1. Introduction

Tamoxifen is a non steroidal compound that can act as an estrogen receptor agonist or antagonist depending on the target tissues. Tamoxifen is a selective estrogen receptor modulator which is the most widely used for the treatment of breast cancer and for the prevention of tumor for women at high risk (Jordan, 1992; Park and Jordan, 2002; Winer et al., 2002). The pharmacological effects of tamoxifen in breast cancer have been ascribed to the antiestrogenic properties of the drug (Coezy et al., 1982; Katzenellenbogen et al., 1984; Lim et al., 2005). As a result, tamoxifen has been viewed as a pro-drug that needs to be metabolized to produce its effects (Furr and Jordan, 1984). For this reason, 4OH-tamoxifen is more suitable than tamoxifen as a model compound for in vitro studies that examine the effects of tamoxifen.

Even though 4OH-tamoxifen has been shown to block the voltage-regulated chloride channels (Monaghan et al., 1997; Zhang et al., 1994) and to activate BK channels (Dick and Sanders, 2001), to our knowledge, there are no reports on the effects of 4OH-tamoxifen on other ion channel function, specifically on cardiac K⁺ currents. Accordingly, in the work reported here we directly examined the effects of 4OH-tamoxifen on cardiac K⁺ currents. We undertook voltage-clamp studies to compare K⁺ current densities under control conditions and after superfusion of the cells with 4OH-tamoxifen. Experiments were also designed to examine the involvement of estrogen receptor and gene transcription in the response to 4OH-tamoxifen.

2. Material and methods

2.1. Ventricular myocytes isolation

Ventricular myocytes were isolated from adult female CD-1 mice (2–3 months) by enzymatic dissociation as previously described.
(Fiset et al., 1997; Trépanier-Boulay et al., 2001). All experiments were conducted in accordance with the Canadian Council Animal Care guidelines. The animals were heparinized (1 U/kg; i.p.) 20 min prior to sacrifice, anesthetized by inhalation of isoflurane and then killed by cervical dislocation. The heart was rapidly excised and retrogradely perfused on a modified Langendorff apparatus through the aorta (2 ml/min) with the following solutions: (1) 5 min with Tyrode solution containing (in mM): 130 NaCl; 5.4 KCl; 1 CaCl₂; 1 MgCl₂; 0.33 Na₂HPO₄; 10 HEPES, 5.5 glucose (pH adjusted to 7.4 with NaOH); (2) 10 min with Tyrode solution with zero Ca²⁺; (3) 20 min with Tyrode solution containing 0.03 mM Ca²⁺, 20 mM taurine, 0.1% bovine serum albumin (BSA; Fraction V, Sigma Chemicals Co., St. Louis, MO, USA); and (4) 5.5 min with Kraftbrühe (KB) solution containing (in mM) 100 K⁺-glutamate; 10 K⁺-aspartate; 25 KCl; 10 KH₂PO₄, 2 MgSO₄; 20 creatine base; 0.5 EGTA; 5 HEPES; 0.1% BSA, 20 glucose (pH to 7.4 with KOH) (Isenberg and Klockner, 1982). During cell isolation solutions were maintained at 37±1 °C and were equilibrated with 100% O₂. At the end of the perfusion the right ventricular free wall was dissected from the heart and placed in “KB” solution. The ventricular tissue was triturated gently with a Pasteur pipette for 10–15 min to free individual ventricular myocytes. Rod-shape single myocytes were then collected and stored in “KB” solution at 4 °C until use.

2.2. Cellular electrophysiology

Isolated cells were placed in a recording chamber (volume ~200µl) on the stage of an inverted microscope, and continuously superfused with oxygenated HEPES-buffered Tyrode solution at 1–2 ml/min. Whole-cell voltage-clamp recordings were made using the ruptured patch-clamp technique with a patch-clamp amplifier, Axopatch 200 B (Axon Instruments, Foster City, USA). Pipettes were made from borosilicate glass (World Precision Instrument, Sarasota, FL, USA; OD: 1 mm; ID: 0.75 mm; length: 6”) and had resistances in the range of 1.5–4 MΩ when filled with pipette solution. The pipette solution was composed of (mmol/l): 110 K⁺-aspartate, 20 KCl, 8 NaCl, 1 MgCl₂, 1 CaCl₂, 10 BAPTA, 4 K₃ATP and 10 HEPES (pH adjusted to 7.2 KOH). Series resistance in the whole-cell mode was in the range of 4–8 MΩ; 80–90% series resistance compensation was always used. Voltage-clamp currents were low-pass filtered at 1–3 kHz (4-pole Bessel), digitized at 4–10 kHz. The data were recorded using Pclamp 8.0 and analyzed with Clampfit (Axon Instruments, Foster City, USA). Current amplitudes were normalized to the cell capacitance and expressed as pA/pF⁻¹. Capacitive transients elicited by a 10 mV depolarizing step from a holding potential of −80 mV were recorded at 25 Hz (filtered at 10 kHz). Cell capacitance was measured by integrating the surface area of the capacitive transient. A correction of −10 mV was applied to compensate for the patch pipette-bath liquid junction potential (K⁺-aspartate). All experiments were carried out at room temperature (20–22 °C).

2.3. K⁺ currents recordings and analysis

We previously reported a biophysical and pharmacological approach for separating the three main time- and voltage-dependent components of the outward K⁺ current expressed in ventricular myocytes isolated from the heart of adult mice (Brouillette et al., 2004). The Ca²⁺-independent transient outward K⁺ current, Iₖiso, can be separated from total outward current using an “inactivating prepulse” (100 ms at −40 mV before the main depolarizing step). The ultrarapid delayed rectifier K⁺ current, Iₖur, can be isolated using micromolar concentrations of 4-aminopyridine (i.e., 100 µM 4-AP). The remaining 4-AP resistant K⁺ current, or the steady-state outward K⁺ current, Iₖss, can be obtained by combining these two procedures (inactivating Iₖiso and eliminating Iₖur by application of low concentration of 4-AP). We previously confirmed that this approach provides an accurate

![Fig. 1. Separation of K⁺ currents in adult mouse ventricular myocytes.](image-url)
description of densities and kinetic properties of these $K^+$ conductances (Brouillette et al., 2004). However, in a study such as the present one where we need to superfuse voltage-clamped cells with pharmacological agents, the time-course of the experiment are substantially prolonged. To deal with this problem, we applied the same approach as the one described above but instead of 500-ms voltage-clamp steps we used depolarizing steps of 5 s which completely inactivated $I_{Kur}$ without the need to use 4-AP to block this current. These protocols were also validated in our previous report (Brouillette et al., 2004) where we showed that $I_{Kur}$ was very similar whether $I_{Kur}$ was removed either by inactivation (5 s steps), or block by 4-AP.

In summary, current–voltage relationships for the total $K^+$ current ($I_{peak}$) was constructed from the current elicited by a series of 5 s test potentials at 10 mV increment from $-110 \text{ mV}$ to $+50 \text{ mV}$ from a holding potential of $-80 \text{ mV}$ at a frequency rate of 0.1 Hz. This voltage protocol was repeated with a 100-ms depolarizing step to $-40 \text{ mV}$ applied immediately before the 5 s test pulses to voltage inactive $I_{Kur}$ (Fig. 1A). The current obtained with the inactivating prepulse protocol was denoted as $I_{KL ShoK}$, and is composed of $I_{Kur}$ and $I_{KL}$ (Fig. 1B). By subtracting the current traces recorded with and without the inactivating prepulse (e.g., by subtracting $I_{Kur}$ and $I_{KL}$), we obtained $I_{KL}$ (Fig. 1C). With these long voltage protocols, $I_{KL}$ as well as the inward rectifier $K^+$ current ($I_{KL}$) can be directly measured at the end of the 5 s voltage steps (between $-40$ and $+50 \text{ mV}$ for $I_{KL}$ and between $-110 \text{ mV}$ and $-40 \text{ mV}$ for $I_{KL}$). Moreover, $I_{KL}$ and $I_{KL}$ could be obtained either from the voltage protocols including (e.g., $I_{KL ShoK}$) or not (e.g., $I_{KL}$) the $I_{Kur}$ inactivating prepulse. $I_{Kur}$ was obtained by subtracting current traces of $I_{KL}$ from those of $I_{KL ShoK}$. The amplitudes of $I_{peak}$, $I_{Kur}$, and $I_{peak}$ were measured at the peak current amplitude. Fig. 1 illustrates how the different $K^+$ currents present in adult mouse ventricular myocytes were obtained using this approach.

2.4. Drugs

4OH-tamoxifen and actinomycin D were obtained from Sigma. ICI 182,780 (7a,17b-[9-[(4,4,5,5,5-pentafluoreopentyl)sulfinyl]nonyl]estra-1,3,5(10)-triene-3,17-diol) was purchased from Tocris (UK). 4OH-tamoxifen, actinomycin D and ICI 182,780 were dissolved in ethanol as stock solution of 1 mM. Aliquots of the stock solution were stored at $-20 \degree \text{ C}$ until use. 4OH-tamoxifen was used at final concentrations of 0.5, 1 and 10 µM. ICI 182, 780 and actinomycin D final concentration was 10 µM for both compounds. The final concentrations of ethanol (0.01%) had no significant effects on the current traces.

2.5. Statistical analysis

$K^+$ currents were normalized for cell capacitance and expressed as current density (in pA/pF$^{-1}$). Data are reported as mean±standard error (S.E.M). Paired Student’s $t$ test or analyses of variance (ANOVA) were used when appropriate. $P$-values less than 0.05 were considered statistically significant.

3. Result

3.1. Effect of 4OH-tamoxifen on total $K^+$ currents ($I_{peak}$)

We examined the effects of three concentrations of 4OH-tamoxifen (0.5, 1 and 10 µM) on the major $K^+$ currents present in
adult mouse ventricle. Fig. 2A illustrates typical examples of a family of total K\(^+\) currents (\(I_{\text{peak}}\)) obtained in the same voltage-clamped mouse ventricular myocyte before and after 30 min exposure to 10 \(\mu\)M of 4OH-tamoxifen. Fig. 2B summarizes the corresponding current–voltage (\(I\text{–}V\)) relationships of \(I_{\text{peak}}\). At this concentration, 4OH-tamoxifen significantly decreased the density of both the inward and outward portions of \(I_{\text{peak}}\). For instance, the current density of \(I_{K1}\) measured at \(-110\) mV was reduced to \(-11.8\pm1.5\) pA\(\text{pF}^{-1}\) with 10 \(\mu\)M of 4OH-tamoxifen compared to the control values \((-15.7\pm1.1\) pA\(\text{pF}^{-1}\), \(n=12\), \(P<0.05\)). The bar graphs illustrated in Fig. 2C show mean values of the percentage of \(k_{1}\) recorded under control conditions and in the presence of 0.5, 1 and 10 \(\mu\)M of 4OH-tamoxifen. These data show that \(k_{1}\) was also decreased by 1 \(\mu\)M of 4OH-tamoxifen \((-13.5\pm1.2\) pA\(\text{pF}^{-1}\)) compared to control \((-17.2\pm1.4\) pA\(\text{pF}^{-1}\), \(n=10\), \(P<0.05\)), but was not significantly affected by the lower concentration of the drug tested (control: \(-16.9\pm1.7\) pA\(\text{pF}^{-1}\); 0.5 \(\mu\)M 4OH-tamoxifen: \(-15.1\pm2.4\) pA\(\text{pF}^{-1}\); \(n=9\); \(P=\text{N.S.}\)). Data presented in Fig. 2B also shows that 10 \(\mu\)M of 4OH-tamoxifen

![Fig. 3](image-url)  
**Fig. 3.** Effects of 10 \(\mu\)M of 4OH-tamoxifen on \(I_{\text{Kslow}}\). A. Typical examples of \(I_{\text{Kslow}}\) recorded under control conditions (CTL) and after exposure to 10 \(\mu\)M of 4OH-tamoxifen (4OH-tamoxifen) in female ventricular mouse myocytes. B. Mean \(I\text{–}V\) relationships for \(I_{\text{Kslow}}\) obtained before and after addition of 10 \(\mu\)M of 4OH-tamoxifen (*\(P<0.05\)). C. Bar graphs presenting the cumulative data of \(I_{\text{Kslow}}\) obtained at +30 mV before and after application of 0.5 \(n=9\), 1 \(n=10\) and 10 \(\mu\)M \(n=12\) of 4OH-tamoxifen. Results are expressed as percent of control. *\(P<0.05\) compared to CTL.

![Fig. 4](image-url)  
**Fig. 4.** Effects of 10 \(\mu\)M of 4OH-tamoxifen on \(I_{\text{to}}\). A. Superimposed current records illustrating \(I_{\text{to}}\) under control conditions (CTL) and in the presence of 10 \(\mu\)M of 4OH-tamoxifen in ventricular myocytes. Current recordings were obtained by subtracting the corresponding current traces recorded with (Fig. 2A) and without (Fig. 3A) the inactivating prepulse. B. Mean (± S.E.M) \(I\text{–}V\) relationships for \(I_{\text{to}}\) under control conditions and with 10 \(\mu\)M of 4OH-tamoxifen (*\(P<0.05\)). C. Bar graphs presenting the cumulative data of \(I_{\text{to}}\) obtained at +30 mV before and after application of 0.5 \(n=9\), 1 \(n=10\) and 10 \(\mu\)M \(n=12\) of 4OH-tamoxifen. Results are expressed as percent of control (*\(P<0.05\), compared to controls).
significantly decreased the density of the outward portion of the total K\(^+\) current for voltages ranging between −30 and +50 mV (at +30 mV, control: 61.3 ± 5.1 pApF\(^{-1}\); 4OH-tamoxifen: 38.2 ± 4.1 pApF\(^{-1}\), n = 12, P < 0.01). The density of \(I_{\text{peak}}\) measured at +30 mV was reduced when the cells were perfused with 0.5 µM of 4OH-tamoxifen from 63.7 ± 4.4 pApF\(^{-1}\) to 49.4 ± 5.4 pApF\(^{-1}\) (n = 9; P < 0.05). Similarly, 1 µM 4OH-tamoxifen reduced \(I_{\text{peak}}\) from 62.9 ± 7.0 pApF\(^{-1}\) to 45.8 ± 5.5 pApF\(^{-1}\) (n = 10; P < 0.01). Data presented in this figure were obtained from ventricular myocytes isolated from 7 different female mice.

3.2. Effect of 4OH-tamoxifen on \(I_{\text{Kslow}}\)

Fig. 3A shows superimposed current records that correspond to \(I_{\text{Kslow}}\) before and after application of 10 µM 4OH-tamoxifen. \(I_{\text{Kslow}}\) was significantly reduced by the application of 10 µM of 4OH-tamoxifen. This is also illustrated in Fig. 3B which compares the corresponding \(I-V\) plots for \(I_{\text{Kslow}}\) before and after addition of 10 µM of the drug (at +30 mV; control: 34.1 ± 2.6 pApF\(^{-1}\); 4OH-tamoxifen: 21.3 ± 2.1 pApF\(^{-1}\), n = 12, P < 0.05). Fig. 3C shows that 4OH-tamoxifen also inhibited \(I_{\text{Kslow}}\) at lower concentrations. At +30 mV, \(I_{\text{Kslow}}\) was reduced from 31.8 ± 3.9 to 26.1 ± 4.7 pApF\(^{-1}\) (n = 10; P < 0.05) in the presence of 1 µM 4OH-tamoxifen and from 32.7 ± 2.6 to 25.8 ± 1.9 pApF\(^{-1}\) (n = 9; P < 0.01) with 0.5 µM of the drug.

3.3. Effect of 4OH-tamoxifen on \(I_{\text{ss}}\)

Fig. 4 illustrates that short term superfusion of myocytes with 0.5, 1, and 10 µM of 4OH-tamoxifen had significant effects on \(I_{\text{ss}}\). Fig. 4A shows that representative current traces of \(I_{\text{ss}}\) were significantly smaller after exposure to 10 µM 4OH-tamoxifen compared to baseline. Pooled data in Fig. 4B illustrate the \(I-V\) relationships that \(I_{\text{ss}}\) was similar whether \(I_{\text{ss}}\) was obtained from \(I_{\text{peak}}\) or \(I_{\text{Kslow}}\). As previously mentioned in Materials and methods, the current density of \(I_{\text{ss}}\) was similar whether \(I_{\text{ss}}\) was obtained from \(I_{\text{peak}}\) or \(I_{\text{Kslow}}\). The current density of \(I_{\text{ss}}\) was significantly reduced by the application of 10 µM of 4OH-tamoxifen for all its activation range. For instance, there was a 41% reduction in the density of \(I_{\text{ss}}\) when the current was measured at +30 mV (control: 28.8 ± 3.5 pApF\(^{-1}\); 10 µM 4OH-tamoxifen: 16.1 ± 2.6 pApF\(^{-1}\), n = 12, P < 0.05). Fig. 5D summarizes the changes of \(I_{\text{ss}}\) density measured at +30 mV following exposure to 0.5, 1 and 10 µM 4OH-tamoxifen. A reduction of 29% of the mean current density of \(I_{\text{ss}}\) was obtained with 1 µM of 4OH-tamoxifen (at +30 mV, control: 26.2 ± 3.2 pApF\(^{-1}\); 4OH-tamoxifen: 18.9 ± 2.3 pApF\(^{-1}\), n = 10; P < 0.05). At 0.5 µM, 4OH-tamoxifen depressed \(I_{\text{ss}}\) density by 21% (at +30 mV, 4OH-tamoxifen compared to baseline).
control: 27.1 ± 2.3 pA\(\text{pF}^{-1}\); 4OH-tamoxifen: 21.4 ± 2.2 pA\(\text{pF}^{-1}\), \(n = 9, P < 0.05\).

Taken together, these results demonstrate that 4OH-tamoxifen significantly reduced \(I_{\text{K1}}, I_{\text{K2}}\) and \(I_{\text{K3}}\), but had no significant effect on \(I_{\text{K2}}\).

### 3.6. Inactivation kinetics of \(I_{\text{K1}}\) and \(I_{\text{K2}}\)

In the next series of experiments we examined the effects of 10 \(\mu\)M of 4OH-tamoxifen on the voltage dependence of steady-state inactivation as well as on the time-course of recovery from inactivation of both \(I_{\text{K1}}\) and \(I_{\text{K2}}\). The voltage-clamp protocols and the analysis methods used to measure the steady-state inactivation and recovery from inactivation of these currents have been described previously (Brouillette et al., 2004). Fig. 6 summarized the results obtained in these experiments. Fig. 6A compares the steady-state inactivation of \(I_{\text{K1}}\) under control conditions and in the presence of 10 \(\mu\)M 4OH-tamoxifen. The normalized current data were fitted to a Boltzmann function. Neither the half-inactivation potentials (\(V_{1/2}\)) nor the mid-point slope factors (\(k\)) were significantly affected by the application of 4OH-tamoxifen (\(V_{1/2}\): control: \(-54.2 ± 0.7\) mV, 4OH-tamoxifen: \(-57.0 ± 0.5\) mV; \(k\): control: \(5.8 ± 0.6\), 4OH-tamoxifen: \(6.0 ± 0.4\) (\(n = 5, P = \text{N.S.}\)). Fig. 6B shows that the recovery from inactivation of \(I_{\text{K1}}\) was similar between control (27.1 ± 2.3 pA\(\text{pF}\)) and 4OH-tamoxifen treated cells (21.4 ± 2.2 pA\(\text{pF}\)). Moreover, the presence of 4OH-tamoxifen (10 \(\mu\)M) did not affect the current density of \(I_{\text{Kpeak}}\) (\(n = 4, P = \text{N.S.}\)). Fig. 6C shows that the voltage dependence of steady-state inactivation of \(I_{\text{K2}}\) was also comparable between the control (\(V_{1/2}\): \(-55.0 ± 0.8\) mV; \(k\): 7.0 ± 0.7) and 4OH-tamoxifen (\(V_{1/2}\): \(-57.0 ± 1.4\) mV; \(k\): 8.2 ± 1.3, \(n = 4, P = \text{N.S.}\)) groups. Similarly, the recovery from inactivation of \(I_{\text{K2}}\) was also comparable in control (1041 ± 111 ms) and 4OH-tamoxifen (1464 ± 121 ms, \(n = 5, P = \text{N.S.}\)) cells as illustrated in Fig. 6D.

### 3.7. Effects of ICI 182,780 and actinomycin D on \(K^{+}\) currents

4OH-tamoxifen acts as an estrogen receptor partial agonist/antagonist. Thus, to rule out the involvement of the nuclear estrogen receptor and gene transcription in the response obtained with 4OH-tamoxifen, cells were pre-treated for 30 min with the pure estrogen receptor antagonist ICI 182,780 (10 \(\mu\)M) or the gene transcription inhibitor actinomycin D (10 \(\mu\)M). Fig. 7A shows that when administered alone ICI 182,780 did not reduce the density of \(I_{\text{Kpeak}}\). Moreover, the presence of ICI 182,780 did not prevent the inhibitory effects of 4OH-tamoxifen (10 \(\mu\)M) on \(I_{\text{Kpeak}}\), suggesting that the effects of 4OH-tamoxifen on \(K^{+}\) currents were produced by a direct action on the ion channel without involving binding to the intracellular estrogen receptor. Similar findings were also obtained with actinomycin D as illustrated in Fig. 7B. Preincubation of the cells with actinomycin D (10 \(\mu\)M) did not affect the current density of \(I_{\text{Kpeak}}\). Furthermore, 4OH-tamoxifen (10 \(\mu\)M) reduced \(I_{\text{Kpeak}}\) density by approximately 37% whether the drug was administered alone or in the presence of actinomycin D. These data further confirm that the inhibitory effect of 4OH-tamoxifen is due to a direct interaction with the \(K^{+}\) channels without involvement of gene transcription and estrogen receptor.

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**Fig. 6.** Comparison of steady-state inactivation and recovery from inactivation of \(I_{\text{K1}}\) and \(I_{\text{K2}}\) between CTL and 4OH-tamoxifen treated ventricular myocytes. A. Left—voltage dependence of steady-state inactivation of \(I_{\text{K1}}\). The plots on the graph presents Boltzmann functions fitted to mean data recorded under control conditions and in the presence of 10 \(\mu\)M of 4OH-tamoxifen. The voltage dependence of steady-state inactivation of \(I_{\text{K1}}\) was identical in both groups. Data were pooled from 5 cells. Right—recovery from inactivation of \(I_{\text{K1}}\). The smooth lines are best-fit single exponential functions obtained before and after the addition of 10 \(\mu\)M of 4OH-tamoxifen. The recovery from inactivation was similar between 4OH-tamoxifen treated cells and CTL cells. Data were pooled from 4 different cells. B. Left—voltage dependence of steady-state inactivation of \(I_{\text{K2}}\). The plots on the graph present the Boltzmann functions fitted to mean data recorded under control conditions and in the presence of 10 \(\mu\)M of 4OH-tamoxifen. The steady-state inactivation of \(I_{\text{K2}}\) was similar in both groups. Data were pooled from 4 cells. Right—recovery from inactivation of \(I_{\text{K2}}\). This panel shows the result of a voltage-clamp experiment comparing the rate of recovery from inactivation in CTL and 4OH-tamoxifen. The smooth lines are best-fit single exponential functions to mean data obtained from both groups. As shown in this panel, CTL and 4OH-tamoxifen myocytes recovered from inactivation in a similar fashion. Data were pooled from 5 cells.
The selective estrogen receptor modulator, tamoxifen is commonly used for treatment and prevention of breast cancer. It is believed that the effects of tamoxifen on K⁺ channels may be responsible for the QT interval prolongation since experimental studies have reported that acute exposure to tamoxifen can reduce cardiac K⁺ currents. However, in vivo, tamoxifen is extensively metabolized and most of its effects are thought to be related to its major metabolites. Among these metabolites, 4OH-tamoxifen has an affinity for the estrogen receptor that has been reported to be more than 100-fold greater than that of tamoxifen (Coezy et al., 1982; Lim et al., 2005; Ruenitz et al., 1991). Therefore, it is probably not appropriate to directly link a prolongation of QT interval to a reduction of cardiac K⁺ currents produced by acute application of tamoxifen. Accordingly, to determine whether the QT prolongation reported with clinical use of tamoxifen could be associated with an effect of 4OH-tamoxifen on K⁺ currents, we directly examined the effects of 4OH-tamoxifen on ventricular K⁺ currents expressed in mouse heart. Using whole-cell voltage-clamp technique, we recorded K⁺ currents in mouse heart. Interestingly, the inhibition of mouse ventricular K⁺ currents by micromolar concentrations of 4OH-tamoxifen is similar to what has been previously reported with tamoxifen in rat heart. In fact, He et al. have shown that the three K⁺ currents present in rat ventricle (I_{K1}, I_0, and the sustained outward delayed rectifier K⁺ current; I_{Kur}) were significantly depressed by superfusion of 3 and 10 µM of tamoxifen (He et al., 2003). In addition, Liu et al. reported that although tamoxifen (1–10 µM) had little effect on I_0 and I_{K1}, the drug significantly blocked the rapid component of the delayed rectifier K⁺ current (I_{Kur}) in rabbit ventricular myocytes (Liu et al., 1998). Of note, these tamoxifen concentrations fall within the plasma concentration range measured during chemotherapy (0.1–10 µM) (Bergan et al., 1999). Moreover, the amounts of metabolites have been shown to be higher than the parent drug in most tissues (MacCallum et al., 1996, 1997). Furthermore, in humans, levels of tamoxifen metabolites have been reported to be 10- to 60-fold higher in tissues than in serum (Lien et al., 1991). Thus, it is expected that acute exposure to the major active tamoxifen metabolite, 4OH-tamoxifen, blocks ventricular K⁺ currents in mouse heart at pharmacologically relevant concentrations.

Taken together these data suggest that 4OH-tamoxifen also could contribute to explain the QT interval prolongation reported with tamoxifen in some clinical studies (Pollack et al., 1997; Trump et al., 1992).

In addition to the similar effects of tamoxifen and 4OH-tamoxifen on K⁺ currents, it has been shown that micromolar concentrations of both drugs markedly reduced voltage-sensitive Ca⁺⁺ currents in rat (Best, 2002). Tamoxifen and 4OH-tamoxifen have also been shown to be potent inhibitors of the Ca⁺⁺-induced contraction in mice (Diaz, 2002) as well as being able to produce a similar increase in BK current density in Xenopus oocytes (Dick et al., 2001).

We reported that the inhibitory effects of 4OH-tamoxifen are still observed in the presence of the selective estrogen receptor antagonist, ICI 182,780, or the inhibitor of RNA synthesis, actinomycin D. These data indicate that 4OH-tamoxifen directly affects K⁺ currents in mouse ventricle without involvement of the nuclear estrogen receptor and gene transcription. In line with this, previous clinical studies reported that some tamoxifen-induced effects are present in different cell types that lack estrogen receptor (Zhang et al., 1994; Lien et al., 1991), suggesting that some pharmacological effects of tamoxifen that are possibly mediated by 4OH-tamoxifen are nuclear estrogen receptor independent (Report from the Breast Cancer Trials Committee, Scottish Cancer Trials Office (MRC), Edinburgh, 1987; Analysis at six years by Nolvadex Adjuvant Trial Organisation, 1985). Furthermore, similar concentrations of 4OH-tamoxifen have been shown to inhibit chloride channels in different tissues as well as Ca⁺⁺ uptake into sarcoplasmic reticulum vesicles isolated from canine cardiac ventricular tissue through mechanisms unrelated to their interaction with the nuclear estrogen receptor (Zhang et al., 1994; Dick et al., 2001; Kargacin et al., 2000). Then overall, 4OH-tamoxifen produced non genomic effects independent of the nuclear estrogen receptor.

4OH-tamoxifen inhibits the current densities of I_0 and I_{Kur} without affecting the steady-state voltage-dependent inactivation or the time-course of recovery from inactivation of both I_0 and I_{Kur}. These data indicate that the reduction of I_0 and I_{Kur} density is not explained by alterations of their inactivation kinetics properties and appears to be mediated by a direct effect of 4OH-tamoxifen on K⁺ channels. Potential mechanisms could contribute to explain a direct interaction of the drug with the K⁺ channels such as inhibition of the maximal channel conductance or reduction of the opening probability of the channels. Further studies are warranted to explore these mechanisms. Altogether, the data presented in this study indicate that 4OH-tamoxifen reduced K⁺ currents in mouse ventricle through a direct action of the drug on the channels without involvement of the nuclear estrogen receptor and without affecting the inactivation kinetics properties of the currents.

It is well recognized that a reduction of cardiac K⁺ currents is associated with a prolongation of ventricular action potential duration; therefore, in the present study we tried to investigate the
effects of 4OH-tamoxifen on the action potential duration. However, in the presence of the three concentrations of 4OH-tamoxifen examined here, we were unable to elicit action potentials. Interestingly, He et al. reported that tamoxifen reduced Na+ currents in rat ventricular myocytes (He et al., 2003) and they were also unable to record action potentials. These observations suggest that similar to tamoxifen, 4OH-tamoxifen may also inhibit Na+ current. Furthermore, Best reported that 4OH-tamoxifen markedly inhibits voltage-sensitive calcium currents and abolished the generation of action potentials (Best, 2002). These data suggest that 4OH-tamoxifen alters ion current responsible for the generation of the action potential waveform. Thus, in addition to its effects on K+ currents, it is possible that 4OH-tamoxifen also reduced sodium and/or calcium currents. This could explain why in this study we were unable to record action potential in the presence of 4OH-tamoxifen in mouse ventricle.

4.1. Conclusion

Although we clearly showed here that acute exposure to 4OH-tamoxifen depresses ventricular K+ currents, it is not possible to conclude that tamoxifen or 4OH-tamoxifen increases the risk of QT prolongation during chronic treatment with tamoxifen. Additional studies are required in order to clearly determine whether long-term exposure to tamoxifen and its active metabolite, 4OH-tamoxifen, could alter K+ currents, QT interval and increase the risk of ventricular arrhythmias. Overall, findings presented here show that 4OH-tamoxifen reduced K+ currents in mouse ventricle and that this effect is not mediated by a genomic mechanism involving intracellular estrogen receptor. Thus the site of action of 4OH-tamoxifen is probably the K+ channel themselves.

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