Cardiac Ion Channel Effects of Tolterodine


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ABSTRACT

Tolterodine is a muscarinic antagonist widely used in the treatment of urinary incontinence. Although tolterodine has not been reported to alter cardiac repolarization, it is chemically related to other muscarinic antagonists known to prolong cardiac repolarization. For this reason, we studied the effects of tolterodine on cardiac ion channels and action potential recordings. Using patch-clamp electrophysiology, we found that tolterodine was a potent antagonist of the human ether-a-go-go-related gene (HERG) K+ channel, displaying an IC50 value of 17 nM. This potency was similar to that observed for the antiarrhythmic drug dofetilide (IC50 of 11 nM). Tolterodine block of HERG displayed a positive voltage dependence, suggesting an interaction with an activated state. Tolterodine had little effect on the human cardiac Na+ channel at concentrations of up to 1 μM. Inhibition of L-type Ca2+ currents by tolterodine was frequency-dependent with IC50 values measuring 143 and 1084 nM at 1 and 0.1 Hz, respectively. Both tolterodine and dofetilide prolonged action potential duration in single guinea pig myocytes over the concentration range of 3 to 100 nM. However, prolongation was significantly larger for dofetilide compared with tolterodine. Tolterodine seems to be an unusual drug in that it blocks HERG with high affinity, but produces little QT prolongation clinically. Low plasma levels after therapeutic doses combined with mixed ion channel effects, most notably Ca2+ channel blockade, may serve to attenuate the QT prolonging effects of this potent HERG channel antagonist.

Voltage-dependent ion channels control the electrical activity of the heart. During the heartbeat, the influx of Na+ and Ca2+, through their respective channels, serves to depolarize the myocardium, whereas K+ efflux through K+ channels repolarizes the heart. Working in concert these channels give rise to the shape and the duration of the action potential on the cellular level and to the electrocardiogram (ECG) waveform measured clinically. Any alteration in the activity of these channels can lead to changes in the ECG wave form and potentially to the development of cardiac arrhythmia. One such proarrhythmic condition is drug-induced (or acquired) long QT syndrome. In this case, administration of a drug slows cardiac repolarization, resulting in a prolongation of the QT interval on the electrocardiogram. This QT prolongation may be associated with the development of the ventricular arrhythmia known as torsades de pointes (Ben-David and Zipes, 1993). It is now believed that most cases of acquired QT prolongation are due to specific inhibition of the human cardiac K+ channel known as human ether-a-go-go-related gene (HERG) (Pearlstein et al., 2003). The HERG channel carries the rapid component of the delayed rectifier K+ current in the human heart (Ikr) (Sanguinetti et al., 1995). HERG channel inhibition is considered to be the mechanism that underlies the QT prolongation and ventricular arrhythmias associated with the administration of drugs such as astemizole (Zhou et al., 1999), terfenadine (Roy et al., 1996), and cisapride (Mohammad et al., 1997; Rampe et al., 1997).

Patch-clamp electrophysiology studies using cloned HERG K+ channels have become an important means for predicting the potential of a drug to cause QT prolongation in humans. Indeed, drugs that display high-affinity block of HERG channel currents almost invariably demonstrate some degree of QT prolongation in clinical practice (De Ponti et al., 2000; Pearlstein et al., 2003; Redfern et al., 2003). One striking exception to this trend is the drug verapamil. Although verapamil blocks HERG with an IC50 value of 143 nM, acquired long QT syndrome and torsades de pointe arrhythmia are not associated with its use (Zhang et al., 1999). This lack of proarrhythmia is presumably due to a concurrent inhibition of cardiac Ca2+ channels by verapamil, an activity that counteracts the QT prolonging effects of HERG channel inhibition (Zhang et al., 1999). Such mixed ion channel effects may

ABBREVIATIONS: HERG, human ether-a-go-go-related gene; APD90, action potential duration at 90%; APD50, action potential duration at 50%; CL, confidence limits; ANOVA, analysis of variance.
therefore mask clinical QT prolongation and confound the interpretation of HERG channel IC\textsubscript{50} data.

Tolterodine (brand name Detrol) is a muscarinic antagonist used in the treatment of overactive bladder. QT prolongation or torsades de pointe arrhythmia are not associated with tolterodine treatment despite widespread clinical use (Hills et al., 1998; Larsson et al., 1999; Millard et al., 1999; Layton et al., 2001). Other drugs in this chemical and pharmacological class (e.g., terodiline) are known to prolong action potential duration, block cardiac K\textsuperscript{+} channels, and produce QT prolongation and arrhythmia in clinical use (Thomas et al., 1995; Jones et al., 1998). However, no data are currently available regarding the effects of tolterodine on cardiac ion channels. For this reason, we examined the effects of tolterodine on a number of cardiac ion channels including HERG, Na\textsuperscript{+}, and Ca\textsuperscript{2+} channels. We also examined the effects of tolterodine on action potentials measured in single cardiac myocytes and compared its activity with that of the potent antiarrhythmic drug dofetilide.

Materials and Methods

Cell Preparation. Chinese hamster ovary cells (American Type Culture Collection, Manassas, VA) were stably transfomed with the cDNA encoding the HERG cardiac K\textsuperscript{+} channel as described previously (Rampe et al., 1997). Cells were maintained in tissue culture incubators at 37°C in a humidified 95% air, 5% CO\textsubscript{2} atmosphere. Stable transfecants were selected by coexpression of the HERG cDNA and neomycin resistance gene incorporated into the expression plasmid. Cells were cultured in Ham’s F-12 medium containing 10% fetal calf serum and 500 \mu g/ml G418. The cDNA encoding SCNSA, the human cardiac Na\textsuperscript{+} channel, was stably transfected into human embryonic kidney-293 cells (American Type Culture Collection) as described previously (Kuryshyev et al., 2000). Cells were grown in Dulbecco’s modified Eagle’s medium supplemented with 10% fetal calf serum and 500 \mu g/ml G418.

Single ventricular myocytes were isolated from guinea pigs using a method modified from that described by Salata et al. (1995). Briefly, male guinea pigs (Hartley) were anesthetized with 5% of isoflurane (Baxter Healthcare Corp., Deerfield, IL) in a mixture of nitrous oxide and oxygen (1:1). Then, a thoracotomy was performed, and the heart was removed and immediately transfused to oxygenated (100% O\textsubscript{2}) saline. The heart was then perfused retrogradely at 20 mln/min through the aorta with an oxygenated Ca\textsuperscript{2+}-free saline at 37°C in three stages: first with standard Ca\textsuperscript{2+}-free saline for 5 min, second with the same solution containing 280 U/ml type II collagenase (Worthington Biochemical, Lakewood, NJ) plus 0.75 U/ml type XIV protease (Sigma-Aldrich, St. Louis, MO) for 8 min, and finally with saline containing 0.2 mM CaCl\textsubscript{2} for additional 7 min. The left ventricle cut into small pieces was gently shaken at room temperature for about 5 min to disperse single myocytes. The isolated myocytes were then maintained at 10°C for electrophysiological recording, usually within 8 h after isolation.

Patch-Clamp Electrophysiology. All ionic currents were recorded using the whole-cell configuration of the patch-clamp technique (Hamill et al., 1981). Electrodes (2–4 MΩ resistance) were made from TW150F glass capillary tubes (WPI, Sarasota, FL). For HERG channel recordings, electrodes were filled with the following solution: 120 mM potassium aspartate, 20 mM KCl, 4 mM Na\textsubscript{2}ATP, 5 mM HEPES, 1 mM MgCl\textsubscript{2}, pH 7.2 with KOH. For Na\textsuperscript{+} channel recordings, the electrode solution contained 130 mM cesium aspartate, 5 mM MgCl\textsubscript{2}, 2 mM Na\textsubscript{2}ATP, 0.1 mM GTP, 10 mM HEPES, pH 7.2 with NaOH. For Ca\textsuperscript{2+} channel recordings, the electrode solution contained 130 mM cesium methanesulfonate, 20 mM tetraethylammonium chloride, 1 mM MgCl\textsubscript{2}, 10 mM EGTA, 10 mM HEPES, 4 mM Tris-ATP, 0.3 mM Tris-GTP, 14 mM phosphocreatine, 50 U/ml creatine phosphokinase, pH 7.2 with CsOH. The external solution for HERG channel recordings contained the following: 130 mM NaCl, 5 mM KCl, 2.8 mM sodium acetate, 1 mM MgCl\textsubscript{2}, 10 mM HEPES, 10 mM Na\textsubscript{2}ATP, 1.0 mM CaCl\textsubscript{2}, pH 7.4 with NaOH. For Na\textsuperscript{+} channel recordings, the external solution contained 40 mM NaCl, 97 mM N-methyl-d-glucamine aspartate, 5.4 mM KCl, 1.8 mM CaCl\textsubscript{2}, 1 mM MgCl\textsubscript{2}, 5 mM HEPES, 10 mM glucose, pH 7.4 with N-methyl-d-glucamine aspartate. The external solution used for recording Ca\textsuperscript{2+} currents contained the following: 137 mM NaCl, 5.4 mM CsCl, 1.8 mM CaCl\textsubscript{2}, 1 mM MgCl\textsubscript{2}, 10 mM HEPES, 10 mM glucose, pH 7.4 with NaOH. All ionic currents were recorded at room temperature using an Axopatch 200B amplifier (Axon Instruments, Foster City, CA). Currents were analyzed using the pCLAMP suite of software (Axon Instruments). IC\textsubscript{50} values were obtained by nonlinear least-squares fit of the data (GraphPad Software, Inc., San Diego, CA).

Action Potential Recording. Myocytes were placed in a temperature-controlled (37 ± 1°C) chamber and perfused with modified Tyrode’s solution (132 mM NaCl, 4 mM KCl, 1.2 mM MgCl\textsubscript{2}, 1.8 mM CaCl\textsubscript{2}, 10 mM HEPES, 10 pH 7.4). Action potentials were recorded using a standard glass microelectrode filled with 3 M KCl (resistance at 20–45 MΩ). Action potentials were amplified using AxoClamp 2B amplifier (Axon Instruments), and data were stored and analyzed using the pCLAMP suite of software (Axon Instruments). Myocytes were allowed to equilibrate at a stimulation rate of 1 Hz for 30 min after which time action potential traces were recorded. The myocytes were then perfused and allowed to equilibrate for 5 min with ascending concentrations of drugs to generate dose-response relationships. Resting membrane potential, action potential amplitude, action potential duration at 90% (APD\textsubscript{90}) and at 50% (APD\textsubscript{50}) of repolarization were determined for each concentration of drug.

Chemicals. (R)-Tolterodine and dofetilide were synthesized at Aventis Pharmaceuticals (Bridge water, NJ). All other chemicals were obtained from Sigma-Aldrich.

Results

Figure 1 shows the effects of tolterodine on HERG K\textsuperscript{+} channel currents. In these experiments, a 2-s depolarization to +20 mV was followed by repolarization of the cell to −40 mV to produce large, slowly inactivating tail currents characteristic of HERG (Sanguinetti et al., 1995). Figure 1A demonstrates that these tail currents were potently blocked by tolterodine. Block was evident over the concentration range of 3 to 100 nM and the IC\textsubscript{50} value measured 17 nM (15–19 nM, 95% CI; Fig. 1B). Tolterodine had no obvious effect on the wave form of the HERG channel currents under these conditions. The effects of tolterodine on HERG currents were mainly reversible upon washout. After exposure to tolterodine (3–100 nM), currents recovered to within 62 ± 5% of predrug levels (n = 3) upon washing the cells with drug-free solution for approximately 30 min. The affinity of tolterodine for blocking HERG was not significantly different from that observed for the potent antiarrhythmic drug dofetilide. The IC\textsubscript{50} value for dofetilide inhibition of peak HERG tail currents measured 11 nM (8–17 nM, 95% CI; Fig. 1B).

Figure 2 shows the effects of tolterodine on HERG channel currents measured over a wide range of test potentials. In these experiments, cells were held at −80 mV, and currents were elicited by 2-s depolarizing pulses to potential ranging from −40 to +30 mV in 10-mV increments. The membrane potential was then returned to −100 mV, and peak inward tail currents were recorded. Current traces in the absence and presence of 10 nM tolterodine are shown in Fig. 2, A and B, respectively. The resultant current-voltage relationship
averaged from seven cells is presented in Fig. 2C. Tolterodine inhibited tail current amplitude in a voltage-dependent manner. When inhibition of HERG current is plotted as a function of test potential, a statistically significant (p < 0.05; ANOVA) correlation between voltage, and drug effect was observed with inhibition ranging from 5 ± 2% at −10 mV to 33 ± 3% at −30 mV (Fig. 2D).

The effects of tolterodine on the human cardiac Na⁺ channel are illustrated in Fig. 3. Sodium channel currents were elicited by depolarizing pulses to −20 mV from a holding potential of −110 mV. Thirty of these depolarizing steps were delivered at a rate of 1 Hz. Tolterodine was then added to the cells and allowed to equilibrate for 2 to 3 min without pulsing. The pulse train was then repeated several times at approximately 1-min intervals to allow the drug to equilibrate with the cells. Typical Na⁺ channel currents under these conditions with a maximal block of 6 ± 2% observed at 1 μM (Fig. 3B).

We used single ventricular myocytes isolated from guinea pigs to examine the effects of tolterodine on L-type Ca²⁺ channel currents. Ca²⁺ currents were elicited by 200-ms depolarizing pulses to 0 mV from a holding potential of −40 mV. As was the case for the Na⁺ channel, 30 depolarizing steps were delivered at a rate of 1 Hz. Tolterodine was then added to the cells and allowed to equilibrate for 2 to 3 min.
without pulsing. The pulse train was then resumed and repeated several more times at approximately 1-min intervals until the current traces from separate trains overlapped. Typical Ca\(^{2+}\)/H11001 channel current traces in the absence and presence of 30 and 300 nM tolterodine are shown in Fig. 4A. Under these conditions tolterodine inhibited Ca\(^{2+}\) channel currents with an IC\(_{50}\) value of 143 nM (98–208 nM, 95% CL; Fig. 4B). We also examined the effects of tolterodine on Ca\(^{2+}\) channels stimulated at a constant frequency of 0.1 Hz. At this frequency the drug was less potent displaying an IC\(_{50}\) value of 1084 nM (818–1437 nM, 95% CL; Fig. 4B). To further examine this frequency dependence, we stimulated cells in absence of drug for 3 min at a rate of either 0.1 or 1 Hz (Fig. 4, C and D, respectively). After this, cells were allowed to equilibrate with 300 nM tolterodine for 3 min without pulsing. After this equilibration period, the pulse trains were repeated. Tolterodine was more effective at blocking Ca\(^{2+}\) currents at 1 Hz compared with 0.1 Hz. When cells were stimulated at 0.1 Hz, Ca\(^{2+}\) current decreased by 4 \(\pm\) 2% in the absence of drug and by 24 \(\pm\) 6% in the presence of 300 nM tolterodine (Fig. 3C). At a 1-Hz stimulation frequency, Ca\(^{2+}\) current declined by 20 \(\pm\) 3% under drug-free conditions and by 60 \(\pm\) 6% in the presence of tolterodine (Fig. 3D). It seems that the drug block observed in Fig. 3, C and D, did not reach a complete steady state. Therefore, the IC\(_{50}\) values reported in Fig. 3B may underestimate, to some degree, the true affinity of tolterodine for cardiac Ca\(^{2+}\) channels, especially at the 1-Hz stimulation rate.

We next examined the effects of tolterodine on action potentials recorded from guinea pig ventricular myocytes and compared these effects to those observed for dofetilide. Figure 5A shows the effects of tolterodine (3–100 nM) on the action potential waveform. Figure 5B illustrates the effects of dofetilide (3–100 nM) on the action potential waveform. Both drugs increased action potential duration in a dose-dependent manner, but the effects of dofetilide were more pronounced. APD\(_{90}\) was increased by 4 \(\pm\) 1, 8 \(\pm\) 1, 16 \(\pm\) 3, and 28 \(\pm\) 6% in the presence of 3, 10, 30, and 100 nM tolterodine, respectively. These same concentrations of dofetilide prolonged APD\(_{90}\) by 8 \(\pm\) 1, 30 \(\pm\) 6, 53 \(\pm\) 13, and 65 \(\pm\) 17%,
respectively (Fig. 6A). A similar pattern was observed for APD$_{50}$ where 100 nM tolterodine produced a maximal prolongation of 25 ± 6%, whereas dofetilide increased APD$_{50}$ by 53 ± 14% at this same concentration (Fig. 6B). Neither drug displayed any significant effects on the resting membrane potential or the action potential amplitude at the concentrations tested.

**Discussion**

This report is the first to detail the effects of tolterodine on cardiac ion channels. We found that tolterodine was a potent inhibitor of the HERG cardiac potassium channel, displaying an IC$_{50}$ value of 17 nM. Some inhibition of HERG was evident at concentrations as low as 3 nM. The effects of tolterodine on HERG displayed a positive voltage dependence, suggesting that tolterodine interacts with an activated state of the HERG channel. Furthermore, we have found that the affinity of tolterodine for blocking HERG is similar to that of the potent class III antiarrhythmic drug dofetilide. In addition to its effects on HERG, tolterodine was also an effective antagonist of cardiac Ca$^{2+}$ channels. When Ca$^{2+}$ channels were stimulated at 1 Hz, tolterodine displayed an IC$_{50}$ value of 143 nM. At a lower stimulation rate (0.1 Hz), the drug was less potent, suggesting the block of Ca$^{2+}$ channels was frequency dependent. Tolterodine had little effect on voltage-dependent Na$^{+}$ channels even at concentrations up to 1 μM. We believe the data support the notion that tolterodine is a potent mixed ion channel antagonist in the heart with prominent effects on both HERG and the L-type Ca$^{2+}$ channel. In contrast, dofetilide is known to be a specific inhibitor of HERG/I$_{Kr}$ with no effects on Ca$^{2+}$ channels up to 1 μM (Paul et al., 2001). A head-to-head comparison of these two drugs on action potential waveforms revealed that tolterodine produced far less APD prolongation relative to dofetilide. We feel that this may reflect the added Ca$^{2+}$ channel blocking properties of tolterodine and that this activity serves to attenuate the APD lengthening that can be observed from pure I$_{Kr}$ inhibition.

Drugs that block HERG/I$_{Kr}$ with high affinity are often associated with QT prolongation on the electrocardiogram and the development of the ventricular arrhythmia known as torsades de pointes. Indeed, HERG channel affinity is now widely used to both predict and to explain drug-induced QT prolongation and attending ventricular arrhythmia (Pearlstein et al., 2003; Redfern et al., 2003). The affinity of tolterodine for HERG is similar not only to dofetilide but also to cisapride (Mohammad et al., 1997; Rampe et al., 1997), terfenadine (Wang et al., 2003), and pimozide (Kang et al., 2000). All of the aforementioned drugs are well known to produce significant QT interval prolongation and, in some cases, torsades de pointes arrhythmia. However, tolterodine, despite widespread clinical use, has not been reported to produce QT prolongation either in controlled clinical trials or during postmarketing surveillance (Larsson et al., 1999; Millard et al., 1999; Layton et al., 2001; Nilvebrant, 2001). After therapeutic doses, $C_{\text{max}}$ levels of tolterodine in normal subjects average about 12 to 16 nM of which 96.3% is bound to serum proteins (Brynne et al., 1998; Olsson and Szamosi, 2001), resulting in free plasma levels of ~1 nM. It is therefore very possible that these plasma levels of tolterodine are simply not high enough to produce QT-prolonging effects. However, in these same studies, poor metabolizers of the drug
(cytochrome P450 2D6 polymorphism) display average $C_{\text{max}}$ values of 51 to 116 nM. Yet even at these high plasma levels, electrophysiographic studies have reportedly revealed no prolongation of the QT interval (Bryrne et al., 1998). In this patient population, pharmacodynamic factors, specifically block of the cardiac L-type calcium channel, could play a role in limiting QT prolongation. In this respect, we feel that tolterodine may be similar to verapamil. Verapamil blocks HERG with an IC$_{50}$ value of 143 nM, but the drug does not produce long QT syndrome despite the fact that plasma levels can reach the micromolar range (Zhang et al., 1999). This apparent discrepancy is explained by verapamil’s block of cardiac L-type Ca$^{2+}$ channels that occurs over a similar concentration range and acts to blunt the QT prolonging effects that would be expected from HERG channel inhibition alone (Zhang et al., 1999). This study is of great interest because it represents one of the only examples where potent block of the HERG channel in vitro did not translate into clinically significant QT prolongation (i.e., a false positive finding in the HERG assay). We believe that tolterodine joins verapamil in this atypical class of drugs that display high affinity for HERG, but no clearly evident clinical QT prolongation. Like verapamil, it is possible that block of the cardiac L-type Ca$^{2+}$ channel, at least in part, serves to counteract the QT-prolonging effects of HERG channel blockade. Detailed clinical electrophysiographic studies, especially in poor metabolizers of the drug, would be valuable for exploring this possibility.

In summary, we have found tolterodine to be a potent blocker of both the L-type Ca$^{2+}$ channel and the HERG K$^+$ channel in vitro. These activities result in a prolongation of action potential duration, but not to the extent observed with channel in vitro. These activities result in a prolongation of HERG with an IC$_{50}$ value of 143 nM, but the drug does not produce long QT syndrome despite the fact that plasma levels can reach the micromolar range (Zhang et al., 1999). This apparent discrepancy is explained by verapamil’s block of cardiac L-type Ca$^{2+}$ channels that occurs over a similar concentration range and acts to blunt the QT prolonging effects that would be expected from HERG channel inhibition alone (Zhang et al., 1999). This study is of great interest because it represents one of the only examples where potent block of the HERG channel in vitro did not translate into clinically significant QT prolongation (i.e., a false positive finding in the HERG assay). We believe that tolterodine joins verapamil in this atypical class of drugs that display high affinity for HERG, but no clearly evident clinical QT prolongation. Like verapamil, it is possible that block of the cardiac L-type Ca$^{2+}$ channel, at least in part, serves to counteract the QT-prolonging effects of HERG channel blockade. Detailed clinical electrophysiographic studies, especially in poor metabolizers of the drug, would be valuable for exploring this possibility.

In summary, we have found tolterodine to be a potent blocker of both the L-type Ca$^{2+}$ channel and the HERG K$^+$ channel in vitro. These activities result in a prolongation of action potential duration, but not to the extent observed with the pure HERG/I$_{Kr}$ antagonist dofetilide. Tolterodine demonstrates that drugs with very high HERG affinity in vitro do not necessarily produce obvious QT prolongation clinically. However, HERG channel testing is now a widely used safety screen in the drug development process and is considered as an important early predictor of clinical QT prolongation. It is therefore difficult to imagine that drugs like tolterodine, with low nanomolar affinity for HERG, will be developed for human use in the hope that some combination of biological factors will ultimately limit their QT-prolonging potential. Just how many marketed drugs currently share these attributes with tolterodine, and how many will be abandoned in the drug development process, remains unclear.

References


Address correspondence to: Dr. David Rampe, Aventis Pharmaceuticals, Route 202-206, P.O. Box 6800, Room JR2-2236, Bridgewater, NJ 08807-0800. E-mail: david.rampe@aventis.com