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Short communication

High affinity blockade of the HERG cardiac K^+ channel by the neuroleptic pimozide

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Abstract

Pimozide is an antipsychotic agent also used to treat facial tics. Pimozide can cause acquired long QT syndrome and ventricular arrhythmias. To elucidate the mechanism behind these clinical findings, we examined the effects of pimozide on the cloned human cardiac K^+ channels HERG (*human ether-a-go-go*-related gene; rapid component of delayed rectifier), Kv1.5 (ultra-rapid delayed rectifier) and KvLQT1/minK (slow component of delayed rectifier). Using patch clamp electrophysiology, we found that pimozide was a potent inhibitor of HERG displaying an IC_{50} value of 18 nM. In contrast, pimozide (10 μM) was a weak inhibitor of KvLQT1/minK and Kv1.5. We conclude that pimozide is a specific, high affinity antagonist of HERG, and that this interaction leads to prolongation of cardiac repolarization.

Keywords: K^+ channel; Pimozide; HERG (human ether-a-go-go-related gene); Arrhythmia; Antipsychotic agent

1. Introduction

Acquired or drug-induced long QT syndrome can be a dangerous side effect that is associated with certain prescription medications. This pro-arrhythmic activity is characterized by a prolongation of the QT interval on the electrocardiogram, and can lead to the life-threatening ventricular arrhythmia torsades de pointes (Ben-David and Zipes, 1993). One mechanism by which drugs can prolong cardiac repolarization is by blocking one or more types of voltage-dependent K^+ channels in the myocardium. Advances in molecular biology have lead to the discovery and cloning of several distinct K^+ channels that contribute to repolarization of the human myocardium. Kv1.5, for example, is the K^+ channel protein that is thought to underlie the ultra-rapid delayed rectifier K^+ current (I_{Kur}) found in the human atria (Fedida et al., 1993). This channel is also found in the ventricular myocardium, but its role there is as yet unknown (Mays et al., 1995). The *human ether-a-go-go*-related gene, HERG, expresses the rapid component of the delayed rectifier current I_{Kr} (Sanguinetti et al., 1995). The KvLQT1 protein complexes with the minK subunit to form the K^+ channel that underlies the slow component of the delayed rectifier current I_{Ks} (Barhanin et al., 1996; Sanguinetti et al., 1996). HERG and KvLQT1/minK may be especially important for repolarizing the heart since mutations in these channels lead to congenital forms of long QT syndrome (for review see Priori et al., 1999). Furthermore, blockade of HERG is believed to cause acquired long QT syndrome associated with the use of drugs like terfenadine (Roy et al., 1996), cisapride (Mohammad et al., 1997; Rampe et al., 1997) and sertindole (Rampe et al., 1998).

Pimozide (ORAP®) is a neuroleptic agent that is widely available and is used to treat Tourette's syndrome as well as various psychiatric disorders (Opler and Feinberg, 1991; Sallee et al., 1997). However, a number of reports have been published linking the use of pimozide with significant prolongation of the QT interval and ventricular arrhythmia (Fulop et al., 1987; Krahenbuhl et al., 1995; Desta et al., 1999). These cardiotoxic events may be associated with high doses of the drug, or with the concomitant use of drugs which slow the metabolism of pimozide, such as macrolide antibiotics (Desta et al., 1999). The very low doses of pimozide required to produce QT interval prolongation (7.5 mg/day, Fulop et al., 1987), suggest a high

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affinity interaction with one or more types of cardiac K^+ channels. To further understand the molecular mechanisms underlying these clinical findings, we examined the effects of pimozide on the cloned human cardiac K^+ channels HERG, KvLQT1/minK and Kv1.5.

2. Materials and methods

2.1. Molecular biology

The cDNA encoding the HERG K^+ channel was isolated as described previously (Rampe et al., 1997) and transfected into Chinese hamster ovary cells (CHO cells, American Type Culture Collection, Rockville, MD, #CRL 61) using Lipofectamine (Gibco, Grand Island, NY). The cDNA encoding the Kv1.5 K^+ channel was transfected into the human embryonic kidney cell line HEK-293 (ATCC #CRL 1573) as previously described (Fedida et al., 1993). KvLQT1 was cloned into the *Nhe*I-(5′-end) and *Bam*HI (3′-end) sites of pcDNA3.1 (Invitrogen, Carlsbad, CA) which also contained the G418 resistance gene. The gene encoding minK was cloned into the same restriction sites of pcDNA3.1 containing the zeocin resistance gene. CHO cells were transfected using Lipofectamine (Gibco) and selection carried out using 400 μg/ml G418 and 350 μg/ml zeocin (Invitrogen).

2.2. Electrophysiology

Cells expressing the cloned human K^+ channels were seeded on glass or plastic coverslips 24–48 h before use. HERG and KvLQT1/minK currents were recorded using the whole-cell patch clamp configuration, while Kv1.5 currents, due to the high expression levels of this channel in this cell line, were recorded from cell-free inside-out membrane patches (Hamill et al., 1981; Rampe et al., 1998). Electrodes (2–5 MΩ resistance) were fashioned from TW150F glass capillary tubes (World Precision Instruments, Sarasota, FL). For whole-cell recordings, electrodes were filled with the following solution (in mM): potassium aspartate, 120; KCl, 20; Na_2ATP, 4.0; HEPES, 5.0; $MgCl_2$, 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 (in mM): NaCl, 130; KCl, 5; sodium acetate, 2.8; $MgCl_2$, 1.0; HEPES, 10; glucose, 10; $CaCl_2$, 1.0; pH 7.4 with NaOH. This served as the internal solution for the inside-out patch recordings. Cells on coverslips were placed in a 0.5-ml chamber. Cells were continuously perfused with external solution, or external solution containing various concentrations of pimozide, at a rate of 3 ml per min. Currents were recorded at room temperature using an Axopatch 1-D or Axopatch 200 B amplifier (Axon Instruments, Burlingame, CA) and were conditioned by a four-pole low pass filter with a cutoff frequency of between one-quarter to one-half the sampling frequency. Currents were analyzed using the pCLAMP suite of software (Axon Instruments).

2.3. Chemicals

Pimozide was obtained from Research Biochemicals International (Natick, MA). All other compounds were obtained from Sigma (St. Louis, MO).

3. Results

Fig. 1 shows the effects of pimozide on HERG channel currents. In these experiments, a 2 s 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., 1995; Roy et al., 1996). Fig. 1A shows that these tail currents were potently blocked by pimozide. The IC_{50} value for pimozide block of peak HERG tail currents measured 18.1 nM (13.7–23.9 nM, 95% C.L., Fig. 1B). Significant ($p < 0.05$, paired *t*-test) inhibition of HERG currents were observed with pimozide concentrations as low as 3 nM (16 ± 2% reduction, $n = 6$). Pi-

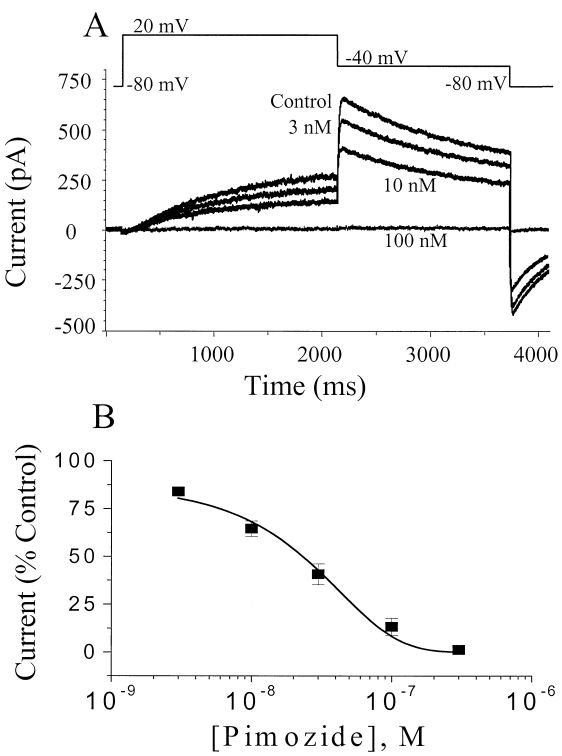

Fig. 1. Effects of pimozide on HERG. (A) Whole-cell HERG currents were elicited by a 2-s depolarizing pulse to +20 mV from a holding potential of −80 mV. The cell was then returned to −40 mV to generate large outward tail currents. The effects of 3, 10, and 100 nM pimozide are shown. (B) Dose–response relationship for pimozide block of peak tail currents at −40 mV. The IC_{50} value and Hill slope measured 18.1 nM and −1.04, respectively. Error bar denote S.E.M. ($n = 6$–9).

mozide had no detectable effect on tail current kinetics. In separate experiments, when cells were returned to a potential of −100 mV, inward HERG tail currents decayed with a time constant of 95 ± 12 ms ($n = 5$). This value was not significantly altered in the presence of 30 nM pimozide and measured 104 ± 11 ms ($n = 5$). The effects of pimozide were not reversible upon washing the cell with drug-free solution for several minutes.

Fig. 2 shows the effects of pimozide on HERG currents during more prolonged voltage steps. Current was activated by 6 s depolarizations to +20 mV from a holding potential of −80 mV. Following several depolarizing pulses, the cell was held at −80 mV for 3 min while 300 nM pimozide was allowed to wash in. The first pulse after this equilibration period showed a reduction in peak current as well as an additional time-dependent block of the current, which developed over the course of the depolarizing step (Fig. 2). Single exponential fit of this block yielded a time constant of 2.01 ± 0.28 s ($n = 5$). Subsequent depolarizing pulses delivered at 45-s intervals showed little or no time-dependent component of block, suggesting that pimozide had not dissociated to any great extent from the channel during the inter-pulse interval.

We also examined the effects pimozide on two other cloned human cardiac K^+ channels, KvLQT1/minK which underlies I_{Ks} in the human heart and Kv1.5 which underlies I_{Kur}. KvLQT1/minK currents were elicited by 4-s depolarizing pulses to +20 mV from a holding potential of −80 mV. Current measured at the end of these pulses was reduced by 27 ± 5% ($n = 5$) by 10 μM pimozide.

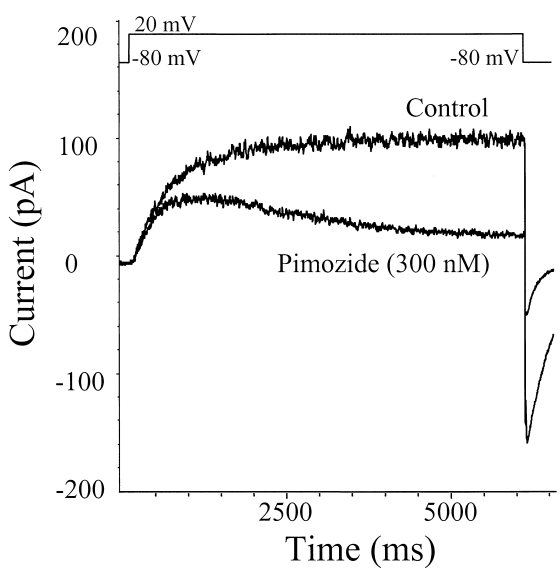

Fig. 2. Effects of pimozide on HERG during prolonged depolarizations. Whole-cell HERG channel currents were elicited by 6-s depolarizations to +20 mV from a holding potential of −80 mV. After control pulses were obtained, the cell was exposed to pimozide for 3 min without pulsing the cell. The first pulse following this equilibration period is pictured and showed a reduction in peak current as well as a time-dependent component of block that developed during the course of the pulse.

Kv1.5 currents were elicited from inside-out membrane patches by 1-s depolarizations to +50 mV from a holding potential of −80 mV (Rampe et al., 1998). Kv1.5 current measured at the end of these pulses was reduced by 29 ± 6% ($n = 5$) by 10 μM pimozide. This drug concentration was the highest obtainable due to solubility limitations.

4. Discussion

This report is the first to detail the effects of the neuroleptic agent pimozide on voltage-dependent K^+ channels cloned from the human heart. We found that pimozide was a potent inhibitor of HERG channel currents displaying an IC_{50} value of 18 nM. Significant inhibition of current was observed at concentrations as low as 3 nM. During prolonged depolarizing pulses to +20 mV, pimozide was shown to enhance the rate of HERG current decay suggesting some interaction with an activated state of the channel. However, since peak current was also reduced, we do not exclude a further interaction of the drug with the closed state of the channel. The effects of pimozide were poorly reversible, suggesting an intracellular site of action for this hydrophobic drug. In contrast to its effects on HERG, pimozide demonstrated little affinity for KvLQT1/minK or Kv1.5, inhibiting these channels by less than 30% at 10 μM. These results suggest that pimozide inhibits HERG with high affinity and is selective for this channel relative to other human cardiac K^+ channels.

Pimozide has been shown to prolong the QT interval and may be associated with torsades de pointes type ventricular arrhythmia (Fulop et al., 1987; Krahenbuhl et al., 1995; Desta et al., 1999). QT prolongation has been observed following therapeutic doses of pimozide, in cases of overdose, and may be enhanced by agents which slow its metabolism including macrolide antibiotics (Fulop et al., 1987; Krahenbuhl et al., 1995; Desta et al., 1999). Recently, the manufacturer of pimozide has issued a ''Dear Doctor'' letter warning of such interactions (ORAP: Important labeling changes, 1999). Pimozide blocks Ca^{2+} channels in the mid-nanomolar range (Luchowski et al., 1984; Enyeart et al., 1990) and this activity has been suggested as the cause of these ECG changes (Fulop et al., 1987; Krahenbuhl et al., 1995). The data presented here strongly suggest that the most likely explanation for these effects is high affinity block of the HERG cardiac K^+ channel. The therapeutic effects of pimozide depend on blockade of the dopamine D_2 receptor that occurs with an affinity of approximately 3–4 nM (Tam and Cook, 1984). Thus, concentrations of pimozide that block dopamine receptors in vitro are similar to those that also produce significant reduction in HERG channel current. Pimozide now joins a growing list of drugs including antihistamines

(Roy et al., 1996), antipsychotics (Rampe et al., 1998) and prokinetic agents (Mohammad et al., 1997; Rampe et al., 1997) that prolong cardiac repolarization via a high affinity interaction with HERG. The present report also underscores the dangers of prescribing pimozide with other drugs that inhibit HERG, where additive effects may be expected to occur.

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