The Antipsychotic Agent Sertindole is a High Affinity Antagonist of the Human Cardiac Potassium Channel HERG

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ABSTRACT

Acquired long QT syndrome is a side effect seen with some pharmacological agents, including antipsychotic drugs, and is associated with the development of ventricular arrhythmias. This syndrome is often caused by the blockade of repolarizing potassium channels the human heart. A new antipsychotic agent, sertindole, has been shown to produce QT prolongation after therapeutic doses in humans. We therefore examined the effects of sertindole on two cloned human cardiac potassium channels, the human ether-a-go-go-related gene (HERG) and Kv1.5, stably transfected into mammalian cell lines. Using patch clamp electrophysiology, we found sertindole blocked HERG currents with an IC50 value of 14.0 nM when tail currents at −40 mV were measured after a 2-sec depolarization to +20 mV. When currents were measured at the end of prolonged (20 sec) depolarizing pulses, the IC50 of sertindole measured 2.99 nM. Sertindole enhanced the rate of current decay during these prolonged voltage steps and displayed a positive voltage dependence. Sertindole was approximately 1000-fold less active at blocking Kv1.5 displaying an IC50 value of 2.12 µM. By comparison, the potent class III antiarrhythmic agent dofetilide blocked HERG with an IC50 value of 9.50 nM but did not enhance HERG current decay or block Kv1.5 channel currents. It is concluded that sertindole is a high affinity antagonist of the human cardiac potassium channel HERG and that this blockade underlies the prolongation of QT interval observed with this drug. Furthermore, the sertindole molecule may provide a useful starting point for the development of very high affinity ligands for HERG.

Prolongation of cardiac repolarization is a side effect that can be associated with some drug therapies. This proarrhythmic activity is characterized by a prolongation of the QT interval on the electrocardiogram and is of particular concern because it may lead to the development of the life-threatening ventricular arrhythmia torsades de pointes (Ben-David and Zipes, 1993). One mechanism by which drugs can prolong QT interval is through blockade of one or more repolarizing potassium channel currents in the human myocardium. Advances in cellular electrophysiology and molecular biology have lead to the discovery of a number of Kv channels the human heart and to the cloning of the proteins which subserved them. For example, the human ether-a-go-go-related gene, HERG, is believed to encode the protein which underlies the rapid component of the delayed rectifier K⁺ current IKr (Sanguinetti et al., 1995; Curran et al., 1995). It has recently been shown that native IKr could also be subserved by a heteroeomer complex of HERG and the protein minK, or by one or by more than one isoform of the channel (McDonald et al., 1997; Lees-Miller et al., 1997; London et al., 1997). Mutations in HERG lead to one form of hereditary long QT syndrome (Sanguinetti et al., 1995; Curran et al., 1995). In addition, blockade of IKr by class III antiarrhythmic agents such as dofetilide is thought to cause acquired long QT syndrome (Colatsky and Argentieri, 1994). Another cloned channel, Kv1.5, is believed to underlie the very rapidly activating delayed rectifier current known as IKur (Fedida et al., 1993) and play an important role in repolarizing the human atria (Wang et al., 1993). The association of Kv1.5 with IKur is supported by the biophysical and pharmacological similarities between the currents generated by heterologously expressed Kv1.5 and those ascribed to IKur in human atrial cells. Kv1.5 protein is also found in human ventricular tissue, but its role here has yet to be determined (Mays et al., 1995).

Many antipsychotic agents have been associated with the development of acquired long QT syndrome. Sertindole (Serdolet) is a new indolypiperidine antipsychotic agent which has nanomolar affinities for dopamine D₂, serotonin 5-HT₂ and alpha-1 adrenergic receptors (Zimbroff et al., 1997). Sertindole is available in several European countries and has recently received an approvable letter from the Food and Drug Administration for marketing in the United States. However, doses of sertindole that produced antipsychotic effects in clinical studies (12–24 mg/day) were also associated with significant increases in the corrected QT interval (van Kammen et al., 1996; Zimbroff et al., 1997). Because the

ABBREVIATIONS: HERG, human ether-a-go-go-related gene; Kv1.5, ultra-rapidly activating delayed rectifier K⁺ channel; HEPES, 4-(2-hydroxyethyl)-1-piperazineethanesulfonic acid.
doses of sertindole that produced acquired long QT were rather low, we theorized that the drug could possess high affinity blockade for one or more types of voltage-dependent K+ channels in the human heart. For this reason we examined the effects of sertindole on the most widely characterized form of the cloned human cardiac K+ channel HERG. In addition we also examined the effects of sertindole on the cloned human cardiac K+ channel Kv1.5.

Methods

Molecular biology. The cDNA encoding the HERG potassium channel was subcloned from a human neuroblastoma cell line [American Type Culture Collection, Rockville (ATCC), MD, no. HTB-11] for stable transfection into mouse L cells (ATCC no. CCL-1) as described previously (Rampe et al., 1997). The cDNA encoding the human heart Kv1.5 potassium channel was stably transfected into the human embryonic kidney cell line HEK-293 (ATCC no. CRL-1573) as described previously (Fedida et al., 1995). Cells were grown in Dulbeco's modified Eagle's medium supplemented with 10% fetal bovine serum (GIBCO BRL, Grand Island, NY) in an atmosphere of 95% air/5% CO2. This media also contained penicillin/streptomycin/ fungizone and G418 (0.5 mg/ml, GIBCO BRL).

Electrophysiology. Cells used for electrophysiological recordings were seeded on glass coverslips 24 to 48 hr before use. HERG currents were recorded using the whole-cell patch clamp configuration and Kv1.5 currents were recorded from cell-free inside-out membrane patches (Hamill et al., 1981). Electrodes (2–4 MΩ resistance) were fashioned from TW150F glass capillary tubes (World Precision Instruments, New Haven, CT). For whole-cell recordings electrodes were filled with the following solution (mmol/liter): potassium asparate, 120; KCl, 20; Na2ATP, 4.0; HEPES, 5.0; MgCl2, 1.0; pH 7.2 with KOH. This served as the external solution for the inside-out patch experiments. The external solution for whole-cell recordings contained (mmol/liter): NaCl, 130; KCl, 5.0; sodium acetate, 2.8; MgCl2, 1.0; HEPES, 10; glucose, 10; CaCl2, 1.0; pH 7.4 with NaOH. This served as the internal solution for the inside-out patch recordings. For some HERG current recordings, the external KCl concentration was increased to 20 mM by equimolar substitution for NaCl. Currents were recorded at room temperature using an axopatch 1-B amplifier (Axon Instruments, Burlingame, CA) and were conditioned by 4-pole low-pass filter with a cutoff frequency of between one-quarter to one-half the sampling frequency. Currents were stored on a hard disk and analyzed using a Compaq Deskpro computer and the pCLAMP suite of software (Axon Instruments). The IC50 values for all compounds were obtained by nonlinear least-squares fits of the data (GraphPAD Software, San Diego, CA).

Chemicals. Sertindole was synthesized at Hoechst Marion Rousell (Strasbourg, France). Dofetilide was obtained from Pfizer Central Research (Sandwich, Kent, England). All other compounds were obtained from Sigma Chemical Co. (St. Louis, MO).

Results

Figure 1 shows the effects of sertindole on HERG current. In these experiments, a 2-sec depolarization to +20 mV from a holding potential of −80 mV was followed by repolarization of the cell to −40 mV to produce large, slowly deactivating tail currents characteristic of HERG (Sanguinetti et al., 1993; Roy et al., 1996). Figure 1A shows that these tail currents were potently blocked by sertindole. The IC50 value for sertindole block of peak HERG tail currents under these conditions was 14 nM (12.6–15.9 nM, 95% C.L. fig. 1B). Sertindole had no detectable effect on HERG tail current kinetics. When cells were returned to a potential of −100 mV, inward HERG tail currents decayed with a time constant of 75.7 ± 8.3 msec (n = 5). This value was not significantly altered (P > .05, paired t test) in the presence of 30 nM sertindole and measured 78.4 ± 13.0 msec (n = 5). With this protocol, the amplitude of these tail currents was reduced by 68 ± 7%.

Figure 2 shows the effects of sertindole on HERG during prolonged depolarizing steps. Current was activated by 20-sec depolarizations to +20 mV in the presence of 20 mM extracellular K+ to enhance current amplitude. HERG currents were stable under these conditions decreasing by 7 ± 3% over a 10-min time period (n = 6). To test the effects of sertindole we held the cell at −80 mV without depolarization and allowed various concentrations of the drug to wash in for 3 min. The first pulse after this equilibration period showed no effect on the initial time course of current activation but did reveal a time-dependent block of the current which developed during the depolarizing step (fig. 2A). Single exponential fit of this blocked yielded time constants of 12.2 ± 1.5 sec (n = 7) at a concentration of 10 nM sertindole and 6.9 ±

![Figure 1](https://example.com/fig1.png)

**Fig. 1.** Effects of sertindole on HERG. A, Whole-cell HERG currents in an L cell were elicited by a 2-sec depolarizing pulse to +20 mV from a holding potential of −80 mV. The cell was then returned to −40 mV to generate large outward tail currents. The effects of 10 and 30 nM sertindole on these tail currents is shown. Inset, Molecular structure of sertindole. B, Dose- response relationship for sertindole block of HERG. Peak tail currents at −40 mV after the test pulse to +20 mV were used to generate the dose-response curve shown. The IC50 value and Hill slope measured 14.0 nM and −1.08, respectively. Error bars denote S.E.M. (n = 5–6).

![Figure 2](https://example.com/fig2.png)

**Figure 2.** Effects of sertindole on HERG during prolonged depolarizing steps. Current was activated by 20-sec depolarizations to +20 mV in the presence of 20 mM extracellular K+ to enhance current amplitude. HERG currents were stable under these conditions decreasing by 7 ± 3% over a 10-min time period (n = 6). To test the effects of sertindole we held the cell at −80 mV without depolarization and allowed various concentrations of the drug to wash in for 3 min. The first pulse after this equilibration period showed no effect on the initial time course of current activation but did reveal a time-dependent block of the current which developed during the depolarizing step (fig. 2A). Single exponential fit of this blocked yielded time constants of 12.2 ± 1.5 sec (n = 7) at a concentration of 10 nM sertindole and 6.9 ±
0.7 sec \((n = 5)\) at 30 nM. Subsequent depolarizing pulses delivered at 40-sec intervals showed little or no time dependent component of block suggesting that sertindole had not dissociated appreciably from the channel during this interpulse interval (fig. 2A). Furthermore, the blocking effects of sertindole on HERG were only slightly reversible upon washing the cell with drug-free solution (fig. 2B). Finally, the apparent affinity of sertindole for HERG was enhanced under these conditions relative to those described in figure 1. Significant inhibition of HERG was seen at sertindole concentrations of 1 nM and higher and yielded an IC\(_{50}\) value of 2.99 nM (2.51–3.55 nM, 95% C.L.; fig. 2C).

Figure 3 shows the effects of sertindole on HERG currents measured over a wide range of test potentials. Selected current traces under control conditions, and in the presence of 10 nM sertindole, are shown in figure 3A and B, respectively. The resultant current-voltage (I-V) relationship for this data is presented in figure 3C. Although sertindole inhibited HERG current throughout most of the I-V relationship, greater inhibition was observed at more depolarized potentials. When inhibition of current is plotted as a function of membrane potential (fig. 3D), a significant \((P < .05\) analysis of variation with least significant difference test) positive correlation between voltage and drug effect was observed.
Fig. 3. Effects of sertindole on HERG current-voltage relationship. Currents were elicited by 20-sec test pulses to various potentials from a holding potential of −70 mV. Selected traces in the absence of sertindole and in the presence of 10 nM sertindole are shown in A and B, respectively. Arrows indicate zero current level. C illustrates the entire I-V relationship in the absence (filled circles) and presence (filled triangles) of 10 nM sertindole. Data at the end of the 20-sec pulses were used to generate the I-V plots. The percent inhibition of HERG channel current is plotted as a function of test potential in D. Error bars denote S.E.M (n = 5).

Fig. 4. Effects of sertindole on Kv1.5 currents. A, Kv1.5 current was elicited from an inside-out membrane patch by a 1-sec depolarization to +50 mV from a holding potential of −80 mV. The effects of 1 and 10 μM sertindole are shown. B, Dose-response relationship for sertindole inhibition of Kv1.5 current yielded an IC₅₀ value and Hill slope of 2.12 μM and −0.97, respectively. Error bars denote S.E.M. (n = 5–6).
with inhibition of HERG current ranging from 25 ± 5% at -10 mV to 79 ± 5% at +40 mV.

Figure 4 shows the effects of sertindole on another human cardiac potassium channel, Kv1.5. Sertindole blocked Kv1.5 current recorded from inside-out membrane patches mainly by enhancing the rate of current decay during depolarization (fig. 4A). The time constant for this effect measured 13.1 ± 1.3 msec (n = 5) at a concentration of 10 μM. However, sertindole was far less potent at inhibiting Kv1.5 relative to HERG displaying an IC50 value of 2.12 μM (1.58–3.16 μM, 95% C.L.; fig. 4B).

For comparative purposes we next examined the effects of the potent class III antiarrhythmic agent dofetilide on both HERG and Kv1.5. Under conditions identical to those described for sertindole in figure 2, we found dofetilide to be a potent blocker of HERG currents displaying an IC50 value of 9.50 nM (5.25–15.8 nM, 95% C.L.; fig. 5B). This value is consistent with those reported previously for the class III antiarrhythmic agent dofetilide tested under identical conditions (IC50 = 15.3 nM, Rampe et al., 1997). Under conditions of elevated extracellular K+ (20 mM), prolonged depolarizations to -120 mV resulted in an IC50 value of approximately 3 nM. This value was about 3-fold more potent than we observed for dofetilide. Sertindole could be shown to enhance the rate of HERG current decay during these prolonged pulses. The effects of sertindole were also strongly voltage-dependent with block being enhanced at more positive potentials. Although sertindole also inhibited Kv1.5 channel currents in a time-dependent fashion, it did so at concentrations approximately 1000-fold higher than those required to inhibit HERG. Taken together, these results demonstrate that sertindole is a potent antagonist of HERG and that the drug appears to block an activated state of the channel. These results also show that indolylpiperidines such as sertindole represent a new structural class of molecule.

Discussion

Our report is the first to detail the effects of the new antipsychotic agent sertindole on voltage-dependent K+ channels cloned from human heart. We found that sertindole was a potent inhibitor of HERG channel current displaying an IC50 value of 14 nM when tail currents were measured after 2-sec test depolarizations, but that the compound had no observable effects on kinetics of current deactivation. These results are similar to those described previously for the class III antiarrhythmic agent dofetilide tested under identical conditions (IC50 = 15.3 nM, Rampe et al., 1997). Under conditions of elevated extracellular K+ (20 mM), prolonged depolarizations to -20 mV resulted in an IC50 value of approximately 3 nM. This value was about 3-fold more potent than we observed for dofetilide. Sertindole could be shown to enhance the rate of HERG current decay during these prolonged pulses. The effects of sertindole were also strongly voltage-dependent with block being enhanced at more positive potentials. Although sertindole also inhibited Kv1.5 channel currents in a time-dependent fashion, it did so at concentrations approximately 1000-fold higher than those required to inhibit HERG. Taken together, these results demonstrate that sertindole is a potent antagonist of HERG and that the drug appears to block an activated state of the channel. These results also show that indolylpiperidines such as sertindole represent a new structural class of molecule.
that can block HERG with affinities similar to those reported for methanesulfonanilides such as dofetilide.

Antipsychotic agents have been associated with acquired long QT syndrome. Drugs such as chlorpromazine (Warner et al., 1996) and haloperidol (Lawrence and Nasraway, 1997) have caused QT prolongation and, in some cases, torsades de pointes type arrhythmias under various clinical settings. It is likely that these effects stem from the inhibition of one or more types of voltage-dependent K\(^+\) channels in the human myocardium. Indeed, haloperidol has recently been shown to inhibit HERG channel currents with an IC\(_{50}\) of approximately 1 \(\mu\)M (Suessbrich et al., 1997). The very low doses of sertindole required to produce significant prolongation in the QT interval (12–24 mg/day; van Kammen et al., 1996; Zimbroff et al., 1997) suggests a high affinity interaction with one or more types of K\(^+\) channels in the myocardium. Sertindole is believed to derive its antipsychotic properties through blockade of serotonin 5-HT\(_2\)A, dopamine D\(_2\) and alpha-1 adrenergic receptors that occur with \(K_i\) values ranging from 0.2 to 1.4 nM (Zimbroff et al., 1997). The affinity of sertindole for HERG is therefore very similar to that of these other receptors. Because HERG was originally cloned from human brain (Warmkne and Ganetsky, 1994) it is tempting to suggest that some of the therapeutic (i.e., antipsychotic) effects of sertindole could result from the blockade of HERG-like channels in the brain. This idea remains speculative since no central nervous system functions have thus far been associated with HERG and mutations in the gene are not accompanied by neurological abnormalities (Curran et al., 1995; Titus et al., 1997). Regardless, it is likely that the prolongation of QT interval observed with therapeutic doses of sertindole result from blockade of HERG in the human myocardium. Because sertindole and dofetilide are approximately equipotent at blocking HERG, it is possible that sertindole may share a proarrhythmic risk similar to that described for the methanesulfonanilides (MacNeil, 1997). However, the exact proarhythmic risk of sertindole relative to other classes of drugs awaits further clinical investigation.

In summary, we have described the effects of the new atypical antipsychotic agent sertindole on the human cardiac K\(^+\) channels HERG and Kv1.5. We found sertindole to be a potent antagonist of the HERG channel. The blocking effects of sertindole on HERG were consistent with an interaction of the drug with an activated state of the channel. Due to its high affinity for HERG, the sertindole molecule could serve as a useful starting point for the development of other high affinity antagonists for this channel. Such ligands could serve as useful tools for characterization of the HERG channel. For example, high affinity ligands in this chemical series could be radiolabeled and used for receptor binding studies. Modifications in the structure resulting in ligands that covalently bind to HERG would be useful for the purification of the channel. Such studies may be necessary for determining the molecular makeup of the HERG channel complex in native tissue.

**References**


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