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# Blockade of HERG human $K^+$ channel and $I_{Kr}$ of guinea pig cardiomyocytes by prochlorperazine

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**Abstract**

Prochlorperazine, a drug for the symptomatic control of nausea, vomiting and psychiatric disorders, can induce prolonged QT, *torsades de pointes* and sudden death. We studied the effects of prochlorperazine on human *ether-a-go-go*-related gene (*HERG*) channels expressed in *Xenopus* oocytes and also in the delayed rectifier $K^+$ current of guinea pig cardiomyocytes. Prochlorperazine induced a concentration-dependent decrease in current amplitudes at the end of the voltage steps and tail currents of HERG. The $IC_{50}$ for a prochlorperazine block of HERG current in *Xenopus* oocytes progressively decreased relative to the degree of depolarization, from 42.1 μM at −40 mV to 37.4 μM at 0 mV to 22.6 μM at +40 mV. The block of HERG by prochlorperazine was use-dependent, exhibiting a more rapid onset and a greater steady-state block at higher frequencies of activation, while there was partial relief of the block with reduced frequencies. In guinea pig ventricular myocytes, bath applications of 0.5 and 1 μM prochlorperazine at 36 °C blocked rapidly activating delayed rectifier $K^+$ current by 38.9% and 76.5%, respectively, but did not significantly block slowly activating delayed rectifier $K^+$ current. Our findings suggest that the arrhythmogenic side effects of prochlorperazine are caused by a blockade of HERG and the rapid component of the delayed rectifier $K^+$ current rather than by a blockade of the slow component.


*Keywords:* Prochlorperazine; Antiemetic drug; HERG channel; $I_{Kr}$; Long QT syndrome; Arrhythmias

## 1. Introduction

A variety of drugs including antiarrhythmics, antihistamines, and antipsychotics has been associated with cardiac arrhythmia and sudden cardiac death (Redfern et al., 2003). Some of these drugs have been shown to prolong the QT interval of the electrocardiogram (ECG) (Tan et al., 1995). The lengthening of the QT interval usually reflects a delayed repolarization of the action potential in ventricular myocytes (Tan et al., 1995). In addition, excessive prolonging of the QT interval can cause a potentially fatal ventricular tachyarrhythmia, termed *torsades de pointes* (Haverkamp et al., 2000), which has led to the withdrawal of some QT-prolonging drugs from the market. Several ion currents (including inward $Na^+$ and $Ca^{2+}$ currents and outward $K^+$ current) contribute to the determination of the cardiac action potential duration; among those, the most common mechanism by which drugs delay repolarization and prolong the QT interval is by blocking one or more outward $K^+$ currents (Barry et al., 1998; Jeron et al., 2000).

In human cardiomyocytes, a specific blockage of rapidly activating components of delayed rectifier cardiac $K^+$ current ($I_{Kr}$) appears to be the main mechanism whereby drugs act to produce acquired long QT syndrome and the associated ventricular arrhythmias (Brown and Rampe, 2000). The human *ether-a-go-go*-related gene (*HERG*) encodes the major protein underlying $I_{Kr}$, the pore-forming unit of the channel (Sanguinetti et al., 1995). Mutations of *HERG* have been shown to cause Chromosome 7-linked inherited long QT syndrome 2 (Curran et al., 1995). Several drugs that block $I_{Kr}$ and HERG cause *torsades de pointes* (Suessbrich et al., 1996), and in many cases, the cardiotoxicity of numerous drugs is due solely to their interaction with HERG $K^+$ channels (Taglialatela et al., 1998). The slowly activating component of the delayed rectifier $K^+$

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current, $I_{Ks}$, is also responsible for terminating the plateau phase of the action potential like $I_{Kr}$ (Sanguinetti and Jurkiewicz, 1990).

Prochlorperazine, a phenothiazine, was introduced into medical practice in 1956 and is widely used in the prevention and symptomatic control of nausea, vomiting, vestibular, and psychiatric disorders (Lapierre et al., 1969). However, phenothiazines were reported to delay repolarization by prolonging Phase 3 of the action potential (Arita and Surawicz, 1973), and they also can produce ECG abnormalities such as QTc prolongation (Lathers and Lipka, 1987). In addition, the inhibition of HERG currents by trifluoperazine, a phenothiazine, has been suggested to underlie the proarrhythmic effects (like long QT syndrome and *torsades de pointes*) of the drug in psychiatric patients (Choi et al., 2005). This raises the possibility that prochlorperazine may prolong the action potential duration in vivo and may cause long QT syndrome by inhibiting $I_{Kr}$, the HERG current, or $I_{Ks}$, which can eventually result in *torsades de pointes* and sudden death. It is necessary to test chemical entities experimentally for the potential to block the HERG channel because the drugs that inhibit HERG channels are structurally diverse (Haverkamp et al., 2000) and it is difficult to predict which drugs are likely to produce a HERG blockade or long QT syndrome purely on the basis of their chemical structures. In this study, we used the HERG channel expressed in *Xenopus* oocytes to test whether prochlorperazine would block the HERG channel. To confirm the hypothesis, we also measured native $I_{Kr}$ in guinea pig ventricular myocytes. Finally, we tested whether the drug could change the slow component of the delayed rectifier $K^+$ current, $I_{Ks}$.

## 2. Materials and methods

### 2.1. Expression of HERG in oocytes

*HERG* (accession no. U04270) cRNA was synthesized by in vitro transcription from 1 μg of linearized cDNA using 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 composition of 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 guidelines found in the Research Guideline of Cheju National University.

### 2.2. Solutions and voltage-clamp recordings from oocytes

A normal Ringer's Solution contained 96 mM NaCl, 2 mM KCl, 1.8 mM $CaCl_2$, 1 mM $MgCl_2$, and 10 mM HEPES (pH adjusted to 7.4 with NaOH). The antipsychotic drug prochlorperazine and all salts were purchased from Sigma (St. Louis, MO, USA). A stock solution of prochlorperazine was prepared in distilled water and added to the external solutions at suitable

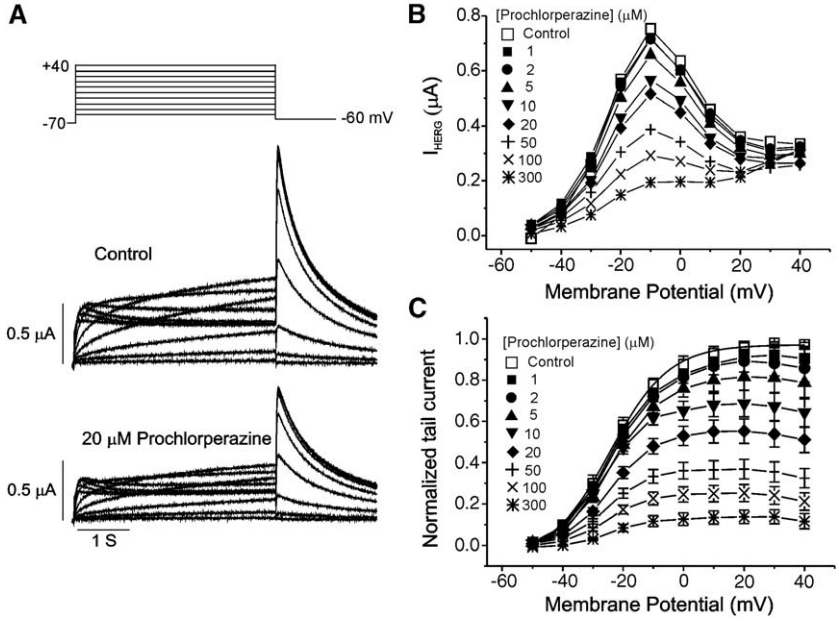


Fig. 1. The effect of prochlorperazine on human *ether-a-go-go*-related gene (*HERG*) currents ($I_{HERG}$) elicited by depolarizing voltage pulses. A: Superimposed current traces elicited by depolarizing voltage pulses (4 s) in 10 mV steps (upper panel) from a holding potential of −70 mV in the absence of prochlorperazine (control, center panel) and in the presence of 20 μM prochlorperazine (lower panel). B: A plot of the HERG current ($I_{HERG}$) measured at the end of depolarizing pulses against the pulse potential in different concentrations of prochlorperazine (obtained from A). C: A 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 taken as 1. *Symbols* with error bars represent mean ± S.E.M. ($n$ = 9). Control data were fitted to the Boltzmann Equation, $y = 1/\{1+\exp[(-V+V_{1/2})/dx]\}$, with $V_{1/2}$ of −24.3 mV.