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Response of I_{Kr} and hERG Currents to the Antipsychotics Tiapride and Sulpiride

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The human *ether-a-go-go*-related gene (*hERG*) channel is important for repolarization in human myocardium and is a common target for drugs that prolong the QT interval. We studied the effects of two antipsychotics, tiapride and sulpiride, on hERG channels expressed in *Xenopus* oocytes and also on delayed rectifier K^+ currents in guinea pig cardiomyocytes. Neither the amplitude of the hERG outward currents measured at the end of the voltage pulse, nor the amplitude of hERG tail currents, showed any concentration-dependent changes with either tiapride or sulpiride (3～300 μM). However, our findings did show that tiapride increased the potential for half-maximal activation ($V_{1/2}$) of HERG at 10～300 μM, whereas sulpiride increased the maximum conductance (G_{max}) at 3, 10 and 100 μM. In guinea pig ventricular myocytes, bath applications of 100 and 500 μM tiapride at 36°C blocked rapidly activating delayed rectifier K^+ current (I_{Kr}) by 40.3% and 70.0%, respectively. Also, sulpiride at 100 and 500 μM blocked I_{Kr} by 38.9% and 76.5%, respectively. However, neither tiapride nor sulpiride significantly affected the slowly activating delayed rectifier K^+ current (I_{Ks}) at the same concentrations. Our findings suggest that the concentrations of the antipsychotics required to evoke a 50% inhibition of I_{Kr} are well above the reported therapeutic plasma concentrations of free and total compound.

Key Words: hERG channel, Rapidly-activating delayed rectifier K^+ channel, Slowly-activating delayed rectifier K^+ channel, Sulpiride, Tiapride

INTRODUCTION

Antipsychotic drugs have been known to cause serious cardiovascular side-effects, including myocarditis, cardiomyopathy and abnormal cardiac rhythm [1]. These drugs share an ability to block the rapid component of the delayed rectifier K^+ current (I_{Kr}), resulting in a delayed repolarization of the cardiac action potential and prolongation of the QT interval, possibly leading to *torsades de pointes* [1]. Several epidemiological studies and case-control reports have shown that the rate of sudden death is increased in psychiatric patients taking antipsychotic drugs [2-5].

Tiapride has been classified as a selective dopamine D_2-receptor antagonist and is used as an atypical neuroleptic drug for the treatment of agitation, aggressiveness, anxiety, and sleep disorders in elderly patients [6]. It is structurally related to the antipsychotic drug sulpiride, also a selective antagonist of dopamine D_2-receptor. Sulpiride is used for the treatment of schizophrenic patients with negative symptoms and as an antiemetic and antidyspeptic [7]. Overdose of sulpiride can cause sinus tachycardia, hypotension, arrhythmia and CNS depression [8]. Tiapride and sulpiride have been reported to induce cardiac ECG changes including prolongation of the corrected QT interval (QT_c) and torsades de pointes at therapeutic doses [1,9-12]. It is known that I_{Kr} - mediated by an ion channel encoded by the human *ether-a-go-go*-related gene (hERG) - is important for the termination of the cardiac action potential and determines the shape of the repolarization phase [13]. The aim of the present study was to investigate the possible electrophysiological mechanisms underlying drug-induced QT prolongation. To determine whether hERG channels would be blocked by antipsychotic compounds we evaluated the effects of tiapride and sulpiride on hERG channels expressed in *Xenopus* oocytes, as well as the rapid (I_{Kr}) and slow (I_{Ks}) components of native delayed rectifier K^+ currents in guinea pig ventricular myocytes.

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METHODS

Expression of hERG in oocytes

hERG (accession no. U04270) cRNA was synthesized by *in vitro* transcription from 1 μg of linearized cDNA using

ABBREVIATIONS: *hERG*, human *ether-a-go-go*-related gene; $V_{1/2}$, the potential for half-maximal activation; G_{max}, the maximum conductance; I_{Kr}, the rapidly activating delayed rectifier K^+ current; I_{Ks}, the slowly activating delayed rectifier K^+ current; QT_c, the corrected QT interval; I_{HERG}, hERG currents; I_{tail}, tail currents; PC12, pheochromocytoma.

T7 message machine kits (Ambion, Austin, TX, USA) and stored in 10 mM Tris-HCl (pH 7.4) at −80°C. Stage V∼VI oocytes were surgically removed from female *Xenopus laevis* (Nasco, Modesto, CA, USA) anesthetized with 0.17% tricane methanesulphonate (Sigma, St. Louis, MO, USA). Using fine forceps, the theca and follicle layers were manually removed from the oocytes, and then each oocyte was injected with 40 nl of cRNA (0.1∼0.5 μg/μl). The injected oocytes were maintained in a modified Barth's Solution. The modified Barth's Solution contained (mM): 88 NaCl, 1 KCl, 0.4 $CaCl_2$, 0.33 $Ca(NO_3)_2$, 1 $MgSO_4$, 2.4 $NaHCO_3$, 10 HEPES (pH 7.4), and 50 μg/ml gentamicin sulphonate. Currents were studied two to seven days after injection. This study was performed according to the Research Guidelines of Kangwon National University IACUC.

Solutions and voltage-clamp recordings from oocytes

Normal Ringer's Solution contained (mM): 96 NaCl, 2 KCl, 1.8 $CaCl_2$, 1 $MgCl_2$ and 10 HEPES (pH adjusted to 7.4 with NaOH). Solutions were applied to oocytes by continuous perfusion of the chamber while recording. Solution exchanges were completed within three min, and the hERG currents were recorded 5 min after the solution exchange. Currents were measured at room temperature (20∼23°C) with a two-microelectrode voltage clamp amplifier (Warner Instruments, Hamden, CT, USA). Electrodes were filled with 3 M KCl and had a resistance of 2∼4 MΩ for voltage-recording electrodes and 0.6∼1 M (for current-passing electrodes. Stimulation and data acquisition were controlled with an AD-DA converter (Digidata 1200, Axon Instruments) and pCLAMP software (v 5.1, Axon Instruments). The antipsychotics tiapride, and sulpiride, and other reagents were purchased from Sigma (St. Louis, MO, USA). Stock solutions of tiapride and sulpiride were prepared in distilled water and DMSO, respectively, and added to the external solutions atat the desired suitable concentrations shortly before each experiment.

Pulse protocols and analysis

To obtain concentration-response curves in the presence of tiapride or sulpiride, concentration-dependent inhibition was fitted with the equation:

$I_{tail} = I_{\text{tail}\cdot\max} / [1+(IC_{50}/D)^n]$,

where I_{tail} indicates peak tail currents, $I_{\text{tail}\cdot\max}$ is the maximum peak tail current, D is the concentration of the small molecule, n is the Hill coefficient, and IC_{50} is the concentration at which the half-maximal peak tail currents were inhibited.

Solutions and voltage clamp recordings from guinea pig ventricular myocytes

Single ventricular myocytes were isolated from guinea pig hearts using a standard enzymatic technique [14]. Isolated cells were superfused at 36°C with normal Tyrode's Solution containing (mM): 140 NaCl, 4.4 KCl, 1.8 $CaCl_2$, 1 $MgCl_2$, 5 HEPES, and 10 glucose (pH 7.4 with 4 M NaOH). Inward-rectifier K^+ currents were inhibited by the addition of 5 mM CsCl. The patch pipettes (outer diameter 1.5 mm, World Precision Instruments, USA) had resistances of around 1∼2 MΩ. The pipette solution for the potassium current measurement contained (mM): 140 KCl, 1 $MgCl_2$, 5 EGTA, 5 MgATP, 2.5 diTris-phosphocreatine and 2.5 disodium phosphocreatine (pH 7.4 with KOH). The 'pipette- to-bath' liquid junction potential was small (−3.5 mV) and was uncorrected. Membrane capacitance (the time integral of the capacitive response to a 10 mV hyperpolarizing pulse from a holding potential of 0 mV, divided by the voltage drop) averaged 121.5±24.5 pF (n=10). Measurements were taken using an Axopatch 200A amplifier (Axon Instruments) and a CV-201 headstage. Voltage-clamp commands were generated using 'WinWCP' (John Dempster, Strathclyde University, UK) or a pClamp (v 5.1, Axon Instruments). The current signals were filtered via a 10 kHz, 8-pole Bessel-type low-pass filter and digitized by an AD-DA converter (Digidata 1200, Axon Instruments) for subsequent analysis (pCLAMP Software 6.0.3.). All chemicals were from Sigma, except for E-4031, which was kindly provided by Eisai Co. (Japan).

Statistical evaluations

All data are expressed as mean±S.E.M. Unpaired or paired Student t tests, or ANOVA were used for statistical comparisons when appropriate, and differences were considered significant at $p<0.05$.

RESULTS

Effect of tiapride or sulpiride on hERG current in Xenopus oocytes

We examined the effect of tiapride or sulpiride on hERG currents (I_{HERG}) using a *Xenopus* oocyte expression system. Throughout these experiments, the holding potential was maintained at −70 mV, and tail currents (I_{tail}) were recorded at −60 mV after depolarizing pulses from −50 to +40 mV (Fig. 1 and 2). The amplitude of the outward currents measured at the end of the pulse (I_{HERG}) increased with increasing positive voltage steps, reaching a maximum at −10 mV. The amplitude of I_{HERG} was normalized to the maximum amplitude of the I_{HERG} obtained under the control conditions, and was plotted against the potential of the step depolarization (Relative I_{HERG}, Fig. 1B and 2B). Depolarizing steps to even greater positive values caused a decrease in current, resulting in a negative slope of the I∼V curve. The amplitude of I_{HERG} showed no concentration-dependent change with increasing either tiapride or sulpiride concentration. After the depolarizing steps, repolarization to −60 mV induced an outward I_{tail}, which had an amplitude even greater than that of I_{HERG} during depolarization, which is due to rapid recovery from inactivation and a slow deactivation mechanism [15]. The amplitude of I_{tail} did not show concentration-dependent changes with increasing either tiapride or sulpiride concentration (Fig. 1A and 2A). The amplitude of I_{tail} was normalized to the peak amplitude obtained under the control conditions at the maximum depolarization, and was plotted against the potential of the step depolarization (n=3, Fig. 1C; n=4, Fig. 2C). In Fig. 1D and 2D, we plotted the values of $V_{1/2}$ and G_{max} against the concentration of tiapride and sulpiride, respectively. Fig. 1D showed that tiapride increased the values of $V_{1/2}$ at 10∼300 μM (n=3, Fig. 1D). On the contrary, sulpiride increased G_{max} at 3, 10, 100 μM (Fig. 2D, n=4, $p<0.05$).

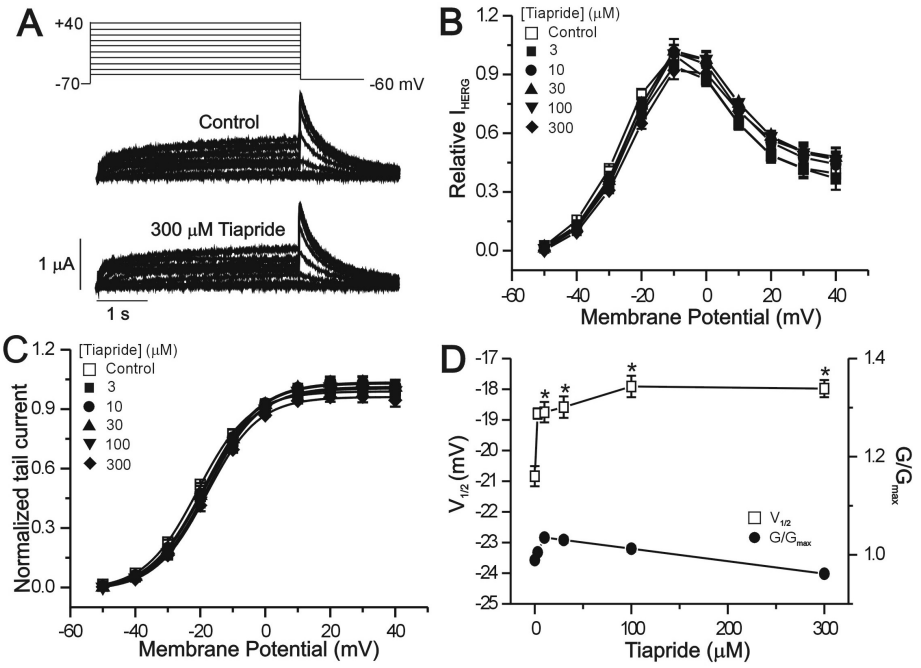

Fig. 1. The effect of tiapride on *human-ether-a-go-go*-related gene (*hERG*) currents (I_{HERG}) elicited by depolarizing voltage pulses. (A) Superimposed current traces elicited by depolarizing voltage pulses (4 s) in 10 mV steps (upper panel) from a holding potential of −70 mV in the absence of tiapride (control, center panel) and in the presence of 300 μM tiapride (lower panel). (B) Plot of the normalized hERG current measured at the end of depolarizing pulses (I_{HERG}) against the pulse potential in the control and tiapride conditions. The maximal amplitude of the I_{HERG} in the control was given a value of 1. (C) Plot of the normalized tail current measured at its peak just after repolarization. The peak amplitude of the tail current in the absence of the drug was set as 1. Control data were fitted to the Boltzmann Equation, $y=1/\{1+\exp[(-V+V_{1/2})/dx]\}$, with $V_{1/2}$ of −20.8 mV. (D) Plot of the values of $V_{1/2}$ (open squares) and G_{max} (closed circles) against the concentration of tiapride. *Symbols* with error bars represent mean±S.E.M. (n=3). *$p<0.05$.

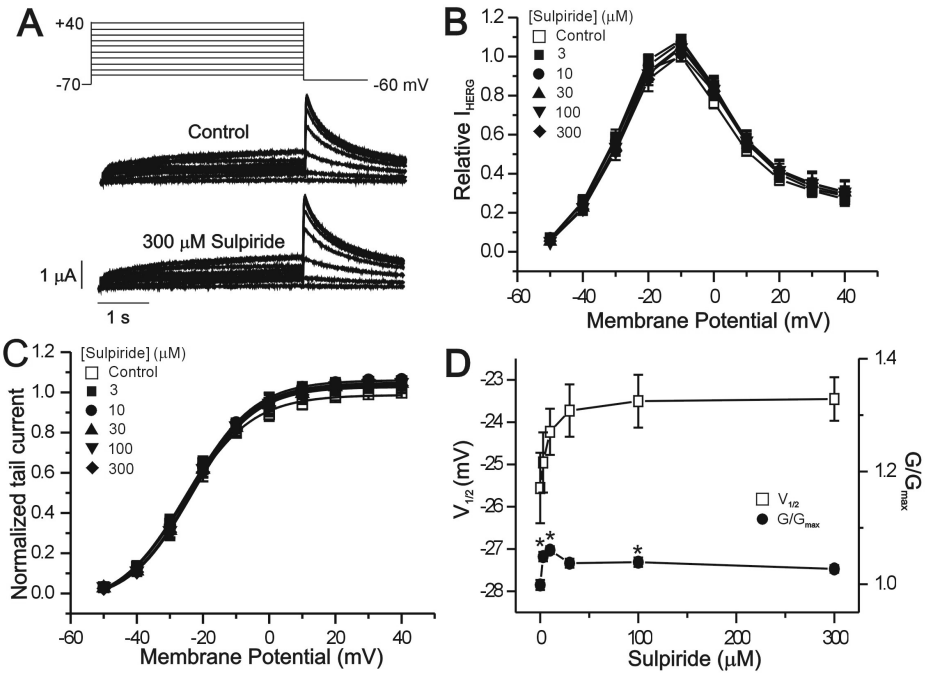

Fig. 2. The effect of sulpiride on *human-ether-a-go-go*-related gene (*hERG*) currents (I_{HERG}) elicited by depolarizing voltage pulses. (A) Superimposed current traces elicited by depolarizing voltage pulses (4 s) in 10 mV steps (upper panel) from a holding potential of −70 mV in the absence of sulpiride (control, center panel) and in the presence of 300 μM sulpiride (lower panel). (B) Plot of the normalized hERG current measured at the end of depolarizing pulses (I_{HERG}) against the pulse potential in the control and tiapride conditions. The maximal amplitude of the I_{HERG} in the control was given a value of 1. (C) Plot of the normalized tail current measured at its peak just after repolarization. The peak amplitude of the tail current in the absence of the drug was set as 1. Control data were fitted to the Boltzmann Equation, $y=1/\{1+\exp[(-V+V_{1/2})/dx]\}$, with $V_{1/2}$ of −25.6 mV. (D) Plot of the values of $V_{1/2}$ (open squares) and G_{max} (closed circles) against the concentration of sulpiride. *Symbols* with error bars represent mean±S.E.M. (n=4). *$p<0.05$.

Effect of tiapride or sulpiride on the rapid and slow components of the delayed rectifier K^+ currents in guinea pig ventricular myocytes

In further experiments, we tested the effects of tiapride and sulpiride on the rapid and slow components of the delayed rectifiers in guinea pig ventricular myocytes at 36°C, using electrophysiological separation of the currents with a voltage clamp protocol [16] (Fig. 3A, inset; stimulation frequency of 0.03 Hz). Depolarization to +40 mV activated both I_{Kr} and I_{Ks}, repolarization to −10 mV revealed I_{Ks} as a deactivating I_{tail}; subsequent repolarization to −50 mV showed a deactivation in I_{Kr}. We confirmed that E-4031 (2 μM), a selective blocker of I_{Kr} [17], blocked the rapid component of the delayed rectifier K^+ current, but had no

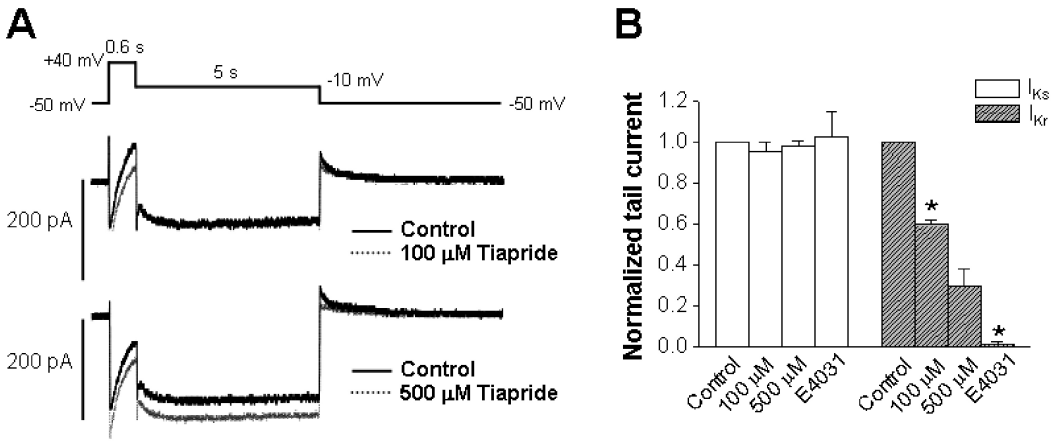

Fig. 3. The effect of tiapride on both slow and rapid components of delayed rectifier K^+ currents in guinea pig ventricular myocytes. (A) Representative traces of the rapid component (I_{Kr}) and the slow component (I_{Ks}) of delayed rectifier K^+ channel tail currents before and after treatment with either 100 μM or 500 μM tiapride. (B) A summary of the effects of 100 μM and 500 μM tiapride and 2 μM E-4031 on I_{Kr} and I_{Ks} tail currents, normalized relative to the control current (n=2~4, *p<0.05). The tail current amplitudes were measured as the difference between the peak outward current and the steady-state current at the end of the repolarizing voltage pulses.

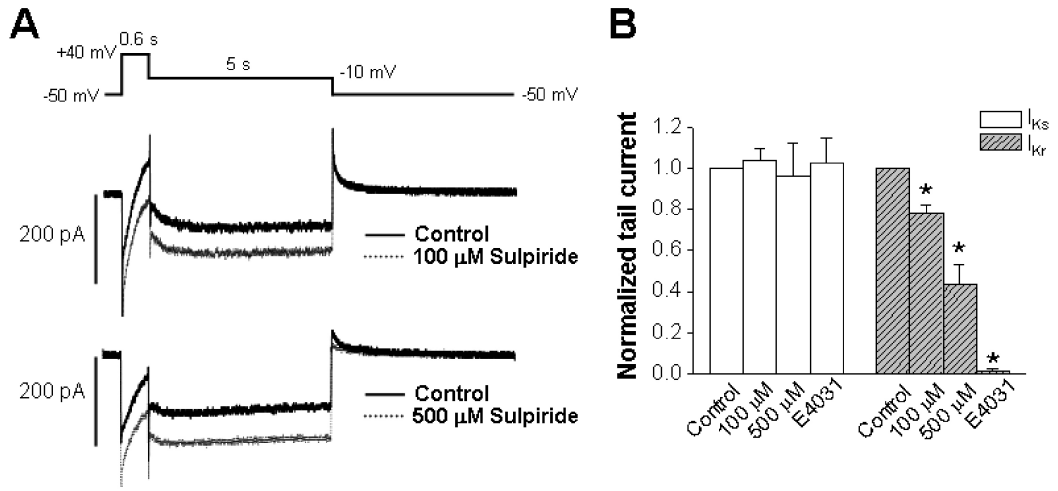

Fig. 4. The effect of sulpiride on both slow and rapid components of delayed rectifier K^+ currents in guinea pig ventricular myocytes. (A) Representative traces of the rapid component (I_{Kr}) and the slow component (I_{Ks}) of delayed rectifier K^+ channel tail currents before and after treatment with either 100 μM or 500 μM sulpiride. (B) A summary of the effects of 100 μM and 500 μM sulpiride and 2 μM E-4031 on I_{Kr} and I_{Ks} tail currents, normalized relative to the control current (n=3~4, *p<0.05). The tail current amplitudes were measured as the difference between the peak outward current and the steady-state current at the end of the repolarizing voltage pulses.

effect on I_{Ks} (n=4, Fig. 3B). We found that tiapride inhibited I_{Kr} in a concentration-dependent manner; 100 μM and 500 μM tiapride inhibited I_{Kr} by 40.3±2.0% and 70.0±8.3%, respectively (n=2~3, Fig. 3), suggesting that native I_{Kr} is more sensitive to the drug than the HERG channels expressed in *Xenopus* oocytes. Neither 100 μM nor 500 μM tiapride had a significant effect on I_{Ks} (n=2~3) under our experimental conditions. These findings indicate that tiapride preferentially blocks the rapid component of the delayed rectifier K^+ current but not the slow component, suggesting that tiapride may prolong action potential duration primarily by blocking I_{Kr} and not I_{Ks}.

Next we tested the effect of sulpiride on the I_{Kr} and I_{Ks} components of the delayed rectifier K^+ current. As shown in Fig. 4, sulpiride also inhibited I_{Kr} in a concentration- dependent manner, with block at 100 μM and 500 μM measured at 21.8±3.8% and 56.6±10.1%, respectively (n=3~4, Fig. 4). This result suggests that the sulpiride sensitivity of native I_{Kr} in ventricular myocytes is greater than that of HERG channels in a *Xenopus* oocyte expression system. Neither 100 μM nor 500 μM sulpiride had a significant effect on I_{Ks} (n=2~4) under our experimental conditions. These findings indicate that sulpiride preferentially blocks the rapid component of the delayed rectifier K^+ current rather than the slow component, and suggests that sulpiride may prolong action potential duration primarily by blocking I_{Kr} and not I_{Ks}.

DISCUSSION

Tiapride and sulpiride are used as antipsychotics based on their ability to inhibit dopamine D_2-receptors [6,7]. Cardiovascular disease is common in men with mental disorders; therefore, it is important to examine the potential for medications used to treat psychotic disorder to prolong ventricular repolarization. It is known that drugs that produce only a slight increase in repolarization time (5～10 msec) increase the risk of sudden death may; however, changes of up to 5 msec may also occur in the QT_c with placebo treatment alone [18,19]. In case reports examining the effects of therapeutic doses of tiapride and sulpiride on the human QT interval, the QT_c increased by 160 msec after tiapride (300 mg) [10] and by 51 msec after sulpiride (150 mg) [20].

The results of this study suggest that tiapride and sulpiride were not potent hERG channel blockers. Both drugs failed to reduce hERG outward currents (measured at the end of the voltage pulse) and hERG tail currents expressed in *Xenopus* oocytes, although tiapride did increase the values of $V_{1/2}$ indicating that it may have altered channel activation gating.

Tiapride and sulpiride are relatively hydrophilic benzamide compounds that are mainly excreted unchanged in the urine, with elimination half-lives usually in the range 3～10 h [22]. The therapeutic plasma concentrations of tiapride and sulpiride have been reported to be up to ～1.9 μM (682 ng/ml) and ～2.8 μM (940 ng/ml), respectively [10,20]. When the rate of plasma protein binding is about 40% [21], the free plasma concentration of tiapride and sulpiride may be expected to be up to 1.1 μM and 1.7 μM, respectively. IC_{50} values for the block of I_{Kr} by tiapride and sulpiride were in the 100～500 μM range, which is 30-fold greater than the highest achievable free or total plasma concentration. It has been suggested that drugs with an IC_{50} at least 30 times greater than the highest achievable free plasma concentration will not cause *torsades de pointes*, a potentially fatal ventricular tachyarrhythmia [23]. In this context, tiapride and sulpiride were unique from other QT_c-prolonging compounds in that they did not produce significant hERG blockade even at 300 μM, which suggests that mechanisms other than hERG channel block could be involved in the ability of tiapride and sulpiride to increase QT_c. Also, the difference in the degree of block of between hERG channel and the I_{Kr} could be attributed to properties specific to the *Xenopus* oocyte expression system. Higher extracellular concentrations of drugs such as dofetilide and BRL-32872 were required to block hERG channels expressed in ooctyes [24,25] due to the vitelline membrane and egg yolk [26]. Otherwise, the reconstitution of the regulatory β-subunit MiRP with HERG in native I_{Kr} could alter the drug sensitivity of hERG and native I_{Kr} channels [27].

Antipsychotics are among the most frequently prescribed medications known to cause QT interval prolongation [28]. Silvestre and Prous reported that the potencies for the hERG channel block were different according to the chemical classes of antipsychotics [29]. Butyrophenones and diphenylbutylpiperidines were most potent hERG channel blockers, all with IC_{50} values in the nM range. In contrast, substituted bezamides and dibenzoxazepines exhibited hERG-blocking activity with IC_{50} values in the μM range, while thioxanthenes and phenothiazines exhibited moderate to low potencies for blocking hERG channels. Our results have shown that tiapride was a more potent inhibitor of activation gating than sulpiride; on the other hand sulpiride altered the G_{max} of hERG current. Altogether, these findings suggest that different antipsychotics may inhibit hERG via specific mechanisms based on their chemical structure.

Our findings are comparable to those reported by Lee et al. where sulpiride blocked hERG currents with an IC_{50} value of ～1 mM but did not significantly affect cardiac I_{Na}, I_{Ca}, I_{Ks} or I_{K1} [30]. Also, Silverstre and Prous reported that sulpiride blocked hERG channels with an IC_{50} value of > 100 μM [29]. On the contrary, sulpiride at 1 and 10 μM inhibited L-type Ca^{2+} current [31] but did not affect voltage-activated K^+ currents in pheochromocytoma (PC12) cells, a rat neuronal cell line [32]. This suggests that sulpiride predominantly blocks the rapid, but not the slow, component of the delayed rectifier K^+ current in cardiac cells. Indeed, the PR interval and QRS width of the surface ECG, as well as AH and HV intervals of His bundle electrograms, were unaffected by sulpiride, suggesting that sulpiride may not affect Na^+ or Ca^{2+} channels of the heart *in situ* [20]. The results of this study, and those of others mentioned above, have shown that sulpiride fails to blocked hERG or I_{Kr} at therapeutic concentrations. However, the combination of overdose with other factors such as heart disease or changes in drug metabolism and protein binding may reduce the IC_{50} value for the sulpiride block of hERG or I_{Kr} and thereby increase the possibility of drug-induced QT prolongation.

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REFERENCES

1. **Stöllberger C, Huber JO, Finsterer J.** Antipsychotic drugs and QT prolongation. *Int Clin Psychopharmacol.* 2005;20:243-251.
2. **Hennessy S, Bilker WB, Knauss JS, Margolis DJ, Kimmel SE, Reynolds RF, Glasser DB, Morrison MF, Strom BL.** Cardiac arrest and ventricular arrhythmia in patients taking antipsychotic drugs: cohort study using administrative data. *BMJ.* 2002;325:1070-1074.
3. **Ray WA, Meredith S, Thapa PB, Meador KG, Hall K, Murray KT.** Antipsychotics and the risk of sudden cardiac death. *Arch Gen Psychiatry.* 2001;58:1161-1167.
4. **Reilly JG, Ayis SA, Ferrier IN, Jones SJ, Thomas SHL.** Thioridazine and sudden unexplained death in psychiatric in-patients. *Br J Psychiatry.* 2002;180:515-522.
5. **Straus SM, Bleumink GS, Dieleman JP, van der Lei J, 't Jong GW, Kingma JH, Sturkenboom MC, Stricker BH.** Antipsychotics and the risk of sudden cardiac death. *Arch Intern Med.* 2004;164:1293-1297.
6. **Steele JW, Faulds D, Sorkin EM.** Tiapride. A review of its pharmacodynamic and pharmacokinetic properties, and therapeutic potential in geriatric agitation. *Drugs Aging.* 1993;3:460-478.
7. **Mucci A, Nolfe G, Maj M.** Levosulpiride: a review of its clinical use in psychiatry. *Pharmacol Res.* 1995;31:95-101.
8. **Capel MM, Colbridge MG, Henry JA.** Overdose profiles of new antipsychotic agents. *Int J Neuropsychopharmacol.* 2000;3:51-54.
9. **Huang BH, Hsia CP, Chen CY.** Sulpiride induced torsade de pointes. *Int J Cardiol.* 2007;118:e100-e102.
10. **Iglesias E, Esteban E, Zabala S, Gascon A.** Tiapride-induced torsade de pointes. *Am J Med.* 2000;109:509.
11. **Lin CH, Chen MC, Wang SY, Lin CY.** Predictive factors for

QTc prolongation in schizophrenic patients taking antipsychotics. *J Formos Med Assoc.* 2004;103:437-441.
12. **Su KP, Shen WW, Chuang CL, Chen KP, Chen CC.** A pilot cross-over design study on QTc interval prolongation associated with sulpiride and haloperidol. *Schizophr Res.* 2003;59:93-94.
13. **Tie H, Walker BD, Valenzuela SM, Breit SN, Campbell TJ.** The Heart of Psychotropic drug therapy. *Lancet.* 2000;355:1825.
14. **Choi SY, Koh YS, Jo SH.** Inhibition of human ether-a-go-go-related gene K^+ channel and IKr of guinea pig cardiomyocytes by antipsychotic drug trifluoperazine. *J Pharmacol Exp Ther.* 2005;313:888-895.
15. **Zhou Z, Gong Q, Ye B, Fan Z, Makielski JC, Robertson GA, January CT.** Properties of HERG channels stably expressed in HEK 293 cells studied at physiological temperature. *Biophys J.* 1998;74:230-241.
16. **Heath BM, Terrar DA.** Separation of the components of the delayed rectifier potassium current using selective blockers of IKr and IKs in guinea-pig isolated ventricular myocytes. *Exp Physiol.* 1996;81:587-603.
17. **Sanguinetti MC, Jurkiewicz NK.** Two components of cardiac delayed rectifier K^+ current. Differential sensitivity to block by class III antiarrhythmic agents. *J Gen Physiol.* 1990;96:195-215.
18. **Malik M.** Problems of heart rate correction in assessment of drug-induced QT interval prolongation. *J Cardiovasc Electrophysiol.* 2001;12:411-420.
19. **Malik M, Camm AJ.** Evaluation of drug-induced QT interval prolongation: implications for drug approval and labeling. *Drug Saf.* 2001;24:323-351.
20. **Sugiyama A, Satoh Y, Shiina H, Takeda S, Hashimoto K.** Torsadegenic action of the antipsychotic drug sulpiride assessed using in vivo canine models. *J Cardiovasc Pharmacol.* 2002;40: 235-245.
21. **Lenhard G, Kieferndorf U, Berner G, Vogtle-Junkert U, Wagener HH.** The importance of pharmacokinetic data on sulpiride: results of a bioequivalence study of two sulpiride 200 mg preparations following oral administration. *Int J Clin Pharmacol Ther Toxicol.* 1991;29:231-237.
22. **Bressolle F, Bres J, Blanchin MD, Gomeni R.** Sulpiride pharmacokinetics in humans after intramuscular administration at three dose levels. *J Pharm Sci.* 1984;73:1128-1136.
23. **Redfern WS, Carlsson L, Davis AS, Lynch WG, MacKenzie I, Palethorpe S, Siegl PK, Strang I, Sullivan AT, Wallis R, Camm AJ, Hammond TG.** Relationships between preclinical cardiac electrophysiology, clinical QT interval prolongation and torsade de pointes for a broad range of drugs: evidence for a provisional safety margin in drug development. *Cardiovasc Res.* 2003;58: 32-45.
24. **Kiehn J, Lacerda AE, Wible B, Brown AM.** Molecular physiology and pharmacology of HERG. Single-channel currents and block by dofetilide. *Circulation.* 1996;94:2572-2579.
25. **Thomas D, Wendt-Nordahl G, Rockl K, Ficker E, Brown AM, Kiehn J.** High-affinity blockade of human ether-a-go-go-related gene human cardiac potassium channels by the novel antiarrhythmic drug BRL-32872. *J Pharmacol Exp Ther.* 2001;297: 753-761.
26. **Madeja M, Musshoff U, Speckmann EJ.** Follicular tissues reduce drug effects on ion channels in oocytes of Xenopus laevis. *Eur J Neurosci.* 1997;9:599-604.
27. **Abbott GW, Sesti F, Splawski I, Buck ME, Lehmann MH, Timothy KW, Keating MT, Goldstein SA.** MiRP1 forms IKr potassium channels with HERG and is associated with cardiac arrhythmia. *Cell.* 1999;97:175-187.
28. **Curtis LH, Østbye T, Sendersky V, Hutchison S, Allen LaPointe NM, Al-Khatib SM, Usdin Yasuda S, Dans PE, Wright A, Califf RM, Woosley RL, Schulman KA.** Prescription of QT-prolonging drugs in a cohort of about 5 million outpatients. *Am J Med.* 2003;114:135-141.
29. **Silvestre JS, Prous JR.** Comparative evaluation of hERG potassium channel blockade by antipsychotics. *Methods Find Exp Clin Pharmacol.* 2007;29:457-465.
30. **Lee HA, Kim KS, Park SJ, Kim EJ.** Cellular mechanism of the QT prolongation induced by sulpiride. *Int J Toxicol.* 2009;28: 207-212.
31. **Ito K, Nakazawa K, Koizumi S, Liu M, Takeuchi K, Hashimoto T, Ohno Y, Inoue K.** Inhibition by antipsychotic drugs of L-type Ca^{2+} channel current in PC12 cells. *Eur J Pharmacol.* 1996;314: 143-150.
32. **Nakazawa K, Ito K, Koizumi S, Ohno Y, Inoue K.** Characterization of inhibition by haloperidol and chlorpromazine of a voltage-activated K^+ current in rat phaeochromocytoma cells. *Br J Pharmacol.* 1995;116:2603-2610.