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Block of hERG channel by ziprasidone: Biophysical properties and molecular determinants

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ABSTRACT

Ziprasidone, an antipsychotic agent, delays cardiac repolarization and, thus, prolongs the QT interval of the cardiac ECG. In this study, we examined the biophysical properties and the molecular determinants of the ziprasidone block of wild-type hERG potassium channels stably expressed in HEK-293 cells or wild-type and mutant hERG channels expressed in *Xenopus* oocytes. In stably transfected HEK-293 cells, ziprasidone blocked wild-type hERG current in a voltage- and concentration-dependent manner (IC_{50} = 120 nM, 0 mV, 37 °C). Ziprasidone showed minimal tonic block of hERG current estimated during a depolarizing voltage (−20 or +30 mV) or evaluated by the envelope of tails test (+30 mV). Rate of the block onset was rapid, but not significantly affected by test potentials ranging from −20 to +30 mV (time constant (τ) = 114 ± 14 ms at +30 mV). The time constant of the slow component of hERG current deactivation (at −50 mV) was significantly increased by ziprasidone (τ = 1776 ± 90 versus 1008 ± 71 ms, $P < 0.01$). Time course of channel inactivation was slowed by ziprasidone in a voltage-dependent manner. The $V_{1/2}$ values for steady-state activation and inactivation of hERG channel in HEK-293 cells were not significantly altered by ziprasidone. In *Xenopus* oocytes, ziprasidone exhibited less potent block of wild-type hERG current (IC_{50} = 2.8 μM, 0 mV, 23 °C). Mutation of the aromatic residues (Tyr-652 or Phe-656) located in the S6 domain of hERG dramatically reduced the potency of channel block by ziprasidone (IC_{50} > 0.4 and 1 mM at 0 mV for Y652A and F656A, respectively). In conclusion, ziprasidone preferentially binds to and blocks open hERG channels. Tyr-652 and Phe-656 are two critical residues in the ziprasidone-binding site.

1. Introduction

The human *ether à-go-go*-related gene (hERG) encodes the pore-forming subunits of the rapid delayed rectifier K^+ channels [1,2]. The K^+ current I_{Kr} conducted by the rapidly activating delayed rectifier K^+ channels in cardiac muscle is one of the most important membrane currents responsible for ventricular action potential repolarization. Reduction of I_{Kr} delays repolarization of cardiac muscle and thus results in prolongation of action potential duration (clinically manifested as QT interval prolongation on the body surface electrocardiogram). Delayed ventricular repolarization predisposes affected individuals to Torsades de pointes (a ventricular tachyarrhythmia) that may degenerate into

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ventricular fibrillation and cause sudden death (long QT syndrome, LQTS) [3,4]. Mutations in hERG are one of the major causes of congenital LQTS [3]. More commonly, LQTS is an adverse effect of many different types of drugs, including antiarrhythmics, antihistamines, antibiotics, gastrointestinal prokinetics, and antipsychotics [5,6]. It has been documented that the drug-induced QT prolongation is mainly due to the drug-mediated inhibition of I_{Kr} although these drugs are structurally diverse [5–8].

Because of their potential proarrhythmic effects, a number of non-cardiac drugs have been withdrawn from the market (e.g. terfenadine, cisapride, sertindole, grepafloxacin, thioridazine) or many have been labeled for restricted use (e.g. mesoridazine, ziprasidone, droperidol, astemizol, arsenic trioxide). Therefore, screening compounds for hERG and/or QT interval liability is now routine in the pharmaceutical industry. To facilitate rational design of safer drugs without hERG liability, it is important to understand the biophysical and molecular mechanisms of hERG block by drugs. In studies of the molecular interactions between the hERG channel and its blockers, it has been found that two aromatic residues (Tyr-652 and Phe-656) in the S6 transmembrane domain are particularly crucial in the block of hERG channels by structurally diverse drugs including MK-499, dofetilide, cisapride, terfenadine, vesnarinone, chloroquine, quinidine, and budipine [9–14]. These studies suggest that the two aromatic residues mediate the interaction of hERG channel with drug molecules in the inner cavity probably by π-stacking or π-cation interactions [10,12,13,15]. Findings from these studies provided part of the explanations for why hERG and not other voltage-dependent potassium channels (Kv1–4) are readily blocked by structurally diverse drugs [9,10].

Ziprasidone, an atypical antipsychotic agent, has been shown to induce a moderate QT interval prolongation at therapeutic doses due to its ability to block hERG currents [16–18]. However, biophysical properties and molecular determinants for ziprasidone block of the hERG channel have not been reported. We hypothesized that ziprasidone could interact with two crucial S6 residues (Tyr-652 and Phe-656) by π-stacking or π-cation interactions based on the fact that ziprasidone has multiple phenyl rings and more than one basic nitrogen. In the present study, therefore, we examined the biophysical properties and the molecular determinants of the ziprasidone block of wild-type hERG potassium channels stably expressed in HEK-293 cells or wild-type and mutant hERG channels expressed in *Xenopus* oocytes.

2. Materials and Methods

2.1. Culture of HEK-293 cells

The stable transfection of HEK-293 cells with wild-type (WT) hERG cDNA has been previously described [19]. Transfected HEK-293 cells were maintained at 37 °C in Minimal Eagle Medium (MEM) supplemented with 10% fetal bovine serum, 1% penicillin–streptomycin, 2 mM L-glutamine, 0.1 mM non-essential amino acids, 1 mM sodium pyruvate, and 0.2 mg/ml geneticin (Invitrogen Corporation, Carlsbad, CA, USA). Cells were passaged weekly and were not allowed to become more than 80% confluent. For electrophysiological recordings, the cells were harvested from the culture dish by trypsinization, washed twice with standard MEM, and maintained in culture medium at room temperature for later use on the same day [20].

2.2. Electrophysiology

HERG potassium current in HEK-293 cells was recorded at 37 °C using a whole-cell patch clamp technique (Axopatch 200A amplifier). Cells were voltage-clamped with single suction pipettes (initial resistances of 1.8–3.4 MΩ) made from borosilicate glass capillary tubing. Series resistance compensation (75–80%) was used in all experiments. Currents were filtered at 5 kHz and digitized at 10 kHz. PCLAMP 8 software (Axon instruments, Union City, CA) was used to generate voltage clamp protocols and acquire data [20].

Isolation of *Xenopus laevis* oocytes and cRNA injection were performed as described previously [21]. Standard two-microelectrode voltage clamp techniques were used to record hERG potassium currents in oocytes with a GeneClamp 500 amplifier (Axon Instruments, Union City, CA). Currents in oocytes were recorded at room temperature 2–3 days after injection of WT or mutant hERG cRNA [21]. Two hERG channel mutants (Y652A and F656A) were chosen for study based on previous finding that these mutations decreased the potency of channel block by MK-499, terfenadine, and cisapride [10].

2.3. Solutions and drugs

For voltage clamp experiments, HEK-293 cells were superfused with a HEPES-buffered Tyrode solution containing (in mM): 140 NaCl, 5 KCl, 1 $MgCl_2$, 2 $CaCl_2$, 5 glucose, 20 HEPES (pH 7.4, adjusted with NaOH). The pipette solution for HEK-293 cells contained (in mM): 125 K aspartate, 20 KCl, 10 EGTA, 1 $MgCl_2$, 5 MgATP, 5 HEPES (pH 7.3, adjusted with KOH) [20]. Oocytes were bathed in a solution containing (in mM): 96 NaMES [2-(morpholino) ethane sulfonic acid], 2 KMES, 2 $CaMES_2$, 5 HEPES, and 1 $MgCl_2$ (pH 7.6 with methane sulfonic acid) [21]. Ziprasidone was prepared as a stock solution in DMSO and then diluted as desired with the HEPES-buffered Tyrode solution. The maximum DMSO concentration in the final perfusate was less than 0.1%.

2.4. Data analysis

Recordings were digitized online with a DigiData 1200 interface (Axon Instruments, Inc.) and stored on a computer hard disk or a local-area network driver. The digitized data were analyzed with pCLAMP8 (Axon Instruments, Inc.) and ORIGIN software (Origin Lab Corporation, Northampton, MA, USA). The concentration–response curves were fitted with a logistic dose–response equation to obtain IC_{50} values, the concentration at which hERG current is inhibited by 50%. Results were expressed as means ± S.E.M. Statistical differences were evaluated by unpaired or paired *t*-tests and were considered significant at $P < 0.05$.

3. Results

3.1. Concentration- and voltage-dependence of WT hERG channel block by ziprasidone in HEK-293 cells

To define the concentration dependence of ziprasidone block of hERG current in HEK-293 cells at 37 °C, we examined the effect of ziprasidone on the tail currents elicited upon repolarization to −50 mV after a 3 s activating step to 0 mV from a holding potential of −80 mV. Each cell was exposed to only one ziprasidone concentration. After control recording (Ctrl pulse), the cell was superfused with ziprasidone while repetitive pulsing was applied once every 15 s. As shown in Fig. 1A, steady-state block was reached within 150 s (i.e. at the 10th pulse). The effects of different ziprasidone concentrations are summarized in Fig. 1B. Ziprasidone blocked the tail current at −50 mV in a dose-dependent manner with an IC_{50} value of 120 nM. To examine whether the steady-state block is influenced by the frequency of the pulses, we compared the extent of tail current block by ziprasidone (100 nM) at two different pulse frequencies (once every 15 or 10 s). Under our experimental conditions, the extent of block was not significantly affected by the pulse rates tested; when the pulsing interval was reduced from 15 to 10 s, the tail current block was essentially unaffected ($39.3 \pm 4.1\%$ versus $43.3 \pm 4.0\%$, $P > 0.5$, $n = 6$).

It should be noted that the rate of the current decay during the early part of the depolarizing step to 0 mV is much faster in the presence than in the absence of ziprasidone (Fig. 1A). This phenomenon could be caused by either ziprasidone blocking open channels or ziprasidone facilitating channel inactivation. These issues are further explored below.

To examine the effects of ziprasidone on the current–voltage relationship of hERG current and the voltage-dependence of ziprasidone block, hERG current was activated by application of depolarizing voltage steps (−60 to +40 mV, 3 s) using 10 mV increments from a holding potential of −80 mV. Following each depolarizing step, the membrane potential was repolarized to −50 mV to elicit outward tail current. Representative hERG current traces for control conditions are shown in Fig. 2A. Traces for steady-state block of hERG current by ziprasidone (0.5 μM) are shown in Fig. 2B. Consistent with data presented in Fig. 1, both activating current measured at the end of the depolarizing step (I_{step}) and peak tail current amplitude (I_{tail}) measured following the step to −50 mV were dramatically reduced by ziprasidone (Fig. 2). The current–voltage plot for tail currents (Ctrl I_{tail} and Zip I_{tail} at −50 mV) were fitted with a Boltzmann function to obtain the mid-point activation voltage ($V_{1/2}$) and slope factor (Fig. 2C). Neither $V_{1/2}$ (-32.6 ± 1.3 versus -32.3 ± 0.7 mV, $n = 7$) nor slope factor (4.9 ± 0.2 versus 5.1 ± 0.2 mV, $n = 7$) was altered by ziprasidone, indicating that ziprasidone had no effect on the current–voltage relationship of hERG K^+ current. The voltage dependence of hERG tail current block by ziprasidone was shown in Fig. 2D, where relative tail current represents the ratio of the peak tail currents measured in the presence and absence of ziprasidone. Blockade of hERG current by ziprasidone increased significantly over the voltage range where hERG channels activated and saturated at voltages where hERG activation was maximal. These results are consistent with open channel blockade.

3.2. Time-dependent block of WT hERG current by ziprasidone in HEK-293 cells

To further examine whether ziprasidone is an open-channel blocker, we evaluated the time course of block development (time-dependent block) using two protocols. First, we examined the onset of ziprasidone block by analyzing in greater detail the change in current during depolarizing pulses (Fig. 3A). HERG current was activated by applying depolarizing voltage pulses (−20 to +30 mV, 3 s) from a holding potential of

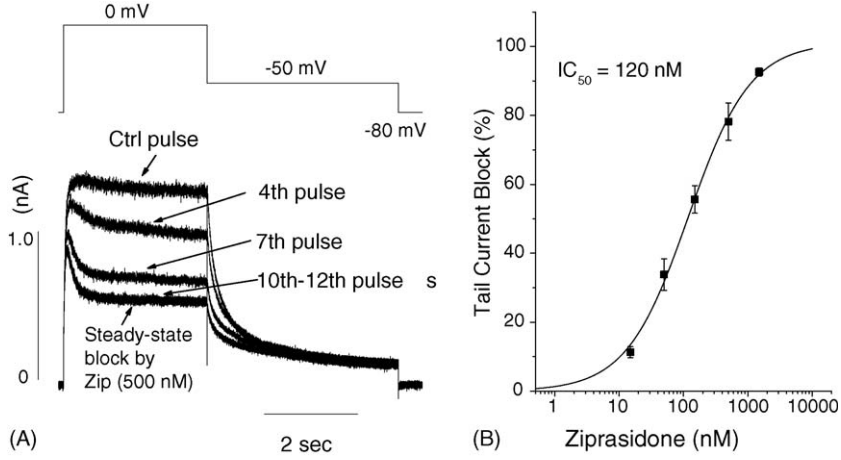

Fig. 1 – Concentration-dependence of ziprasidone block of hERG tail current in HEK-293 cells. Panel A, hERG activating and outward tail currents obtained in the absence and presence of 0.5 μM ziprasidone (Zip). HERG current was activated by stepping to 0 mV and outward tail current was recorded at a repolarization step of −50 mV. Repetitive pulsing was applied once every 15 s during superfusion with ziprasidone. Panel B, concentration–response curve fitted with a logistic dose–response equation. The IC_{50} was 120 nM and the fitting coefficient was 0.903. Data were presented as mean ± S.E.M. (n = 4–8 for each concentration).

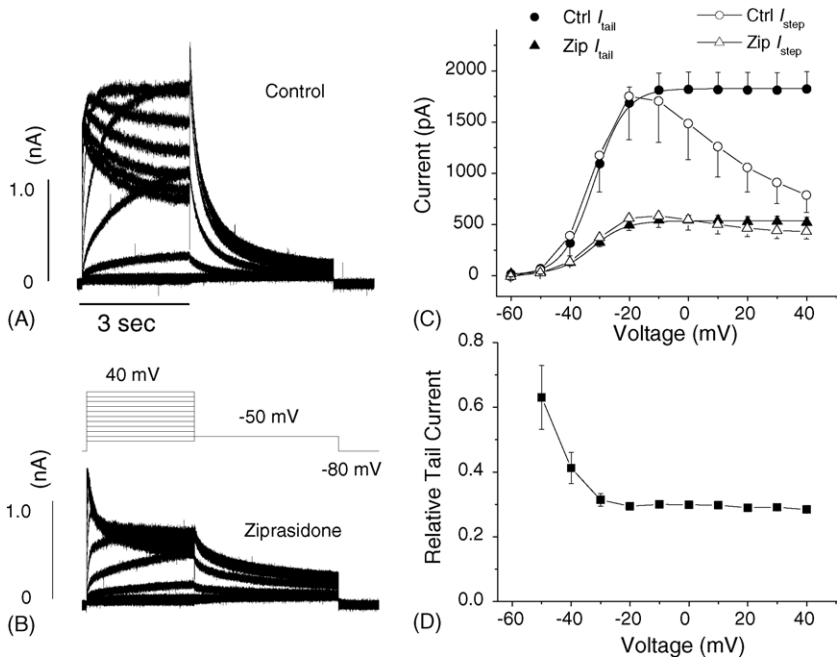

Fig. 2 – Effect of ziprasidone on current–voltage relationship of hERG K^+ current in HEK-293 cells. HERG current was activated by applying voltage pulses for 3 s from −60 to +40 mV (10 mV increments) with a holding potential of −80 mV. Outward tail current was recorded upon repolarization to −50 mV for 4 s. Panel A, representative traces showing the baseline currents (control). Panel B, representative traces showing the currents in the same cell after exposure to 0.5 μM ziprasidone (Zip). Panel C, current–voltage plots for the activating currents measured at the end of depolarizing voltage steps (open symbols) and peak outward tail currents (closed symbols). The current–voltage plot for peak tail current (activation curve) was fitted with a Boltzmann function. Panel D, voltage dependence of tail current blockade. Relative tail current is the ratio of tail currents measured in the presence and absence of ziprasidone. Data were expressed as mean ± S.E.M. (n = 7).

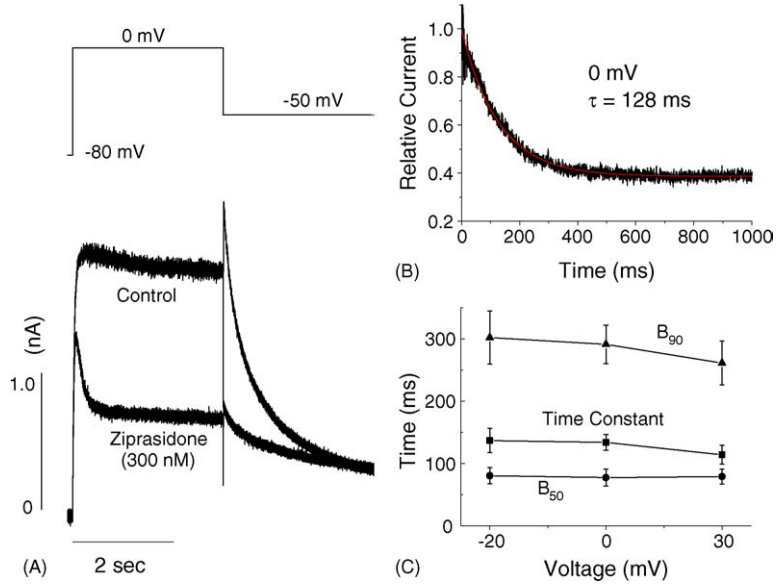

Fig. 3 – Onset of hERG K^+ current block by ziprasidone assessed during a depolarizing voltage step. Panel A, representative recordings of hERG currents elicited by a 3 s pulse to 0 mV before and after 300 nM ziprasidone. Portion of the tail currents recorded at −50 mV is also shown. Panel B, representative onset of ziprasidone block shown by the time course of decay of the relative activating current, which is the ratio of activating current (shown in panel A) recorded before and after exposure to ziprasidone. Panel C, kinetic parameters describing the onset of hERG current block at different test potentials. The time constant was determined by a single exponential fit of the current ratio (see panel B). B_{50} and B_{90} are the times at which current block reached 50 and 90% of the steady-state block, respectively. Data were expressed as mean ± S.E.M. (n = 6–7).

−80 mV. After control recordings, the membrane potential of the same cell was held at −80 mV to maintain hERG channels in the closed state, and the cell was perfused with 0.3 μM ziprasidone for 3 min. The same depolarizing pulse was then repeated in the presence of ziprasidone. Each cell was used to test one depolarizing voltage. Relative activating current plotted as a function of time during the depolarizing pulse was used to estimate initial block and the rate of block development (Fig. 3B). The relative activating current was the ratio of the activating current measured after and before ziprasidone. The development of ziprasidone block was rapid, with 50% of final block (B_{50}) achieved within 80 ms at all voltages tested and 90% of block (B_{90}) achieved within 302 ms (Fig. 3C). The time course of the relative activating current was well fitted to a single exponential function (Fig. 3B), with a mean time constant (τ) of 114 ± 14 ms at +30 mV (Fig. 3C). There was no voltage-dependency for all time parameters (B_{50}, B_{90}, and τ). In the presence of ziprasidone, the relative activating current at 0 mV was 96 ± 2% at zero time (extrapolated by exponential fitting). The minimal tonic block (initial block at zero time of the depolarization pulse) is a strong argument for open-channel block.

The time course for the development of ziprasidone block of hERG channels was also assessed using an envelope of tails test [11,20]. Cells were held at a holding potential of −80 mV and pulsed to +30 mV for a variable duration from 10 to 400 ms in a 20 ms increment. HERG tail currents were recorded upon repolarization to −100 mV (data not shown). The onset of the hERG block by 0.3 μM ziprasidone developed rapidly with a time constant of 95 ± 21 ms at +30 mV. The tonic block of hERG channel by ziprasidone is minimal (2.2%). Results obtained from the envelope of tails test at +30 mV indicated that ziprasidone did not bind to hERG channels in the rested state and activation was required in order for ziprasidone to block hERG channels.

3.3. *Effects of ziprasidone on inactivation of WT hERG channels in HEK-293 cells*

Two different protocols were employed to examine the effects of ziprasidone on the inactivation of hERG channels. The voltage dependence of steady-state inactivation was assessed using a protocol described previously [20]. After a 1 s pulse to +20 mV, the membrane potential was clamped to various test voltages for 20 ms to allow inactivation to relax to a steady-state (Fig. 4A). The 20 ms test pulse was followed by a return step to +20 mV. Peak current elicited by the second step to +20 mV provides a relative number of open conducting channels. At negative potentials, the current declined because channel closing occurred through deactivation. Thus, peak current amplitudes were corrected for channel closing and then plotted as a function of the voltage of the 20 ms test pulses (steady-state inactivation curve) and fitted to a Boltzmann function (Fig. 4B). Peak current amplitudes were prominently reduced by ziprasidone (0.3 μM). However, $V_{1/2}$ (−67.3 ± 5.0 versus −68.8 ± 3.2 mV, $n = 7$) and slope factors (25.4 ± 1.6 versus 25.1 ± 2.2 mV, $n = 7$) for the steady-state inactivation were not significantly altered by ziprasidone, indicating that the voltage-dependence of steady-state inactivation of hERG channels was not modified by ziprasidone.

A three-pulse protocol (Fig. 5A and B) was used to assess the time course of hERG channel inactivation [19,20]. Following a 500 ms pulse to +60 mV to allow the current to fully activate and inactivate, a 2 ms pulse was applied to −100 mV to remove inactivation without allowing deactivation to occur. Test pulses to potentials ranging from −20 to +60 mV were then applied to elicit channel inactivation. Time constants of inactivation were determined by a single exponential fitting of the inactivating currents. As shown in Fig. 5C, the inactivation time constants were significantly increased by ziprasidone at voltages equal or positive to 0 mV. The relative

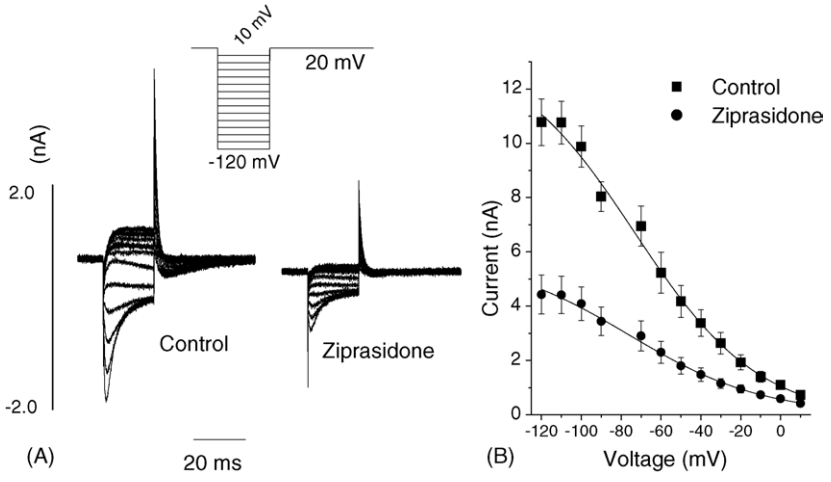

Fig. 4 – Effect of ziprasidone on voltage dependence of steady-state inactivation of hERG channel in HEK-293 cells. Panel A, representative recordings showing the effect of ziprasidone (300 nM) on the steady-state inactivation of hERG channels. Currents were elicited with a steady-state inactivation protocol (see inset) in which inactivation was allowed to relax to steady-state during 20 ms test pulses to potential ranging from −120 to +10 mV. Panel B, steady-state inactivation curves. Peak outward currents (from left panel) elicited by the second step to 20 mV were corrected for closing and then plotted as a function of the preceding test pulse potentials. The inactivation curve was fitted with a Boltzmann function. The $V_{1/2}$ and slope factor were not significantly altered by ziprasidone. Data were expressed as mean ± S.E.M. ($n = 7$).

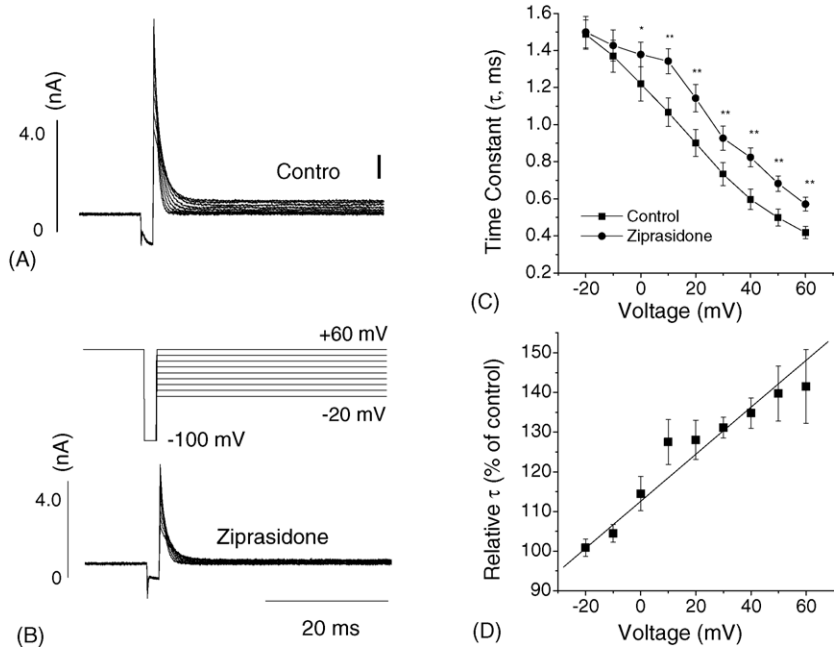

Fig. 5 – Effect of ziprasidone on hERG channel inactivation kinetics in HEK-293 cells. Panels A and B, representative traces showing the effect of ziprasidone on the inactivating rate of hERG channel currents. A three-pulse protocol was used to assess the inactivation (see inset in panel B). Panel C, inactivation time constant plotted as a function of test voltages. The time constants were determined by fitting the inactivating currents (panels A and B) to a single exponential function. At voltages equal or positive to 0 mV, the time constants were significantly increased by ziprasidone ($^{*}P < 0.05$; $^{**}P < 0.01$). The relative increase in time constants was voltage dependent (panel D). The relative increase was calculated as the ratio of the τ values measured in the presence and absence of ziprasidone. Data were expressed as mean ± S.E.M. ($n = 7$).

increase in time constants was voltage dependent (Fig. 5D). The relative increase was calculated as the ratio of the τ values measured in the presence and absence of ziprasidone.

3.4. *Effect of ziprasidone on WT hERG channel deactivation kinetics in HEK-293 cells*

Outward tail currents were recorded during a repolarization pulse to −50 mV following a depolarization step to 0 mV (Fig. 6). The time course of the deactivating tail currents was fitted with a double exponential function. The slow component of the deactivating tail currents was significantly slower in the presence of ziprasidone at 0.3 μM (Fig. 6). The slowed deactivation in the presence of ziprasidone suggested that closure of the activation gate is delayed when drug is bound to the channel. This is similar to the so-called ''foot in the door'' effect, which has been described for other drugs [12,20].

3.5. *Ziprasidone block of WT and mutant hERG channels expressed in oocytes*

Previous studies have shown that two aromatic residues, Tyr-652 and Phe-656 located in S6 domain and facing the pore cavity of the channel, are important components of the binding site for a number of compounds [9–13]. To determine whether these key residues are also important for hERG block by ziprasidone, we compared the potency of channel block for wild-type and two mutant hERG channels (Y652A and F656A). Currents were measured in the presence of 2 mM extracellular K^+ (for WT and Y652A channels) or 96 mM extracellular K^+ (for F656A mutant channel). The effect of ziprasidone on WT and Y652A was quantified during a 5 or 2.5 s activating pulses to 0 mV from a holding potential of −90 mV. Because F656A hERG expressed poorly in oocytes, the effect of drug on this mutant channel was quantified during pulses to −140 mV after the 2.5 s activating pulses. As shown in Fig. 7, wild type hERG channel current in oocytes was inhibited by ziprasidone in a concentration-dependent manner (IC_{50} = 2.8 μM, 0 mV, 23 °C). The potency of channel block by ziprasidone was dramatically reduced in two hERG mutant channels (IC_{50} was increased by >140 and 357-fold at 0 mV for Y652A and F656A, respectively). These results indicate that Tyr-652 and Phe-656 are critical for the interaction between ziprasidone and hERG channel.

4. Discussion

Ziprasidone is a new atypical antipsychotic recently marketed in the United States and several European countries. Its main advantage over other atypical and typical drugs is its low propensity for causing weight gain. However, ziprasidone has been shown to cause moderate prolongation of corrected cardiac QT (QTc) interval, a property shared by other antipsychotics [16]. The QTc prolongation observed clinically is well supported by preclinical studies in which ziprasidone has been shown to block hERG current and prolong action

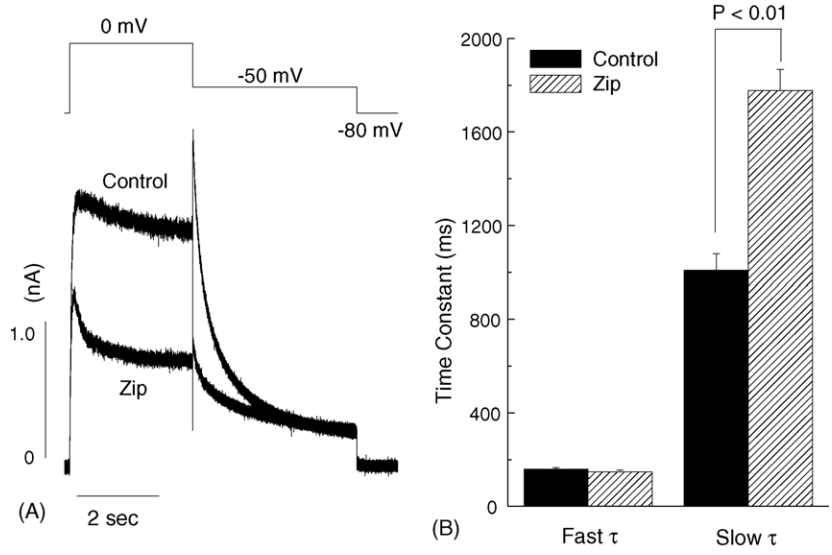

Fig. 6 – Ziprasidone alters hERG tail current kinetics in HEK-293 cells. Panel A, tail currents recorded at −50 mV following a depolarizing step to 0 mV were fitted with a double exponential function. Panel B, pooled data showing that the slow component of tail current deactivation was significantly slower in the presence of ziprasidone (Zip). Data were expressed as mean ± S.E.M. (n = 7).

potential duration of rabbit Purkinje fiber in a concentration-dependent manner [17,18, this study]. The IC_{50} value (0.12 μM) for hERG block observed in our study in HEK-293 cells at 37 °C is very close to the peak serum concentration (0.1 μM or 45 ng/ml) of ziprasidone in patients administered the lowest oral therapeutic dose of 20 mg [23]. The IC_{50} values reported by Kongsamut et al. (0.17 μM in CHO cells at room temperature) and Ducrop et al. (0.24 μM in HEK-293 cells at 20 °C) are slightly

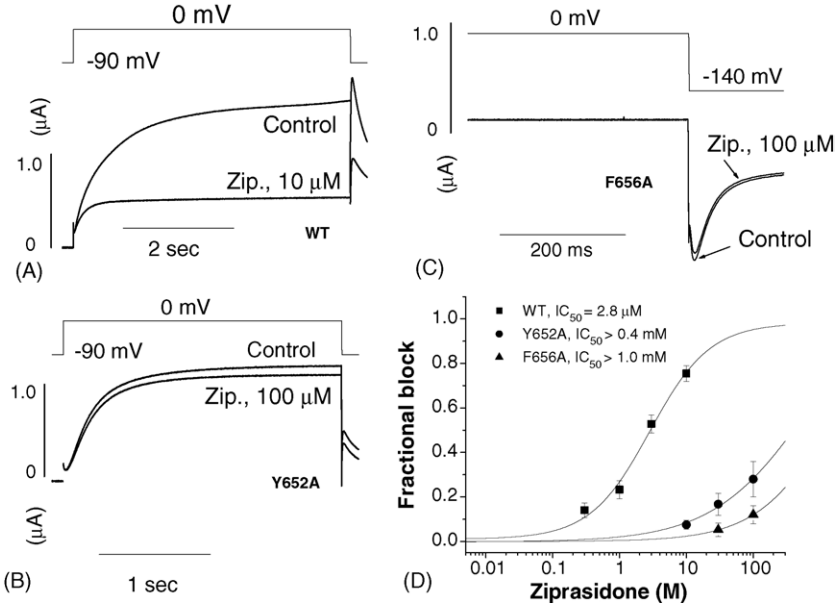

Fig. 7 – Concentration-dependent block of WT and mutant hERG channels expressed in oocytes. Panels A–C, representative traces for WT and mutant hERG channel currents in the presence and absence of indicated concentrations of ziprasidone (Zip). Currents were measured in the presence of 2 mM extracellular K^+ (for WT and Y652A channels) or 96 mM extracellular K^+ (for F656A mutant channel). The effect of the drug on WT and Y652A was quantified during a 5 or 2.5 s activating pulses to 0 mV (respectively) from a holding potential of −90 mV. Because F656A hERG expressed poorly in oocytes, the effect of drug on this mutant channel was quantified during pulses to −140 mV after 2.5 s activating pulses. The concentration–response curves were fitted with a logistic dose–response equation to obtain the IC_{50} values (panel D). Data were expressed as mean ± S.E.M. (n = 5–8). The potency of ziprasidone block was dramatically reduced in the mutant channels.

higher than that observed in our study. This difference could be caused by the different experimental conditions under which the concentration-dependent effect of ziprasidone was examined. In regard to experimental conditions, temperature could be the major factor contributing to the observed difference in IC_{50} values, because temperature has been shown to influence the potency of other hERG channel blockers [24]. It should be noted that the IC_{50} value for WT hERG block observed in oocytes are 11–28 fold higher than that observed in mammalian cells [Figs. 1 and 7 of this study, 17,18]. The reduced potency of drug block in oocytes compared with mammalian cells is a common finding and may be related to the lipophilic yolk sac in oocytes that acts to sequester the drug [5,11].

In addition to the important confirmatory observation on the concentration-dependent blockade of hERG channel currents, this study also addressed the biophysical properties and the molecular mechanism of the ziprasidone block, which were not studied in the two previous reports by Kongsamut et al. [17] and Ducrop et al. [18]. The current study demonstrates that ziprasidone preferentially blocks open hERG channels, exhibiting several features that are typical of other open-channel blocker, such as dofetilide, cisapride, vesnarinone, ambasilide, chloroquine, cocaine, and mesoridazine [11,12,20,22,25–27]. First, tonic block of hERG current by ziprasidone was minimal (Fig. 3), suggesting that ziprasidone does not block hERG channels in the rested states. Second, block increased significantly over the voltage range (negative to −20 mV) where hERG channel activates and became saturated at voltages positive to −20 mV (eliciting maximal hERG channel activation, Fig. 2). These results suggest that channel activation is required for ziprasidone block, and channel inactivation has little effect on drug affinity. Third, block development in response to a depolarizing pulse was highly pulse duration-dependent (Fig. 3). Fourth, the apparent rate of hERG channel deactivation was significantly slowed, which is consistent with the so-called ''foot in the door'' effect.

In defining the biophysical properties of the ziprasidone block of hERG channel expressed in HEK-293 cells, we also found that in the presence of ziprasidone, the mid-point voltages and slope factors were not altered either in the steady-state activation curve or the steady-state inactivation curve, indicating that ziprasidone did not alter the voltage-dependence of either activation or inactivation gating of the hERG channel.

To examine whether key molecular determinants of hERG blockade for previously investigated drugs are also important for hERG channel block by ziprasidone, we compared the potency of channel block for WT and two mutant hERG channels (Y652A and F656A) expressed in oocytes. Ziprasidone blocked WT hERG current in a concentration-dependent manner with an IC_{50} value of 2.8 μM at 0 mV and room temperature (Fig. 7). The potency of channel block by ziprasidone was reduced by over 100-fold in these mutant hERG channels ($IC_{50} > 0.4$ and 1 mM at 0 mV for Y652A and F656A, respectively). These results indicate that Tyr-652 and Phe-656 are critical for ziprasidone-induced block of the hERG channel. Our results are consistent with previously reported studies, in which all tested hERG-blocking drugs (except fluvoxamine) have been shown to block the mutant hERG channel (F656A) with potency being reduced by over 100-fold compared to their blockade of the WT hERG channel. Similarly, mutation of Tyr-652 to alanine also leads to dramatic attenuation of hERG blockade by most studied drugs except vesnarinone and fluvoxamine [9–14,28]. The lack of profound attenuation of fluvoxamine's blockade of hERG channel by mutation of F-656 is unique among all drugs tested so far, indicating that fluvoxamine may have a distinct binding site outside the pore region [28]. To the best of our knowledge, ziprasidone is the first antipsychotic drug that has been investigated in studies that are designed to examine the importance of the molecular interactions between the key S6 residues and drug molecules. A large number of drugs have been shown to block hERG channel current [5,6]. So far, however, the hERG channel-binding site has only been investigated for a small number of drugs [9–14,28, this study]. More data (well-characterized hERG blockers) are needed to implement the database, which can be used to generate a pharmacophore model [29]. The pharmacophore model may be useful in the presynthetic virtual screening of discovery compounds for hERG activity. Taken together, all studies dealing with the biophysical and molecular mechanisms of drug blockade of hERG channel will facilitate rational design of new compounds devoid of hERG channel binding and/or blocking activity.

In conclusion, the present study demonstrates that ziprasidone preferentially binds to and blocks open hERG channels. Tyr-652 and Phe-656 are critical molecular determinants for block of hERG channel by ziprasidone. Results observed by us [this study] and others [17,18] would explain the clinical QTc prolongation observed with ziprasidone at therapeutic concentration [23].

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