 mmol/l NaOH. Modified KB solution for cell storage contained (in mmol/l) 100 K-glutamate, 10 K-aspartate, 25 KCl, 20 glucose, 10 KH\(_2\)PO\(_4\), 5 HEPES, 2 MgSO\(_4\), 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 \(I_{Na}, I_{NaS}, \text{and} I_{K1}\). 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 \(I_{Na}\). The currents derived by subtracting these two protocols are the isolated \(I_{Na}\) values. \(I_{Na}\) amplitude was measured as the peak current amplitude.

To measure \(I_{K1}\), 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 \(\mu\)mol/l Ba\(^{2+}\). Ba\(^{2+}\) blocks \(I_{K1}\), whereas it does not inhibit either \(I_{Na}\) or \(I_{NaS}\) (25, 33). Subtraction of the current evoked in the presence and absence of Ba\(^{2+}\) yields the isolated \(I_{K1}\) current. Values obtained at the end of the 500-ms pulse were used for analysis.

\(I_{NaS}\) 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 \(\mu\)mol/l BaCl\(_2\) was applied to block \(I_{Na}\) and \(I_{K1}\), respectively. \(I_{NaS}\) amplitude was measured as the residual current at the end of the 500-ms pulses.

Whole cell \(I_{Na}\) 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 \(\mu\)mol/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 \(I_{Na}\). \(I_{Na}\) was isolated using techniques outlined in MATERIALS AND METHODS; results are illustrated in Fig. 2D. The effects of tamoxifen at 1, 3, and 10 \(\mu\)mol/l on \(I_{Na}\) were assessed with cumulative...
Concentration-dependent effects of tamoxifen on action potential parameters of isolated rat ventricular myocytes. 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>Vmax, V/s</th>
<th>APD50, ms</th>
<th>APD90, 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; Vmax, maximum upstroke velocity; APD50 and APD90, action potential duration measured at 50 and 90% of repolarization, respectively. *P < 0.05, data significantly different from pretreatment values.

Effects of tamoxifen on IK1 current. Figure 2, A and C, shows representative current traces of K+ currents before and after exposure to 200 μmol/l Ba2+. The Ba2+-sensitive current, IK1, was obtained by subtracting the current in the presence and absence of Ba2+ as shown by Fig. 2E. Superfusion of myocytes with 1, 3, and 10 μM tamoxifen for 10 min had significant concentration-dependent effects on IK1 (Fig. 4). From a control current density of −13.8 ± 1.4 pA/pF, cumulative additions of tamoxifen decreased IK1 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-
tionships for $I_{K_1}$ before and after exposure to tamoxifen are shown in Fig. 4D.

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

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

Effects of tamoxifen on $I_{Na}$. As noted above and shown in Table 1, tamoxifen at concentrations of 1 and 3 $\mu$mol/l significantly decreased the maximum upstroke velocity of the AP in rat ventricular myocytes. This suggests that the $I_{Na}$ responsible for depolarization of the myocytes is also affected by tamoxifen. To...
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

---

**AJP-Heart Circ Physiol** • VOL 285 • AUGUST 2003 • www.ajpheart.org
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 $I_{\text{K1}}$ inhibition. Inhibition of $I_{\text{K1}}$ 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 $\mu$M/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 current channels (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 $I_{\text{to}}$, $I_{\text{K1}}$, 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 $I_{\text{to}}$, $I_{\text{K1}}$, 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_{Na} is inhibited, because the AP upstroke velocity was significantly depressed in the presence of tamoxifen. Also consistent with strong I_{Na} inhibition is our inability to stimulate APs in the presence of 10 umol/l tamoxifen. The effects of tamoxifen on I_{Na} were verified by direct measurement of peak inward I_{Na}. Our results demonstrating that tamoxifen inhibits I_{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

This study was supported by the Heart and Stroke Foundation of Ontario (Grant T4180 to C. A. Ward), the Heart and Stroke Foundation of Alberta, the American Heart Association, and the Canadian Institutes for Health Research. G. J. Kargacin is an Alberta Heritage Foundation for Medical Research Senior Scholar. C. A. Ward is a Heart and Stroke Foundation of Canada Research Scholar.

REFERENCES


