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Inhibition of human ether-a-go-go-related gene potassium channels by α 1-adrenoceptor antagonists prazosin, doxazosin, and terazosin

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Abstract Human ether-a-go-go-related gene (HERG) potassium channels are expressed in multiple tissues including the heart and adenocarcinomas. In cardiomyocytes, HERG encodes the α -subunit underlying the rapid component of the delayed rectifier potassium current, I_{Kr} , and pharmacological reduction of HERG currents may cause acquired long QT syndrome. In addition, HERG currents have been shown to be involved in the regulation of cell proliferation and apoptosis. Selective α 1-adrenoceptor antagonists are commonly used in the treatment of hypertension and benign prostatic hyperplasia. Recently, doxazosin has been associated with an increased risk of heart failure. Moreover, quinazoline-derived α 1-inhibitors induce apoptosis in cardiomyocytes and prostate tumor cells independently of α 1-adrenoceptor blockade. To assess the action of the effects of prazosin, doxazosin, and terazosin on HERG currents, we investigated their acute electrophysiological effects on cloned HERG potassium channels heterologously expressed in *Xenopus* oocytes and HEK 293 cells.

Prazosin, doxazosin, and terazosin blocked HERG currents in *Xenopus* oocytes with IC_{50} values of 10.1, 18.2, and 113.2 μ M respectively, whereas the IC_{50} values for HERG channel inhibition in human HEK 293 cells were 1.57 μ M, 585.1 nM, and 17.7 μ M. Detailed biophysical studies revealed that inhibition by the prototype α 1-blocker prazosin occurred in closed, open, and inactivated channels. Analysis of the voltage-dependence of block displayed a reduction of inhibition at positive membrane

potentials. Frequency-dependence was not observed. Prazosin caused a negative shift in the voltage-dependence of both activation (-3.8 mV) and inactivation (-9.4 mV). The S6 mutations Y652A and F656A partially attenuated (Y652A) or abolished (F656A) HERG current blockade, indicating that prazosin binds to a common drug receptor within the pore-S6 region.

In conclusion, this study demonstrates that HERG potassium channels are blocked by prazosin, doxazosin, and terazosin. These data may provide a hypothetical molecular explanation for the apoptotic effect of quinazoline-derived α 1-adrenoceptor antagonists.

Keywords Apoptosis · Arrhythmia · Doxazosin · HERG · Long QT syndrome · Prazosin · Potassium channel · Terazosin

Introduction

Selective α 1-adrenoceptor antagonists such as prazosin, doxazosin, and terazosin are established antihypertensive agents, lowering blood pressure by reducing vascular tone in resistance and capacitance vessels (Grimm 1989). Several studies have shown that they may improve other cardiovascular risk factors as well, including lipid profile, insulin sensitivity, left ventricular hypertrophy, platelet aggregation and fibrinolysis (Pool 1996). Moreover, α 1-inhibitors are generally associated with a reasonably low incidence of serious adverse effects (Grimm 1989). The short-acting α 1-antagonist prazosin, and the long-acting compounds terazosin and doxazosin are members of the quinazoline chemical class. Recently, serious concerns have been raised by data from the Antihypertensive and Lipid Lowering Treatment to Prevent Heart Attack Trial (ALLHAT 2000). The risk of congestive heart failure (CHF) was twice as high with doxazosin as with chlorthalidone, forcing discontinuation of the doxazosin arm of ALLHAT and suggesting that doxazosin or even all α -blockers should no longer be used as first-line antihypertensive therapy. Doxazosin and prazosin have been shown to in-

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duce apoptosis in cultured cardiomyocytes at concentrations of 1 to 40 μM (Gonzalez-Juanatey et al. 2003). This might explain the adverse cardiovascular effect observed in the ALLHAT study, since apoptosis is known to occur in myocardial dysfunction and heart failure (Kang and Izumo 2000).

Alpha1-inhibitors are also widely used in the treatment of symptomatic benign prostatic hyperplasia (BPH), where α 1-receptors mediate increased tension in prostatic smooth muscle, thereby producing lower urinary tract symptoms. Selective α 1-adrenoceptor antagonists relax prostatic smooth muscle, relieve bladder outlet obstruction, and enhance urine flow (Akduman and Crawford 2001). Interestingly, quinazoline-derived α 1-adrenoceptor antagonists exert long-term clinical responses that are not fully explained by relaxation of prostate smooth muscle. It has been demonstrated that doxazosin (15 μM) and terazosin (25 μM) induce apoptosis in prostate tumor epithelial cells and prostate tissue obtained from patients with benign prostatic hyperplasia, while the structurally unrelated, sulfonamide-based α 1-blocker tamsulosin was ineffective (Kyprianou and Benning 2000; Benning and Kyprianou 2002; Kyprianou 2003).

This apoptotic effect of α 1-blockers on cardiomyocytes and prostate cells was independent of antiadrenergic action (Benning and Kyprianou 2002; Gonzalez-Juanatey et al. 2003; Kyprianou 2003). The exact molecular mechanism, however, remains to be investigated. Considering the clinical significance of quinazoline-induced apoptosis in CHF, benign prostatic hyperplasia and prostate cancer, elucidation of the underlying molecular mechanism is of particular interest.

Voltage-gated potassium channels are increasingly recognized as modulators of cell proliferation and apoptosis (Wonderlin and Strobl 1996). In particular, human ether-a-go-go-related gene (HERG) potassium channels (Warmke and Ganetzky 1994) are expressed in a variety of tumor cells including adenocarcinoma (Bianchi et al. 1998; Cherubini et al. 2000; Crociani et al. 2003). In heart tissue, the HERG channel is the molecular counterpart of the rapid component of the repolarizing delayed rectifier potassium current, I_{Kr} (Sanguinetti et al. 1995). Mutations in HERG account for chromosome 7-linked inherited long QT syndrome (LQT-2), a potentially lethal cardiac repolarization disorder (Viskin 1999; Thomas et al. 2003a). Pharmacological blockade of I_{Kr} causes lengthening of the cardiac action potential, which may produce a beneficial class III antiarrhythmic effect (Kiehn et al. 1999; Thomas et al. 2001). On the other hand, excessive prolongation of the cardiac action potential may lead to acquired long QT syndrome and life-threatening "torsade de pointes" arrhythmias (Napolitano et al. 1994; Thomas et al. 2002, 2003b). In tumor cells, HERG expression facilitates cell proliferation (Wang et al. 2002), and inhibition of HERG currents has been shown to reduce proliferation (Smith et al. 2002). Furthermore, HERG channels are involved in the regulation of tumor cell apoptosis (Wang et al. 2002).

These findings prompted the hypothesis that quinazoline-derived α 1-adrenoceptor antagonists cause direct block

of HERG potassium channels. To assess the pharmacological action of these drugs on HERG currents, the inhibitory effects of the prototype α 1-blocker prazosin on HERG currents were elucidated in detail in the present study. In addition, HERG current block by doxazosin and terazosin was investigated.

Materials and methods

Molecular biology. The HERG cDNA clone was generously donated by Dr. M.T. Keating, and the hMiRP1 clone was kindly provided by Dr. S.A. Goldstein. Procedures for in vitro transcription and oocyte injection have been published previously (Kiehn et al. 1999). Briefly, HERG wild type (Warmke and Ganetzky 1994), hMiRP1 (Abbott et al. 1999), HERG Y652A, and HERG F656A (Scholz et al. 2003) cRNAs were prepared with the mMACHINE mMACHINE kit (Ambion, Austin, TX, USA) using SP6 RNA polymerase after linearization with *Eco*RI (Roche Diagnostics, Mannheim, Germany). Stage V-VI defolliculated *Xenopus* oocytes were injected with 46 nl of cRNA per oocyte.

The cDNA encoding the HERG potassium channel cloned in pCDNA3 was stably transfected into the human embryonic kidney cell line HEK 293 as described previously (Thomas et al. 2001). Cells were cultured in Dulbecco's Modified Eagle Medium/Nutrient Mixture F-12 (D-MEM/F12, Gibco BRL, Rockville, USA) supplemented with 10% fetal bovine serum (FBS, Gibco BRL), 100 U/ml penicillin G sodium, 100 $\mu\text{g}/\text{ml}$ streptomycin sulphate and 500 $\mu\text{g}/\text{ml}$ geneticin (G418, Gibco BRL) in an atmosphere of 95% humidified air and 5% CO_2 at 37°C.

The investigation conforms to the Guide for the Care and Use of Laboratory Animals published by the US National Institute of Health (NIH Publication No. 85-23, revised 1996). All experiments followed the European Community guidelines for the use of experimental animals.

Electrophysiology and statistics. Two-microelectrode voltage-clamp recordings from *Xenopus laevis* oocytes were carried out as published previously (Thomas et al. 1999). In brief, recordings were performed using a Warner OC-725A amplifier (Warner Instruments, Hamden, CT, USA) and pClamp software (Axon Instruments, Foster City, CA, USA) for data acquisition and analysis. Microelectrodes had tip resistances ranging from 1 to 5 M Ω . The recording chamber was continually perfused. All experiments were carried out at room temperature (20–22°C), and no leak subtraction was done during the experiments. HERG current recordings from HEK 293 cells were performed by use of the whole-cell patch clamp configuration (Hamill et al. 1981) as previously reported (Thomas et al. 2001). All experiments were carried out at room temperature (22–25°C).

Concentration-response relationships for drug-induced block were fit with a Hill equation of the following form: $I_{\text{drug}}/I_{\text{control}} = 1/[1+(D/IC_{50})^{nH}]$ where I indicates current, D is the drug concentration, nH is the Hill coefficient, and IC_{50} is the concentration necessary for 50% block. Activation curves were fit with a single-power Boltzmann distribution of the form $I_{\text{tail}} = I_{\text{tail,max}}/[1+e^{(V_{1/2}-V)/k}]$ where V is the test pulse potential, $V_{1/2}$ is the half-maximal activation potential, and k is the slope of the activation curve. Inactivation curves were fit to the following single-power Boltzmann equation: $I = I_{\text{max}}/[1+e^{(V-V_{1/2})/k}]$.

All data are expressed as mean \pm standard deviation, unless otherwise specified. We used paired and unpaired Student's t -tests (two-tailed tests) to compare the statistical significance of the results: $p < 0.05$ was considered statistically significant. Multiple comparisons were performed using one-way ANOVA. If the hypothesis of equal means could be rejected at the 0.05-level, pairwise comparisons of groups were made and the probability values were adjusted for multiple comparisons using the Bonferroni correction.

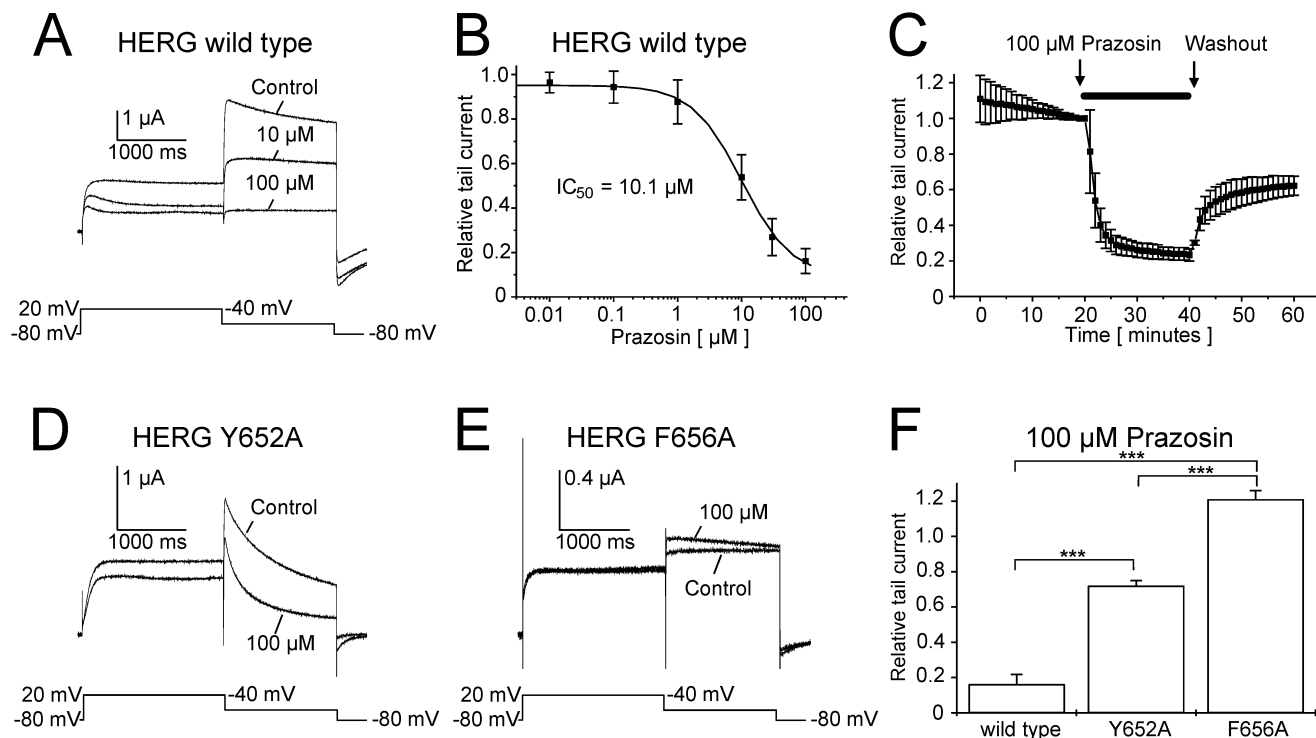


Fig. 1A–F Inhibition of HERG channels by prazosin. **A** Representative current traces recorded from the same cell under control conditions and after superfusion with prazosin (10 μM and 100 μM). Current amplitudes were monitored during control periods and during 10 min of drug application at 0.1 Hz pulsing frequency. **B** concentration-response relationship for the effect of prazosin on HERG peak tail currents ($n=6-10$ oocytes). The IC_{50} yielded 10.1 μM. **C** Time course of HERG tail current inhibition by 100 μM prazosin ($n=5$). For simplicity, not all current measurements are displayed. After a control period of 20 min, currents decreased rapidly upon perfusion with the drug solution within 5 min. Subsequent 15 min of drug application caused weak additional block (<9%). **D, E** Original current traces illustrating the effects of 100 μM prazosin on mutant HERG Y652A and HERG F656A currents. **F** Mean relative tail current amplitudes after application of 100 μM prazosin (10 min) are shown for HERG wild type ($n=10$), HERG Y652A ($n=7$), and HERG F656A currents ($n=7$) respectively. The inhibitory effects of prazosin were attenuated (Y652A) or completely abolished (F656A) (** $p < 0.001$; see text for voltage protocols)

Solutions and drug administration. Voltage clamp measurements of *Xenopus* oocytes were performed in a solution containing (in mM): 5 KCl, 100 NaCl, 1.5 CaCl₂, 2 MgCl₂, and 10 HEPES (pH 7.4 with NaOH). Current and voltage electrodes were filled with 3 M KCl solution. For whole-cell patch clamp recordings from HEK 293 cells electrodes were filled with the following solution (in mM): 130 K-aspartate, 5.0 MgCl₂, 5 EGTA, 4 ATP, 10 HEPES, (pH adjusted to 7.2 with KOH). The external solution for these experiments contained (in mM): 137 NaCl, 4.0 KCl, 1.0 MgCl₂, 1.8 CaCl₂, 10 HEPES, 10 glucose (pH adjusted to 7.4 with NaOH). Prazosin (Sigma) was prepared as 10 mM stock solution in methanol and stored at -20°C. Doxazosin (Sigma) was prepared as 10 mM stock solution in DMSO and stored at -20°C. Terazosin (Sigma) was prepared as 10 mM stock solution in water and stored at -20°C. To prepare a 1 mM solution, terazosin was dissolved directly with the bath solution. On the day of experiments, aliquots of the stock solutions were diluted to the desired concentrations with the bath solution. HERG current amplitudes recorded from *Xenopus* oocytes, as well

as the half-maximal voltages for activation and inactivation were not significantly altered during a control period of 10 min ($n=8-9$; data not shown) or upon application of 1% (v/v) methanol (maximum bath concentration; $n=5$; data not shown). In addition, superfusion with 1% (v/v) DMSO (maximum bath concentration) for 10 min did not significantly affect HERG current amplitudes ($n=8$; data not shown).

Results

Prazosin blocks HERG potassium currents

Prazosin blocked HERG potassium channels expressed in *Xenopus laevis* oocytes in a concentration-dependent manner, as displayed in Fig. 1. Currents were elicited by a 2 s depolarizing step to +20 mV followed by a repolarizing step to -40 mV for 1.6 s to produce large, slowly decaying outward tail currents which are a characteristic of HERG potassium channels (Sanguinetti et al. 1995). The holding potential was -80 mV in all experiments performed in this study, unless indicated otherwise. Pulses were applied at a frequency of 0.1 Hz during superfusion with the drug solution for 10 min. After the monitoring period, pulses were applied to determine the degree of block (Fig. 1A). To study the concentration dependence of HERG current block by prazosin, HERG peak tail currents were normalized to the respective control values and plotted as relative current amplitudes in Fig. 1B ($n=6-10$ oocytes were investigated at each concentration). The half-maximal inhibition concentration (IC_{50}) for block of tail currents was 10.1 μM with a Hill coefficient n_H of 1.12.

The time course of block is shown in Fig. 1C ($n=5$). The onset of block was fast. After a control period of 20 min, HERG channel block by 100 μM prazosin occurred

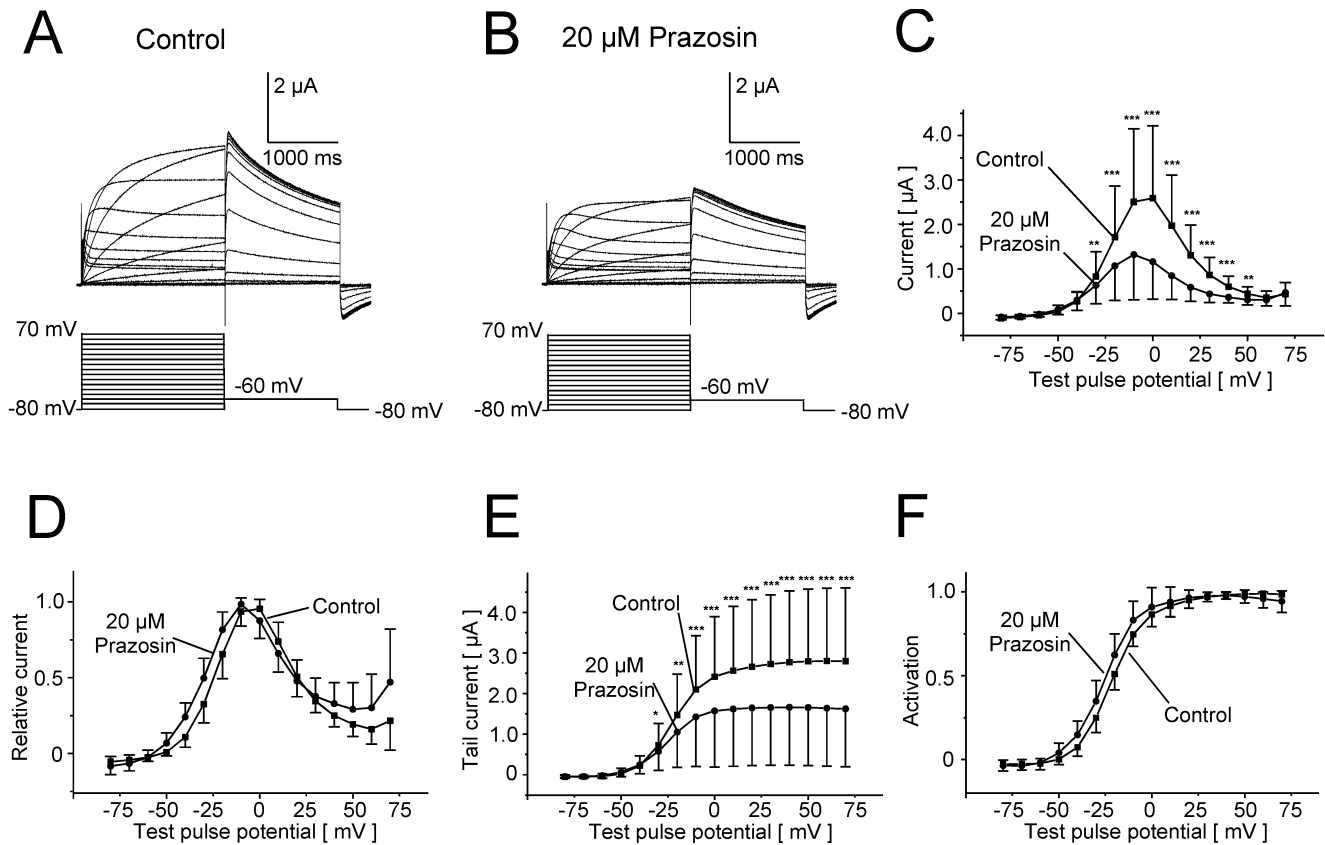


Fig. 2A–F Effects of prazosin on the voltage dependence of HERG activation. **A** Control measurement and **B** the inhibitory effects of 20 μ M prazosin (10 min) are shown in one representative oocyte. **C**, **D** Resulting current amplitudes at the end of the test pulse as a function of the preceding test pulse potential under control conditions and after incubation with prazosin (**C**, original current amplitudes; **D**, values normalized to peak step currents; $n=12$). **E**, **F** Activation curves, i.e., the peak tail current amplitudes as function of the preceding test pulse potential during the first step of the voltage protocol, recorded under isochronal conditions (**E**, original current amplitudes; **F**, values normalized to peak tail currents; $n=9$). The half-maximal activation potential $V_{1/2}$ was shifted by -3.8 mV (** $p<0.001$; ** $p<0.01$; * $p<0.05$; see text for voltage protocols)

rapidly within approximately 5 min. During the following 15 min of drug application a slight increase in the degree of block (less than 9%) could be observed. Upon washout, the blocking effects on HERG were partially reversible within 20 min (Fig. 1C).

Incomplete attenuation of prazosin block by Y652A mutation

It has been demonstrated that the aromatic residues Y652 and F656 located in the S6 domain are key determinants of drug binding to HERG channels (Mitcheson et al. 2000a). Thus, the effect of prazosin on mutant HERG Y652A and HERG F656A channels was investigated to assess the significance of these residues in prazosin blockade of HERG currents. Voltage protocols were applied as described

above to record currents under control conditions and after application of 100 μ M prazosin, reducing HERG wild type currents to $16.1\pm 5.6\%$ ($n=10$; Fig. 1F). As illustrated in Fig. 1F, the inhibitory effect of prazosin was significantly attenuated (Y652A) or even abolished (F656A) by replacement of aromatic channel pore residues. Mean relative current amplitudes measured after prazosin application yielded $71.9\pm 3.0\%$ (Y652A; $n=7$) and $120.9\pm 5.1\%$ (F656A; $n=7$) of control currents, respectively.

Effects of prazosin on HERG current activation

The effect of prazosin on HERG current voltage (I-V) relationship was investigated under isochronal recording conditions. Depolarizing pulses were applied for 2 s to voltages between -80 and $+70$ mV in 10 mV increments (0.2 Hz), and tail currents were recorded during a constant repolarizing step to -60 mV for 1.6 s. Families of current traces from one cell are shown for control conditions and after exposure to 20 μ M prazosin (10 min) in Fig. 2A, B. The currents activated at potentials greater than -50 mV, reached a peak at 0 mV and then decreased at more positive potentials due to inactivation (Sanguinetti et al. 1995), giving the I-V relationship its typical bell-shaped appearance (Fig. 2C, D). Figure 2E, F display peak tail currents as a function of the preceding test pulse potential, resulting in activation curves. The peak tail current, measured during the repolarizing second step of the voltage protocol, increased with voltage steps from -40 mV to

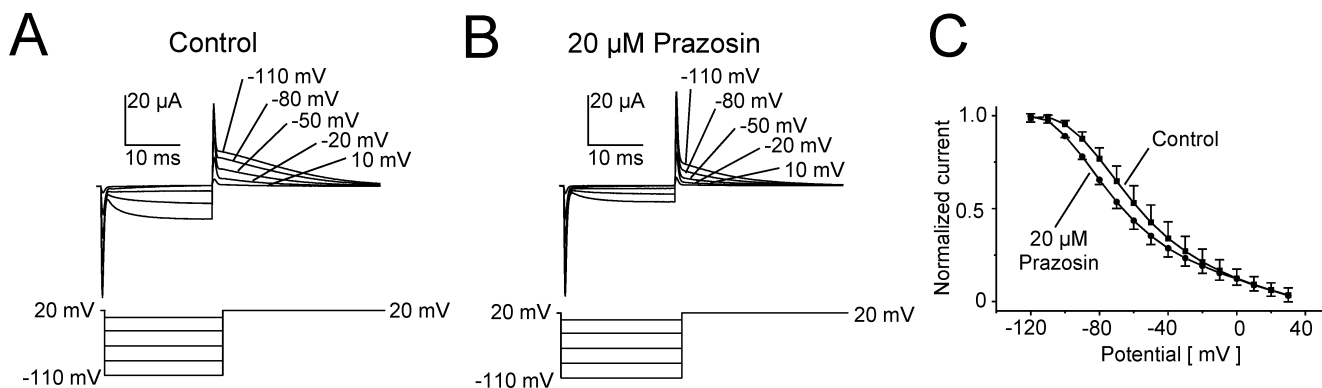


Fig. 3A–C Effects of prazosin on HERG current inactivation. **A, B** Measurements of the steady-state inactivation at constant 20 mV after various potentials from -120 to 30 mV (increment 10 mV). Note that, for clarity, not all original current traces are displayed. **C** The normalized mean inactivating current amplitudes at 20 mV, giving the steady-state inactivation curves. The mean half-maximal inactivation voltage was shifted by 9.4 mV towards more negative potentials ($n=6$; see text for voltage protocols)

$+20$ mV and then plateaued for test pulse potentials positive to $+20$ mV (Fig. 2E). HERG currents at the end of the test pulse to 0 mV were reduced by $53.3 \pm 11.3\%$, and peak tail currents were blocked by $40.2 \pm 12.5\%$ ($n=12$). The half-maximal activation voltage $V_{1/2}$ (Fig. 2F) was shifted by -3.8 ± 3.1 mV from -20.9 ± 3.9 mV under control conditions to -24.7 ± 4.5 mV after prazosin incubation ($n=9$). This difference was also statistically significant when compared with time and solvent controls.

Effects of prazosin on HERG channel inactivation

To measure steady-state inactivation relationships, channels were inactivated at a holding potential of 20 mV, before being recovered from inactivation at various potentials from -120 to 30 mV (increment 10 mV) for 20 ms. Finally, the resulting peak outward currents at constant 20 mV as a measure of steady-state inactivation were recorded. After having obtained the control measurements (Fig. 3A), we applied $20 \mu\text{M}$ prazosin to the oocytes. The holding potential was -80 mV during the monitoring period of 10 min to avoid destruction of the cell, as it would occur when holding the cell at 20 mV. One typical recording in the presence of the drug is displayed in Fig. 3B. The inactivating outward current amplitude measured at 20 mV was normalized and plotted against the test pulse potential, giving the steady-state inactivation curve (Fig. 3C). Mean values for the half-maximal inactivation voltage yielded -58.3 ± 6.8 mV for control and -67.8 ± 3.9 mV for prazosin measurements ($n=6$), displaying a shift of -9.4 ± 6.4 mV. This difference was also statistically significant when compared with time and solvent controls.

The biophysical mechanism of HERG current inhibition by prazosin

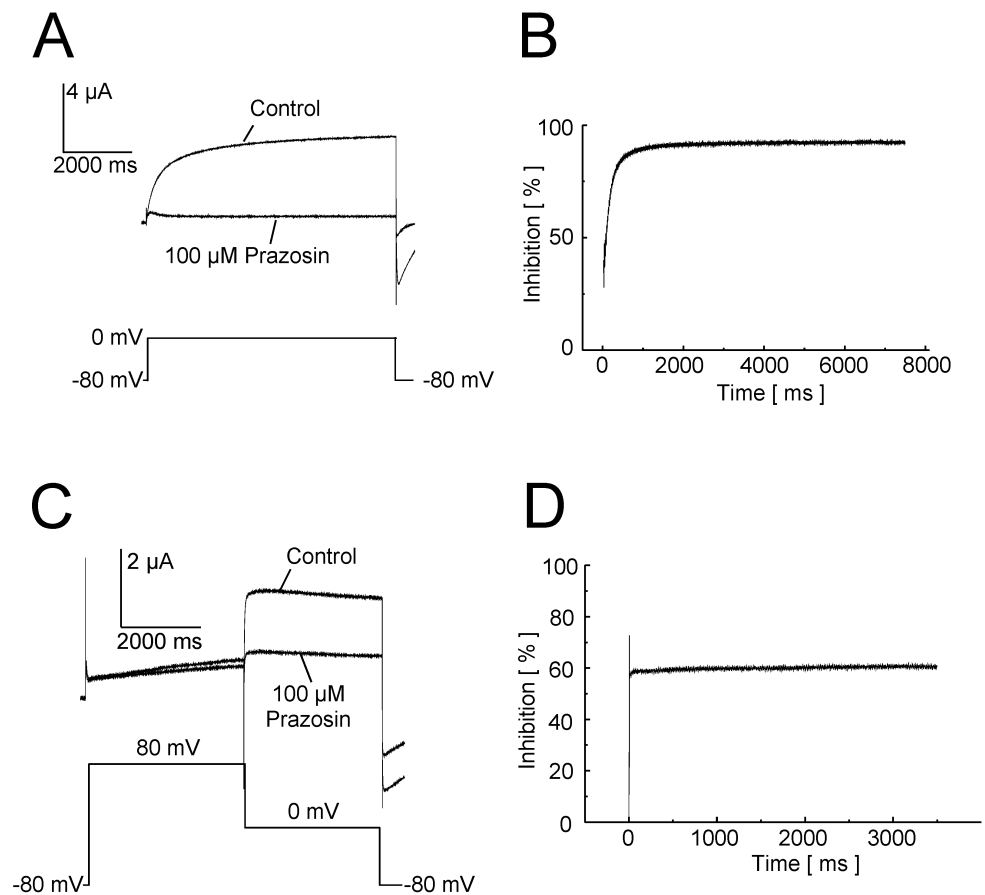
To investigate whether the channel is blocked in the closed or activated (i.e., open and/or inactivated) state, we activated currents using a protocol with a single depolarizing step to 0 mV for 7.5 s. After having obtained the control measurement, we allowed $100 \mu\text{M}$ of the drug to wash in for 10 min while holding all channels in the closed state at -80 mV. Then, measurements with prazosin were performed (Fig. 4A). The degree of inhibition, i.e., $((1 - \text{current in the presence of prazosin} / \text{control current}) \times 100)$ after the incubation period is displayed in Fig. 4B. Analysis of the test pulse after prazosin application revealed a time-dependent increase of block to 87.5% at 500 ms (Fig. 4B), which is consistent with block of activated HERG potassium channels. Some degree of closed state block cannot be ruled out by this protocol. In this series of experiments, prazosin reduced HERG outward currents at the end of the 0 -mV pulse by $78.5 \pm 15.7\%$ ($n=8$).

To address the question whether HERG channels are blocked by prazosin in the inactivated state, a long test pulse to $+80$ mV (4 s) was applied to inactivate the channels, followed by a second voltage step (0 mV, 3.5 s) to open HERG channels ($n=8$). Typical current traces under control conditions and after application of $100 \mu\text{M}$ prazosin for 10 min while holding the cell at -80 mV are displayed in Fig. 4C. Figure 4D depicts the normalized relative block upon channel opening during the second voltage pulse (0 mV), illustrating that pronounced inhibition of HERG channels had already been obtained during the preceding inactivating $+80$ -mV pulse, and virtually no additional time-dependent block of open channels was observed during the 0 -mV pulse. It is concluded from these experiments that prazosin inhibits HERG channels predominantly in the open and inactivated state.

Voltage-dependence of HERG channel block by prazosin

To determine the voltage-dependence of HERG channel block, we applied the following methodical approach. Since unblocking was slow, only one experiment at each potential could be carried out with one individual oocyte. Currents were elicited by 34 s depolarizing pulses ranging

Fig. 4A–D Block of activated HERG channels by prazosin. After having recorded the control measurement, the oocyte was held at -80 mV for 10 min during superfusion with the drug solution ($100 \mu\text{M}$ prazosin). **A** The control recording and the first pulse measured immediately after the incubation period. **B** Degree of inhibition in %. Current inhibition increased time-dependently to 87.5% at 500 ms in this representative experiment, indicating that mainly open and/or inactivated channels were blocked. **C** Inhibition of inactivated channels by $100 \mu\text{M}$ prazosin. HERG channels were inactivated by a first voltage step to $+80$ mV, followed by channel opening at 0 mV. **D** The corresponding relative block during the 0-mV step. Maximum inhibition was already achieved in the inactivated state, and weak further time-dependent development of block occurred upon channel opening after the inactivating voltage step (see text for voltage protocols)



from -40 mV to 80 mV, and peak inward tail currents were measured during a second step to -120 mV (400 ms). First, control currents were recorded. Then the oocyte was superfused with the drug solution ($20 \mu\text{M}$ prazosin) while holding the cell at constant -80 mV for 10 min where HERG channels are in the closed state. After this, measurements at the test pulse potential were performed. Relative inhibition of peak tail currents was plotted as function of the preceding test pulse potential in Fig. 5A ($n=5-6$ cells studied at each potential). Prazosin reduced HERG currents in a voltage-dependent manner, with block being more pronounced at negative membrane potentials, indicating that drug block was prevented by strong channel inactivation.

Lack of frequency-dependence of prazosin block

The frequency-dependence of block was investigated in the following series of experiments. HERG potassium channels were rapidly activated by a depolarizing step to 20 mV for 300 ms, followed by a repolarizing step to -40 mV (300 ms) to elicit outward tail currents. Pulses were applied at intervals of 1 or 10 s under control conditions and in the presence of $20 \mu\text{M}$ prazosin, with each cell studied only at one stimulation rate. Six oocytes were used at each rate. The development of current reduction was plotted

versus time (Fig. 5B), with the resulting level of steady-state block being a measure of the frequency-dependence of block. There were no pronounced changes in the amount of steady-state block at both rates. Thus, block was not frequency-dependent.

Inhibition of HERG currents by the $\alpha 1$ -adrenoceptor antagonists doxazosin and terazosin

HERG channel blockade by terazosin and doxazosin was investigated using the *Xenopus* oocyte system as described above for prazosin (Fig. 1). Both drugs inhibited HERG currents in a concentration-dependent manner (Fig. 6A, C), revealing IC_{50} values of $18.2 \mu\text{M}$ (doxazosin; $n=4-8$ cells; Fig. 6B) and $113.2 \mu\text{M}$ (terazosin; $n=4-11$ cells; Fig. 6D) with respective Hill coefficients n_H of 1.11 (doxazosin) and 0.94 (terazosin).

Quinazoline-derived $\alpha 1$ -adrenoceptor antagonists block HERG channels in a human cell line

To demonstrate block of HERG by prazosin, doxazosin, and terazosin in human cells, we expressed HERG potassium channels heterologously in human embryonic kidney (HEK 293) cells. Channels were activated by a 2 s de-

Fig. 5 **A** Prazosin block of HERG currents is voltage-dependent. The fraction of blocked peak tail currents is plotted as function of various test pulse potentials. HERG channel block was reduced by channel inactivation at increasing potentials ($n=5-6$ cells). **B** Lack of frequency-dependence of HERG channel block by prazosin. The resulting mean relative tail current amplitudes (1 Hz and 0.1 Hz stimulation rate) are plotted versus time ($n=6$ oocytes were studied at each rate). For the purpose of clear presentation, not all measurements are displayed (see text for voltage protocols)

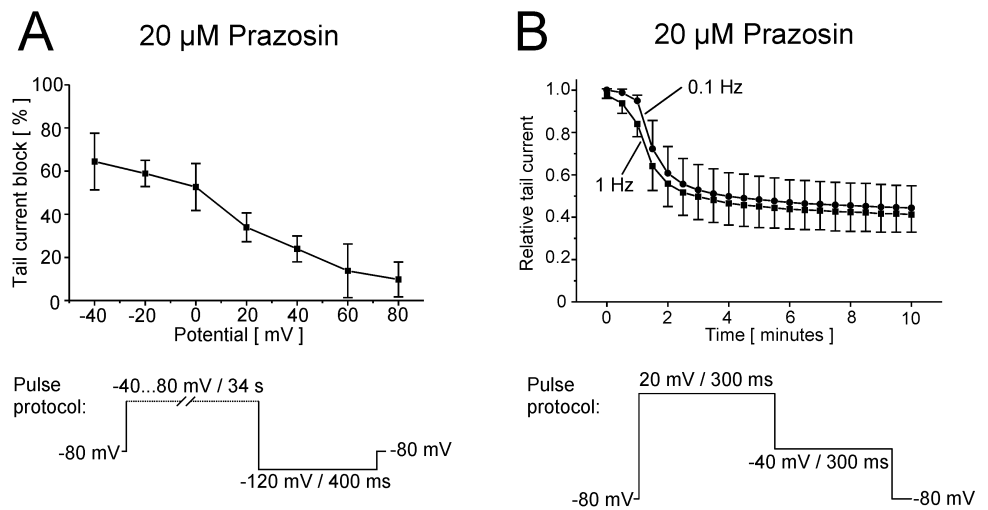
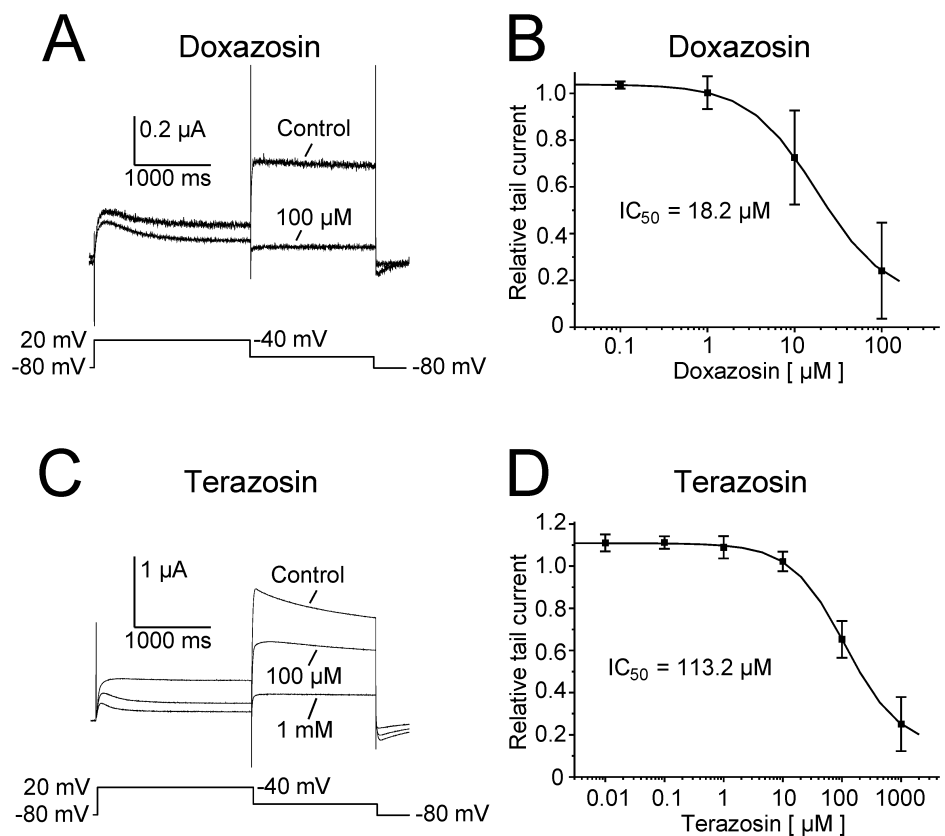


Fig. 6A-D Inhibition of HERG currents by doxazosin and terazosin in *Xenopus* oocytes. **A, C** Currents were elicited as described above for prazosin (Fig. 1). **B, D** Concentration-response relationships for the effects of doxazosin ($n=4-8$) and terazosin ($n=4-11$) on HERG peak tail currents. The IC_{50} values were 18.2 μM (doxazosin) and 113.2 μM (terazosin) respectively



polarization to +20 mV, and outward tail currents were recorded during a step to -50 mV for 2 s (Fig. 7A-C). During the wash-in of the drug we applied the protocol as described above (frequency 0.1 Hz), until a steady state block was maintained for at least 30 s. HERG currents were blocked by quinazoline-derived α_1 -adrenoceptor antagonists in a concentration-dependant manner. The IC_{50} values for block of HERG tail currents were 1.57 μM (prazosin; $n=3$), 585.1 nM (doxazosin; $n=3$ cells (10 μM : 2 cells) studied at each concentration), and 17.7 μM (terazosin; $n=3$), with Hill coefficients n_H of 1.00, 1.12, and 0.95 respectively (Fig. 7D-F).

Discussion

Acute effects of prazosin, doxazosin, and terazosin on HERG currents

Our results indicate that quinazoline-derived α_1 -blockers inhibit HERG potassium channels in *Xenopus* oocytes and human HEK 293 cells. Application of prazosin resulted in a concentration-dependent HERG current decrease at moderate drug concentrations ($\text{IC}_{50}=10.1 \mu\text{M}$), and blockade of HERG channels by doxazosin and terazosin dis-

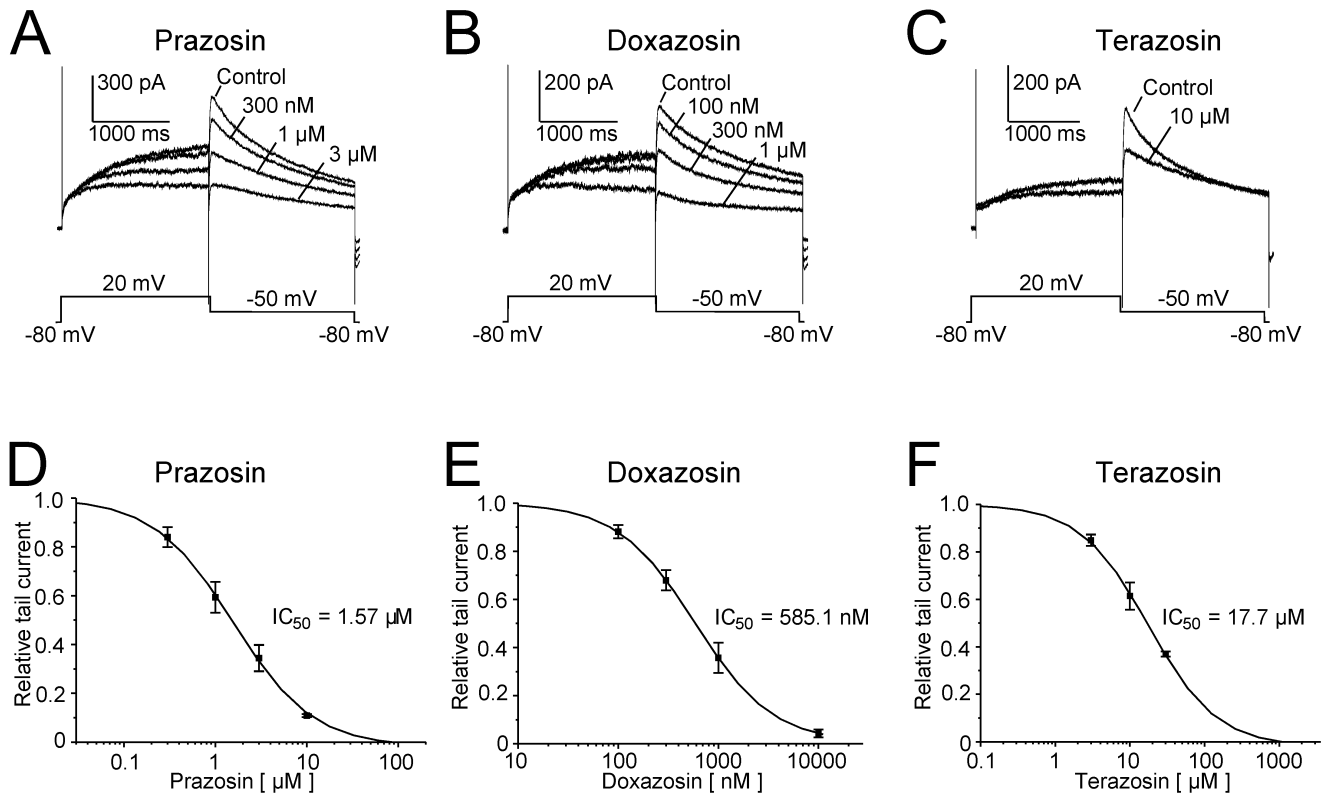


Fig. 7A–F Blockade of HERG channels expressed in human HEK 293 cells by prazosin, doxazosin and terazosin. **A–C** Typical whole cell patch clamp recordings from single HEK cells under control conditions and after application of prazosin (**A**), doxazosin (**B**), and terazosin (**C**). **D–F** Concentration–response curves for inhibition of HERG peak tail currents in HEK 293 cells, yielding IC₅₀ values of 1.57 μM (prazosin; $n=3$ cells), 585.1 nM (doxazosin; $n=2–3$ cells) and 17.7 μM (terazosin; $n=3$ cells). Error bars denote SD (see text for voltage protocols)

played IC₅₀ values of 18.2 μM and 113.2 μM in *Xenopus* oocytes. HERG currents recorded from HEK 293 cells were inhibited with IC₅₀ values of 1.57 μM (prazosin), 585.1 nM (doxazosin) and 17.7 μM (terazosin). This 6.4 to 31.1-fold difference corresponds to previous reports comparing drug block of HERG currents in different expression systems. IC₅₀ values for HERG channel block have been shown to be approximately 10 to 20-fold higher when the drug is applied to the extracellular surface of *Xenopus* oocytes compared to patch clamp experiments using mammalian cells (Thomas et al. 2001, 2003b, 2003c), which is due to specific properties of the *Xenopus* oocyte expression system (e.g., the vitelline membrane and the yolk) that reduce the actual concentration of drugs at the cell membrane.

Therapeutic plasma concentrations in humans yield 58–98 nM for prazosin (Chaignon et al. 1981; Pitterman et al. 1981), 42–177 nM for doxazosin (Shionoiri et al. 1987; Fawzy et al. 1999), and 44–235 nM for terazosin (Patterson 1985; Taguchi et al. 1998), respectively. Protein binding has been reported to be 92–97% (prazosin; Jaillon 1980), 98.3% (doxazosin; Elliott et al. 1987), and

90–94% (terazosin; Sonders 1986). In conclusion, taking into consideration that the IC₅₀ values for block of HERG in human cells are close to the range of therapeutic plasma concentrations, it is reasonable to assume that HERG current inhibition by prazosin, terazosin, and particularly doxazosin may be of physiological relevance. In the present study, *Xenopus* oocytes, which do not express endogenous adrenoceptors, were used to further assess the inhibitory effects of the prototype quinazoline-derived α₁-blocker prazosin on HERG potassium currents. This approach allowed detailed pharmacological and biophysical studies, without the necessity to discriminate between different native ion currents on the basis of kinetics or pharmacology.

It has been suggested that co-assembly of the regulatory β-subunit MiRP1 with HERG is required in order to reconstitute native I_{Kr} (Abbott et al. 1999). This hypothesis has been investigated by Weerapura et al. (2002) in detail, revealing that co-expression of HERG with wild type MiRP1 (in contrast to long QT syndrome-linked mutant MiRP1; Abbott et al. 1999) does not alter its sensitivity to HERG-blocking drugs. In the present study, the IC₅₀ value for HERG channel inhibition by prazosin in *Xenopus* oocytes (10.1 μM) did not markedly differ from the value obtained from HERG coexpressed with MiRP1 under similar experimental conditions (12.4 μM; $n=7$ cells studied at each concentration; data not shown). This is in line with other reports, where drug effects on HERG currents were not altered by co-expression with MiRP1 WT (for summary, see Thomas et al. 2003c). Thus, it is suggested that co-expression with MiRP1 does not provide additional information about the pharmacological and biophysical mechanisms of HERG channel block.

The biophysical mechanism of HERG channel blockade by prazosin

The inhibitory effects of the prototype α 1-blocker prazosin were investigated in detail. One important finding of this study is that prazosin blocks HERG channels predominantly in the open and inactivated state. In addition, the pronounced block at negative membrane potentials supports the hypothesis that prazosin binding to closed channels occurs as well. Prazosin application caused a -3.8 mV shift in the HERG activation curve and a -9.4 mV shift in the half-maximal inactivation voltage, which may cause a net increase of current if other biophysical parameters remained unchanged. However, due to pronounced pharmacological HERG channel block, current increase could not be detected in our study.

HERG channel block by prazosin was reduced at positive membrane potentials, probably due to channel inactivation. This observation could be explained by the following mechanism: The drug binding site is more likely to be accessible for prazosin when the channel is in the open (in contrast to inactivated) state, similar to the block of HERG potassium channels by BRL-32872 and fluoxetine (Thomas et al. 2001, 2002). This suggests that preferentially open channels are blocked by prazosin, although the voltage protocols do not clearly distinguish between open and inactivated states. Unblocking upon repolarization, which allows HERG channels to become available for opening, occurred rather slowly, and a complete washout could not be achieved. The lack of frequency-dependence can be interpreted as the result of fast blocking and slow unblocking kinetics, possibly due to a trapping mechanism of the drug at its binding site (Mitcheson et al. 2000b).

The structural requirements for the drug binding site in HERG as a basis for the unusual susceptibility of this potassium channel to block by structurally diverse drugs have recently been studied in detail. It has been demonstrated that the aromatic rings of Y652 and particularly F656 located in the S6 domain are key determinants of drug binding (Mitcheson et al. 2000a), since mutation of these residues to alanine dramatically reduced the potency of most drugs tested to date (Mitcheson 2003). The lack of HERG F656A current inhibition clearly shows that prazosin predominantly binds to a drug receptor within the pore-S6 region. However, few drugs including prazosin are relatively insensitive to mutation of Y652, indicating that additional mechanisms of HERG channel block exist (Mitcheson 2003).

Physiological and clinical significance of HERG current inhibition by quinazoline-derived α 1-adrenoceptor antagonists

In comparison to other HERG channel inhibitors that cause acquired long QT-syndrome, quinazoline-derived α 1-antagonists seem to have virtually no proarrhythmic potential, suggesting that HERG current block does not neces-

sarily lead to severe cardiac arrhythmias. Similar observations have been made during clinical or experimental application of several antiarrhythmic drugs which are known to block HERG potassium channels, such as amiodarone, verapamil, BRL-32872, and carvedilol, suggesting that HERG current block does not generally lead to severe cardiac arrhythmias with the risk of sudden cardiac death (Kiehn et al. 1999; Zhang et al. 1999; Thomas et al. 2001; Karle et al. 2001). The relatively mild proarrhythmic potential might be due to additional pharmacological effects on ion currents and adrenergic receptors, as suggested earlier (Bril et al. 1996; Chouabe et al. 1998; Zhang et al. 1999; Thomas et al. 2001, 2002). In particular, the possible proarrhythmic action of HERG current inhibition might be counteracted by the prevention of arrhythmias via α 1A-adrenoceptor blockade, since α 1A-adrenergic stimulation may induce arrhythmias through modification of HERG channel activation (Jiang et al. 1999; Bian et al. 2001; Thomas et al. 2003d).

Taking into consideration the antiproliferative effect of HERG channel inhibition observed in tumor cells (Smith et al. 2002), it is reasonable to hypothesize that HERG current block by quinazoline-derived α 1-antagonists might lead to apoptosis in cardiomyocytes, in prostate tissue obtained from patients with benign prostatic hyperplasia, and in prostate tumor epithelial cells (Kyprianou and Benning 2000; Benning and Kyprianou 2002; Gonzalez-Juanatey et al. 2003; Kyprianou 2003). The selective estrogen modulator tamoxifen, which is widely used in the treatment of breast cancer, has been shown to block HERG currents as well (Thomas et al. 2003c), which might contribute to the antiproliferative action of tamoxifen. Considering this evidence, it is tempting to speculate on a general, clinically relevant concept that quinazoline-derived α 1-antagonists exert apoptotic effects via inhibition of HERG potassium currents.

Conclusion

Our results demonstrate that prazosin, doxazosin, and terazosin are inhibitors of cloned HERG potassium channels. We speculate that these data might provide a possible molecular explanation for the apoptotic effect of quinazoline-derived α 1-adrenoceptor antagonists. Furthermore, this mechanism might represent an exciting starting point for future development of novel compounds targeting cancer cell apoptosis.

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References

- Abbott GW, Sesti F, Splawski I, Buck ME, Lehmann MH, Timothy KW, Keating MT, Goldstein SA (1999) MiRP1 forms IKr potassium channels with HERG and is associated with cardiac arrhythmia. *Cell* 97:175–187
- Akduman B, Crawford ED (2001) Terazosin, doxazosin, and prazosin: current clinical experience. *Urology* 58:49–54
- ALLHAT Research Group (2000) Major cardiovascular events in hypertensive patients randomized to doxazosin vs chlorthalidone: the Antihypertensive and Lipid-Lowering Treatment to Prevent Heart Attack Trial (ALLHAT). *JAMA* 283:1967–1975
- Benning CM, Kyprianou N (2002) Quinazoline-derived α 1-adrenoceptor antagonists induce prostate cancer cell apoptosis via an α 1-adrenoceptor-independent action. *Cancer Res* 62:597–602
- Bian J, Cui J, McDonald TV (2001) HERG K(+) channel activity is regulated by changes in phosphatidyl inositol 4,5-bisphosphate. *Circ Res* 89:1168–1176
- Bianchi L, Wible B, Arcangeli A, Tagliatela M, Morra F, Castaldo P, Crociati O, Rosato B, Faravelli L, Olivotto M, Wanke E (1998) HERG encodes a K+ current highly conserved in tumors of different histogenesis: a selective advantage for cancer cells? *Cancer Res* 58:815–822
- Bril A, Gout B, Bonhomme M, Landais L, Faivre JF, Linee P, Poyser RH, Ruffalo RR (1996) Combined potassium and calcium channel blocking activities as a basis for antiarrhythmic efficacy with low proarrhythmic risk: experimental profile of BRL-32872. *J Pharmacol Exp Ther* 276:637–646
- Chaignon M, Le Roux E, Aubert P, Lucsko M, Safar M, Flouvat B, Guedon J (1981) Clinical pharmacology of prazosin in hypertensive patients with chronic renal failure. *J Cardiovasc Pharmacol* 3:151–160
- Cherubini A, Taddei GL, Crociani O, Paglierani M, Buccoliero AM, Fontana L, Noci I, Borri P, Borrani E, Giachi M, Becchetti A, Rosati B, Wanke E, Olivotto M, Arcangeli A (2000) HERG potassium channels are more frequently expressed in human endometrial cancer as compared to non-cancerous endometrium. *Br J Cancer* 83:1722–1729
- Chouabe C, Drici MD, Romey G, Barhanin J, Lazdunski M (1998) HERG and KvLQT1/IsK, the cardiac K+ channels involved in long QT syndromes, are targets for calcium channel blockers. *Mol Pharmacol* 54:695–703
- Crociani O, Guasti L, Balzi M, Becchetti A, Wanke E, Olivotto M, Wymore RS, Arcangeli A (2003) Cell-cycle-dependent expression of HERG1 and HERG1B isoforms in tumor cells. *J Biol Chem* 278:2947–2955
- Elliott HL, Meredith PA, Reid JL (1987) Pharmacokinetic overview of doxazosin. *Am J Cardiol* 59:78G–81G
- Fawzy A, Vashi V, Chung M, Dias N, Gaffney M (1999) Clinical correlation of maximal urinary flow rate and plasma doxazosin concentrations in the treatment of benign prostatic hyperplasia. Multicenter Study Group. *Urology* 53:329–335
- Gonzalez-Juanatey JR, Iglesias MJ, Alcaide C, Pinero R, Lago F (2003) Doxazosin induces apoptosis in cardiomyocytes cultured in vitro by a mechanism that is independent of α 1-adrenergic blockade. *Circulation* 107:127–131
- Grimm RH Jr (1989) Alpha 1-antagonists in the treatment of hypertension. *Hypertension* 13:1131–1136
- Hamill OP, Marty A, Neher E, Sakman B, Sigworth FJ (1981) Improved patch clamp techniques for high resolution current recording from cells and cell free membrane patches. *Pflügers Arch Eur J Physiol* 391:58–100
- Jailion P (1980) Clinical pharmacokinetics of prazosin. *Clin Pharmacokinet* 5:365–376
- Jiang M, Dun W, Fan JS, Tseng GN (1999) Use-dependent 'agonist' effect of azimilide on the HERG channel. *J Pharmacol Exp Ther* 291:1324–1336
- Kang PM, Izumo S (2000) Apoptosis and heart failure: a critical review of the literature. *Circ Res* 99:6252–6256
- Karle CA, Kreye VAW, Thomas D, Rockl K, Kathofer S, Zhang W, Kiehn J (2001) Antiarrhythmic drug carvedilol inhibits HERG potassium channels. *Cardiovasc Res* 49:361–370
- Kiehn J, Thomas D, Karle CA, Schöls W, Kübler W (1999) Inhibitory effects of the class III antiarrhythmic drug amiodarone on cloned HERG potassium channels. *Naunyn-Schmiedeberg Arch Pharmacol* 359:212–219
- Kyprianou N (2003) Doxazosin and terazosin suppress prostate growth by inducing apoptosis: clinical significance. *J Urol* 169:1520–1525
- Kyprianou N, Benning CM (2000) Suppression of human prostate cancer cell growth by α 1-adrenoceptor antagonists doxazosin and terazosin via induction of apoptosis. *Cancer Res* 60:4550–4555
- Mitcheson JS (2003) Drug binding to HERG channels: evidence for a 'non-aromatic' binding site for fluvoxamine. *Br J Pharmacol* 139:883–884
- Mitcheson JS, Chen J, Lin M, Culberson C, Sanguinetti MC (2000a) A structural basis for drug-induced long QT syndrome. *Proc Natl Acad Sci USA* 97:12329–12333
- Mitcheson JS, Chen J, Sanguinetti MC (2000b) Trapping of a methanesulfonanilide by closure of the HERG potassium channel activation gate. *J Gen Physiol* 115:229–240
- Napolitano C, Priori S, Schwartz P (1994) Torsade de pointes: mechanism and management. *Drugs* 47:51–65
- National Institutes of Health (1996) NIH publication no. 85–23, revised edn. NIH, Bethesda, MD
- Patterson SE (1985) Terazosin kinetics after oral and intravenous doses. *Clin Pharmacol Ther* 38:423–427
- Pitterman AB, Rollins DE, Shen DD, Hurwitz A, Hassanein KM (1981) Alpha adrenoceptor blockade with oral prazosin. *Clin Pharmacol Ther* 29:143–148
- Pool JL (1996) Doxazosin: a new approach to hypertension and benign prostatic hyperplasia. *Br J Clin Pract* 50:154–163
- Sanguinetti MC, Jiang C, Curran ME, Keating MT (1995) A mechanistic link between an inherited and an acquired cardiac arrhythmia: HERG encodes the I_{Kr} potassium channel. *Cell* 81:299–307
- Scholz EP, Zitron E, Kiesecker C, Lueck S, Kathöfer S, Thomas D, Weretka S, Peth S, Kreye VAW, Schoels W, Katus HA, Kiehn J, Karle CA (2003) Drug binding to aromatic residues in the HERG channel pore cavity as possible explanation for acquired long QT syndrome by antiparkinsonian drug bupropion. *Naunyn-Schmiedeberg Arch Pharmacol* 368:404–414
- Shionoiri H, Yasuda G, Yoshimura H, Umemura S, Miyajima E, Miyakawa T, Takagi N, Kaneko Y (1987) Antihypertensive effects and pharmacokinetics of single and consecutive administration of doxazosin in patients with mild to moderate essential hypertension. *J Cardiovasc Pharmacol* 10:90–95
- Smith GAM, Tsui HW, Newell EW, Jiang X, Zhu XP, Tsui FWL, Schlichter LC (2002) Functional up-regulation of HERG K+ channels in neoplastic hematopoietic cells. *J Biol Chem* 277:18528–18534
- Sonders RC (1986) Pharmacokinetics of terazosin. *Am J Med* 80:20–24
- Taguchi K, Schafers RF, Michel MC (1998) Radioreceptor assay analysis of tamsulosin and terazosin pharmacokinetics. *Br J Clin Pharmacol* 45:49–55
- Thomas D, Zhang W, Karle CA, Kathofer S, Schols W, Kiehn J (1999) Deletion of protein kinase A phosphorylation sites in the HERG potassium channel inhibits activation shift by protein kinase A. *J Biol Chem* 274:27457–27462
- Thomas D, Wendt-Nordahl G, Röckl K, Ficker E, Brown AM, Kiehn J (2001) High-affinity blockade of HERG human cardiac potassium channels by the novel antiarrhythmic drug BRL-32872. *J Pharmacol Exp Ther* 297:753–761
- Thomas D, Gut B, Wendt-Nordahl G, Kiehn J (2002) The antidepressant drug fluoxetine is an inhibitor of human ether-a-go-go-related gene (HERG) potassium channels. *J Pharmacol Exp Ther* 300:543–548

- Thomas D, Kiehn J, Katus HA, Karle CA (2003a) Defective protein trafficking in hERG-associated hereditary long QT syndrome (LQT2): molecular mechanisms and restoration of intracellular protein processing. *Cardiovasc Res* 60:239–245
- Thomas D, Wu K, Kathöfer S, Katus HA, Schoels W, Kiehn J, Karle CA (2003b) The antipsychotic drug chlorpromazine inhibits HERG potassium channels. *Br J Pharmacol* 139:567–574
- Thomas D, Gut B, Karsai S, Wimmer AB, Wu K, Wendt-Nordahl G, Zhang W, Kathofer S, Schoels W, Katus HA, Kiehn J, Karle CA (2003c) Inhibition of cloned HERG potassium channels by the antiestrogen tamoxifen. *Naunyn-Schmiedeberg Arch Pharmacol* 368:41–48
- Thomas D, Zhang W, Wu K, Wimmer AB, Gut B, Wendt-Nordahl G, Kathofer S, Kreye VAW, Katus HA, Schoels W, Kiehn J, Karle CA (2003d) Regulation of HERG potassium channel activation by protein kinase C independent of direct phosphorylation of the channel protein. *Cardiovasc Res* 59:14–26
- Viskin S (1999) Long QT syndromes and torsade de pointes. *Lancet* 354:1625–1633
- Wang H, Zhang Y, Cao L, Han H, Wang J, Yang B, Nattel S, Wang Z (2002) HERG K⁺ channel, a regulator of tumor cell apoptosis and proliferation. *Cancer Res* 62:4843–4848
- Warmke JW, Ganetzky B (1994) A family of potassium channel genes related to eag in *Drosophila* and mammals. *Proc Natl Acad Sci USA* 91:3438–3442
- Weerapura M, Nattel S, Chartier D, Caballero R, Hebert TE (2002) A comparison of currents carried by HERG, with and without coexpression of MiRP1, and the native rapid delayed rectifier current. Is MiRP1 the missing link? *J Physiol* 540:15–27
- Wonderlin WF, Strobel JS (1996) Potassium channels, proliferation and G1 progression. *J Membr Biol* 154:91–107
- Zhang S, Zhou Z, Gong Q, Makielski JC, January CT (1999) Mechanism of block and identification of the verapamil binding domain to HERG potassium channels. *Circ Res* 84:989–998