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Block of the human *ether-a-go-go*-related gene (hERG) K^+ channel by the antidepressant desipramine

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ABSTRACT

Desipramine is a tricyclic antidepressant for psychiatric disorders that can induce QT prolongation, which may lead to *torsades de pointes*. Since blockade of cardiac human *ether-a-go-go*-related gene (hERG) channels is an important cause of acquired long QT syndrome, we investigated the acute effects of desipramine on hERG channels to determine the electrophysiological basis for its pro-arrhythmic potential. We examined the effects of desipramine on the hERG channels expressed in *Xenopus* oocytes using two-microelectrode voltage-clamp techniques. Desipramine-induced concentration-dependent decreases in the current amplitude at the end of the voltage steps and hERG tail currents. The IC_{50} for desipramine needed to block the hERG current in *Xenopus* oocytes decreased progressively relative to the degree of depolarization. Desipramine affected the channels in the activated and inactivated states but not in the closed states. The S6 domain mutations, Tyr-652 located in the S6 domain of the hERG channel reduced the potency of the channel block by desipramine more than a mutation of Phe-656 in the same region. These results suggest that desipramine is a blocker of the hERG channels, providing a molecular mechanism for the arrhythmogenic side effects during the clinical administration of desipramine.

1. Introduction

It has long been recognized that some psychotropic drugs, including neuroleptics, antipsychotics, antidepressants, stimulants, and antianxiety agents, can be associated with risks of cardiac arrhythmia and sudden death [1]. Desipramine was the first successful antidepressant and has been widely used for the treatment of depression and other psychiatric disorders, such as panic and obsessive–compulsive disorders, bulimia nervosa, and chronic pain disorder [2]. Inhibition of norepinephrine and/or serotonin (5-hydroxytryptamine (5-HT)) transporters by antidepressants in the brain is generally thought to have important implications in their therapeutic effects [2]. Desipramine belongs to a group of psychotropic compounds called tricyclic antidepressants that have similar chemical structures and share a variety of pharmacologic actions [3]. Tricyclic antidepressants are known to inhibit calcium currents in heart myocytes and in neurons [4]. Neuronal sodium channels are ubiquitous and are crucial in dendritic integration, action potential initiation, and conduction. The potency of tricyclic antidepressants as sodium channel inhibitors was shown by the low micromolar IC_{50} values in vitro, and by their in vivo efficiency against neuropathic pain [5]. The cardiovascular effects and toxicity of tricyclic antidepressants have been well documented in depressed patients without pre-existing cardiac disease [6]. The most common manifestation of such effect are the slowing of intraventricular conduction, manifested by prolonged PR, QRS, and QT intervals on the standard ECG, and orthostatic hypotension [6].

During the last years, hERG channel blockade has become a major topic in pharmacological research [7]. The human *ether-a-go-go*-related gene (hERG) encodes the pore-forming subunits of the rapidly-activating delayed rectifier K^+ channel (I_{kr}) in the heart [8]. The function of hERG was unknown, but it was strongly expressed in the heart and was hypothesized to play an important role in repolarization of cardiac action potentials [9]. Mutations in hERG reduce I_{kr} and cause type 2 long QT syndrome (LQT2), a disorder that predisposes individuals to life-threatening arrhythmias [10]. Acquired and inherited LQTs are both associated with a distinct arrhythmia known as *torsades de pointes* are polymorphic ventricular tachycardia associated with abnormal cardiac repolarizations, as detected by QT prolongation on the electrocardiogram, and characterized by sinusoidal twisting of the QRS axis around the isoelectric line [11]. Because of their potential pro-arrhythmic effects, a number of non-cardiac drugs have been withdrawn from the market (e.g. terfenadine, cisapride, and thioridazine) and many have been labeled for restricted use (e.g. mesoridazine, droperidol,

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and arsenic trioxide) [12]. Therefore, the drug-induced QTc interval prolongation is strongly associated with the blockade of hERG channels, suggesting that drug-screening against recombinant hERG channels is now an important component of cardiac safety pharmacology during drug development.

There are no reports about the electrophysiological characteristic of interaction of desipramine with the hERG channel. The aim of this study is to examine the effects of desipramine on the cloned hERG potassium channels heterologously expressed in *Xenopus laevis* oocytes, which approach would be helpful to reveal the detailed insights into the biophysical mechanism of hERG block by the drug.

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. The amino acid mutations where generated by polymerase chain reaction (PCR) with synthetic mutant oligonucleotide primers. The mutations Y652A and F656A were verified by sequencing (ABI3100). Stage V and VI oocytes were surgically removed from female *Xenopus laevis* (Nasco, Modesto, CA, USA) anesthetized with 0.17% tricaine 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 performed according to the Research Guidelines of Kangwon National University IACUC.

2.2. Solution and voltage-clamp recording from oocytes

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). Solutions were applied to oocytes by continuous perfusion of the chamber while recording. Solution exchanges were completed within 3 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 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 antidepressant desipramine and other reagents ware purchased from Sigma (St. Louis, MO, USA). A stock solution of desipramine was prepared in DMSO and added to the external solution at suitable concentrations shortly before each experiment.

The fractional electrical distance (δ), i.e. the fraction of the transmembraneous electrical field sensed by a single positive charge at the binding site, was determined with half-blocking concentrations (K_D) obtained from the fractional current (f_o) as the current with 50 μM desipramine and under control conditions at

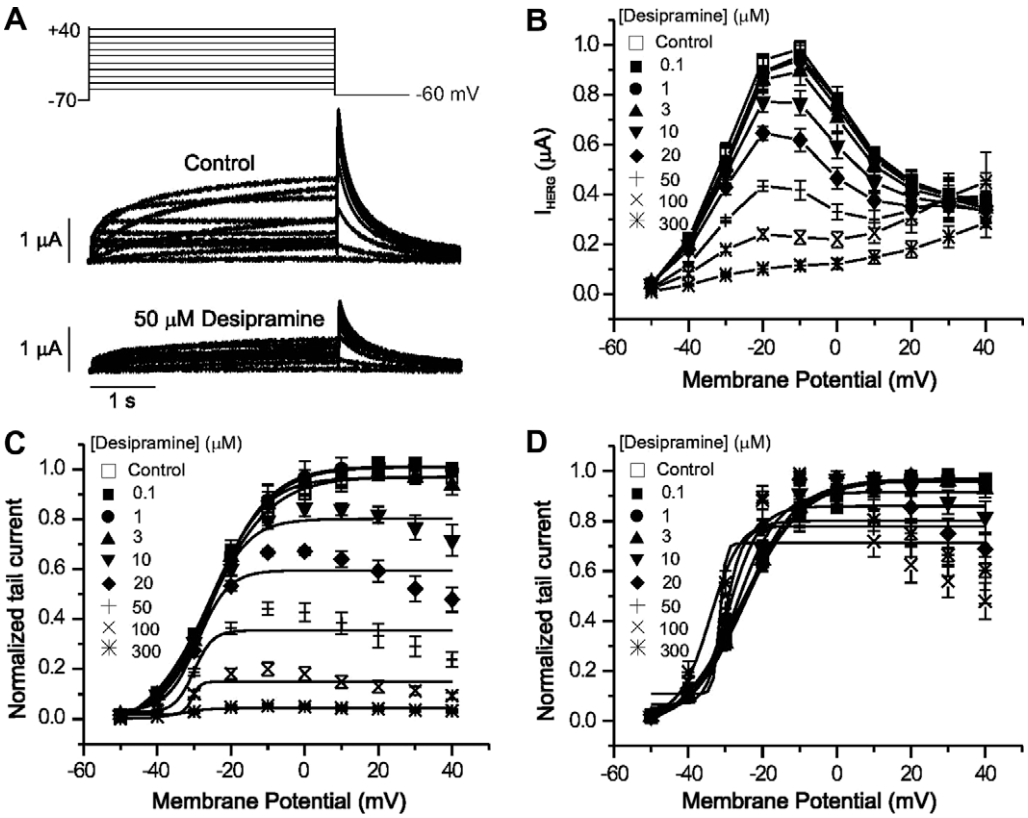

Fig. 1. The effect of desipramine on human *ether-a-go-go*-related gene (hERG) currents (I_{HERG}) elicited by depolarizing voltage pulses. (A) Superimposed current traces elicited by depolarizing voltage pulses (4 s) in 10 mV steps (upper panel) from a holding potential of −70 mV in the absence of desipramine (control, center panel) and in the presence of 50 μM desipramine (lower panel). (B) Plot of the normalized hERG current measured at the end of depolarizing pulses ($I_{HERG,\ nor}$) against the pulse potential in the control and desipramine 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 −26.1 mV. (D) Activation curves with values normalized to the respective maximum value at each concentration of desipramine. Symbols with error bars represent mean ± SEM ($n = 5$).