

Effects of L-type Calcium Channel Antagonists Verapamil and Diltiazem on fKv1.4ΔN Currents in *Xenopus oocytes*

Hui Chen^{a,b}, Dong Zhang^{b*}, Jiang Hua Ren^b and Sheng Ping Chao^b

^aDepartment of Cardiology, Pu Ai Hospital of Wuhan City, Wuhan 430034, China.

^bDepartment of Cardiology, Zhongnan Hospital of Wuhan University, Wuhan 430071, China.

Abstract

The goal of this study was to determine the effects of the L-type calcium channel blockers verapamil and diltiazem on the currents of voltage-gated potassium channel (fKv1.4ΔN), an N-terminal-deleted mutant of the ferret Kv1.4 potassium channel. Measurements were made using a two electrode voltage clamp technique with channels expressed stably in *Xenopus oocytes*. The fKv1.4ΔN currents displayed slow inactivation, with a half-inactivation potential of -38.38 mV and slow recovery from inactivation ($\tau = 1.90$ seconds at -90 mV). The fKv1.4ΔN currents exhibited state-dependent blockade by both drugs, and the inhibition was frequency-, voltage-, and concentration-dependent, consistent with open channel block. Verapamil and diltiazem blocked fKv1.4ΔN currents with 50% inhibitory concentration (IC_{50}) values of 260.71 ± 18.50 $\mu\text{mol/L}$ and 241.04 ± 23.06 $\mu\text{mol/L}$, respectively. Verapamil accelerated the C-type inactivation rate and slowed recovery of the fKv1.4ΔN channel, while shifting the steady activation curve to the right. Blockade of fKv1.4ΔN currents by diltiazem was similar to that of verapamil, but diltiazem accelerated the decay rate of inactivation of fKv1.4ΔN currents without modifying the kinetics of current activation. The present results suggest that verapamil and diltiazem accelerate the C-type inactivation and slow the recovery of the fKv1.4ΔN channel in the open state.

Keywords: Kv1.4ΔN; Potassium channels; Activation; Inactivation; Verapamil; Diltiazem.

Introduction

The L-type calcium channel antagonists verapamil and diltiazem have been used widely for the treatment of hypertension, cardiac arrhythmia, and coronary vasospasm, and are effective in the treatment of angina pectoris (1, 2). In addition to L-type calcium channels, several voltage-gated potassium (Kv) channels are blocked by these antagonists. Verapamil has been reported to produce potent use- and frequency-dependent block of hERG channels,

(3) and to inhibit the hKv1.5 channel in low micromolar concentrations (4). Diltiazem may inhibit hKv1.5 and Kv4.3 currents by binding to the open and inactivated states (5-7). Verapamil has recently been shown to inhibit Kv1.4 currents expressed in *Xenopus oocytes* (6, 8). We also found that diltiazem may block the Kv1.4 channel currents, (9) but the detailed characteristics of this effect have not been studied.

Kv1.4 channels are expressed in the subendocardium of human and ferret heart and in the rat ventricular septum (10-12). This channel conducts transient outward potassium currents (I_{to}) that contribute to the early repolarization

* Corresponding author:

E-mail: highlightzhd@yahoo.com.cn

phase of the cardiac action potential. Kv1.4 expression in the mammalian endocardium is upregulated during hypertrophy and heart failure, (13-16) and this channel plays an important role in repolarization of cardiac myocytes. Kv1.4 inactivation is controlled by N- and C-type inactivation (17) N-type inactivation is faster, and C-type inactivation is slower, which results from conformational changes on the extracellular side of the pore (18, 19). Recovery from inactivation is governed by the slower C-type mechanism (20). Therefore, it is important to examine the effects of drugs on C-type inactivation.

In the present study, we studied the inhibitory effects verapamil and diltiazem on Kv1.4ΔN, an N-terminal deletion construct of Kv1.4 that lacks rapid N-type inactivation but exhibits robust C-type inactivation, (21) in order to provide a more detailed understanding of their mechanisms of action.

Experimental

Molecular biology

fKv1.4ΔN cDNA (GenBank accession no.U06156) was a gift from professor Randall L. Rasmusson (University at Buffalo, SUNY). The constructs and sequences have been described previously (20-22). Removal of residues 2-146 from the N-terminal domain of Kv1.4 results in the loss of the fast N-type inactivation but leaves slow C-type inactivation intact (20-22). Transcribed fKv1.4ΔN cRNA was prepared in vitro using an mMessage mMachine kit (T3 kit, Ambion, USA).

Isolation of oocytes and incubation

Mature female *Xenopus laevis* frogs were provided by the Chinese Academy of Science, Beijing, China. The frogs were cared for using standards approved by the Institutional Animal Care and Use Committee of the Wuhan University of China. Mature female frogs were anesthetized by immersion in tricaine solution (1.5 g/L, Sigma) for 30 min, and oocytes were removed surgically through a lateral incision in the lower abdomen, as described previously (9). Oocytes were digested by placing the ovarian lobes in a collagenase-containing, Ca²⁺-free oocyte Ringer's (OR₂) solution (mmol/L): 82.5

NaCl, 2 KCl, 1 MgCl₂, and 5 HEPES at pH 7.4, with 1 to 1.5 mg/mL collagenase (Type I, Sigma, USA). The oocytes were then shaken gently for about 1 h and washed several times with Ca²⁺-free OR₂ solution as described previously (23). Finally, defolliculated (stage IV) oocytes were selected and placed in ND96 solution (mmol/L): 96 NaCl, 2 KCl, 1 MgCl₂, 1.8 CaCl₂, and 5 HEPES at pH 7.4. Each oocyte was injected with about 25 to 50 nL fKv1.4ΔN cRNA using a microinjector (WPI, Sarasota) and incubated at 18°C in ND96 solution with 100 IU/mL penicillin for a min of 16 h.

Electrophysiology

The two-electrode voltage clamp technique was used for electrophysiological recordings. Microelectrodes were pulled with a two-stage puller (Narishige, Japan) and had tip resistances of 0.5 to 1.0 MΩ when filled with 3 mol/L KCl. The currents in voltage-clamp mode were recorded at room temperature using a preamplifier CA-1B (Dagan, USA) and were filtered at 2.5 KHz. Recordings were made in 2 mmol/L K_o⁺. Verapamil and diltiazem were dissolved in distilled water to give stock solutions of 100 mmol/L. All drugs were purchased from Sigma Chemical Co. (St Louis, MO, U.S.A). Drugs were perfused for 10 min before testing in order to allow equilibration with the oocytes. After this wash-on period, a series of 500-ms depolarizing pulses (from -90 to +50 mV, 1 min) were given to ensure steady-state block before beginning the experimental protocols (22).

Data analysis

Data were digitized and analyzed by using pCLAMP 9.0 (Axon, USA). Further analysis was performed using Clampfit 9.0 (Axon, USA) in combination with Microsoft Excel (Microsoft, USA) and Origin 6.0 software (Microcal Software, USA). Concentration-response curves were generated using the Hill equation. Inactivation time constants were obtained using a single or double exponential decay model fitted to the raw current tracings. Steady-state activation and inactivation were fitted to the Boltzmann equation, and the time course of recovery from inactivation was fitted to a single exponential equation. A paired Student's t-test

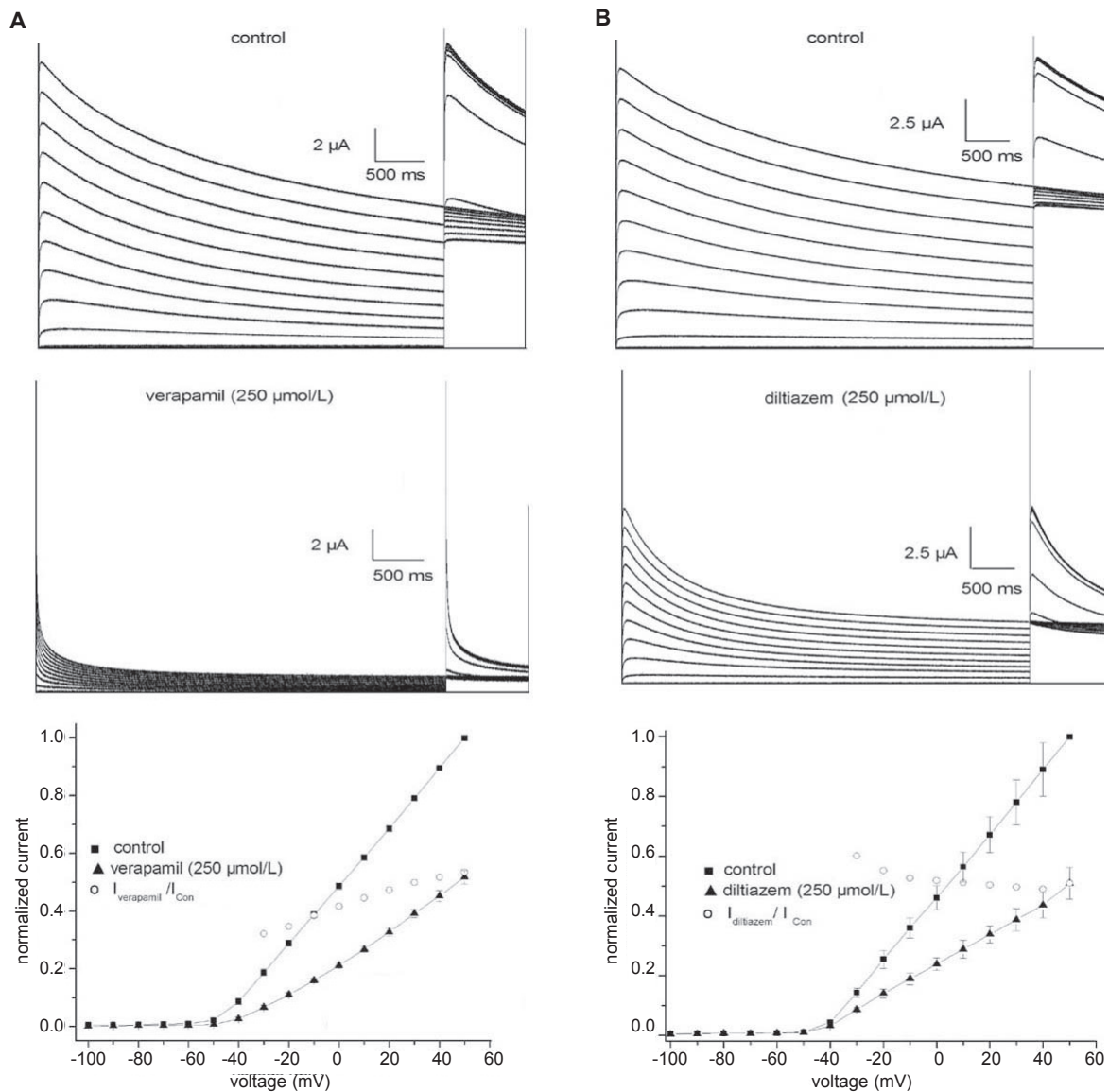


Figure 1. Effects of verapamil (A) and diltiazem (B) on fKv1.4AN channels expressed in *Xenopus* oocytes. Representative curves are shown for 5-second depolarizing pulses from -90 mV to voltages between -100 and $+50$ mV in steps of 10 mV. Upper panels: traces recorded under control conditions. Middle panels: current traces obtained in the presence of 250 $\mu\text{mol/L}$ verapamil or 250 $\mu\text{mol/L}$ diltiazem. The peak currents in the presence of verapamil and diltiazem were poorly resolved from the capacitance transient on this time scale. Bottom panels: effects of 250 $\mu\text{mol/L}$ verapamil and 250 $\mu\text{mol/L}$ diltiazem on the peak current-voltage (I-V) relationships. Currents were normalized to the peak current at $+50$ mV under control conditions. The $I_{\text{Drug}}/I_{\text{Control}}$ ratio was plotted as a function of the membrane potential. Data are shown as mean \pm SEM ($n = 5$).

was used for statistical analysis of the data. The results are expressed as mean \pm SEM, and values of $p < 0.05$ were considered significant.

Results

Inhibition of the fKv1.4AN currents by verapamil and diltiazem

Figure 1 shows traces of currents from

fKv1.4AN channels expressed in *Xenopus* oocytes under control conditions and in the presence of verapamil (A) or diltiazem (B). The cells were held at -90 mV and given 5-sec depolarizing pulses from -100 to $+50$ mV. Outward currents were followed by outward tail currents upon repolarization to $+50$ mV. Under control conditions (Figure 1, upper panels), positive depolarization to -40 mV elicited

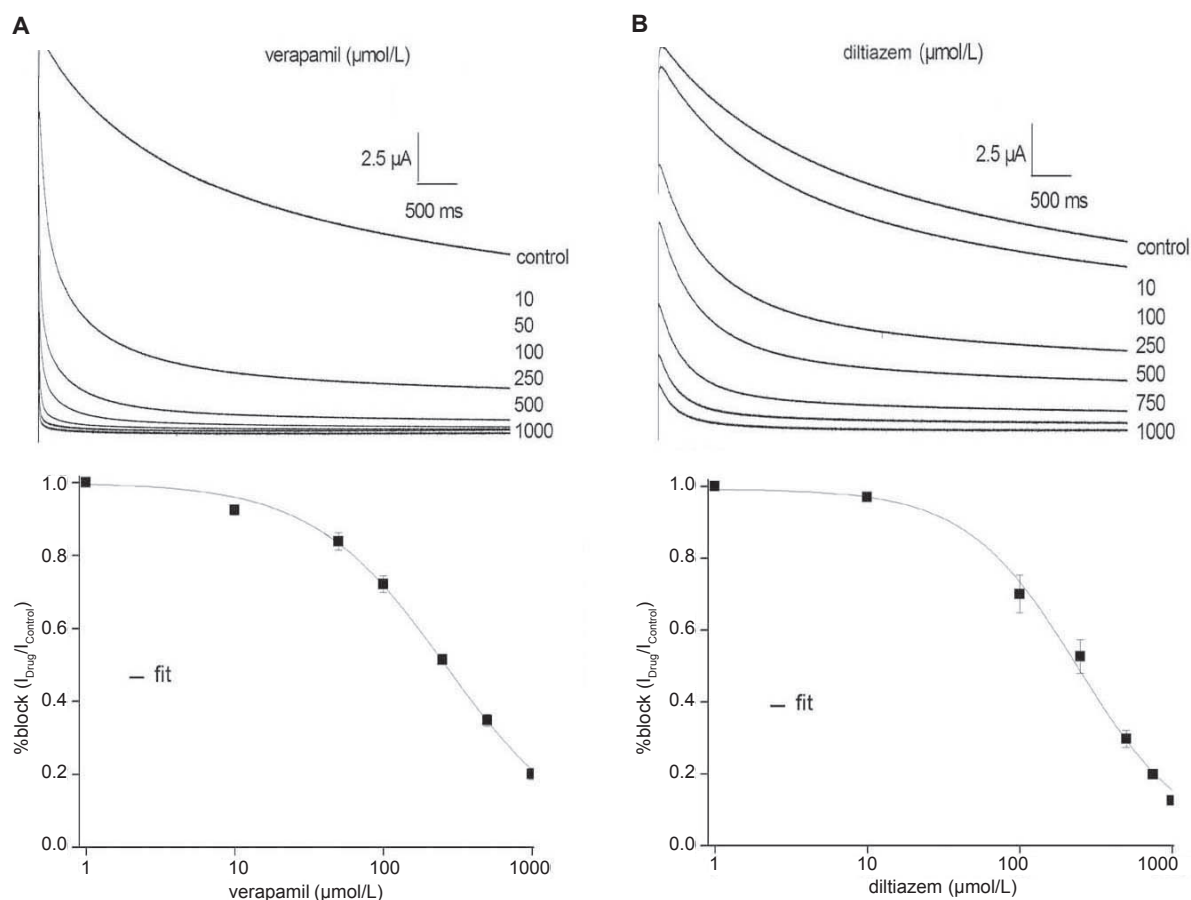


Figure 2. Concentration-response relationships for the inhibition of the fKv1.4ΔN currents by verapamil (A) and diltiazem (B). Upper panels: Representative current traces were elicited in the absence and presence of increasing concentrations of verapamil (A) and diltiazem (B). Currents were recorded by depolarizing pulses to +50 mV from a holding potential of -90 mV. Lower panels: The peak currents were normalized the maximum peak current under control conditions and plotted against verapamil (A) and diltiazem (B) concentrations. The curves were derived by fitting data to the Hill equation: $f = K_D / (K_D + D)$, where f is fractional current, K_D is the apparent dissociation constant, and D is the drug concentration. Symbols and error bar are mean \pm SEM ($n = 5$).

outward currents that declined slowly during the maintained depolarization (slow C-type inactivation). The middle panels of Figure 1 show the differential effectiveness of verapamil and diltiazem at an identical concentration (250 μmol/L). Both drugs not only reduced the current amplitude, but also accelerated the inactivation. Verapamil and diltiazem reduced by $53.5 \pm 6.2\%$ and $52.2 \pm 4.6\%$ ($n = 5$), respectively, the peak current elicited by pulses to +50 mV. The peak currents in the presence of verapamil and diltiazem were poorly resolved from the capacitance transient on the time scale. Verapamil and diltiazem showed similar potencies in inhibiting fKv1.4ΔN currents, and the inhibitory effects were completely abrogated by a 10 min washout of drug (data

not shown).

The effects of 250 μmol/L verapamil and 250 μmol/L diltiazem on the peak current-voltage (I-V) relationships for the fKv1.4ΔN channel are shown in Figure 1 (bottom panel). The I-V relationships were constructed by plotting the normalized currents as a function of the membrane potential. Verapamil and diltiazem induced a voltage-dependent inhibition of the fKv1.4ΔN currents. To quantify the voltage dependency of fKv1.4ΔN block, the relative current (I_{Drug}/I_{Con}) was plotted as a function of the membrane potential. The current begins to activate at the activation threshold (between -40 and -20 mV), and both drugs decreased the peak current at potentials positive to the

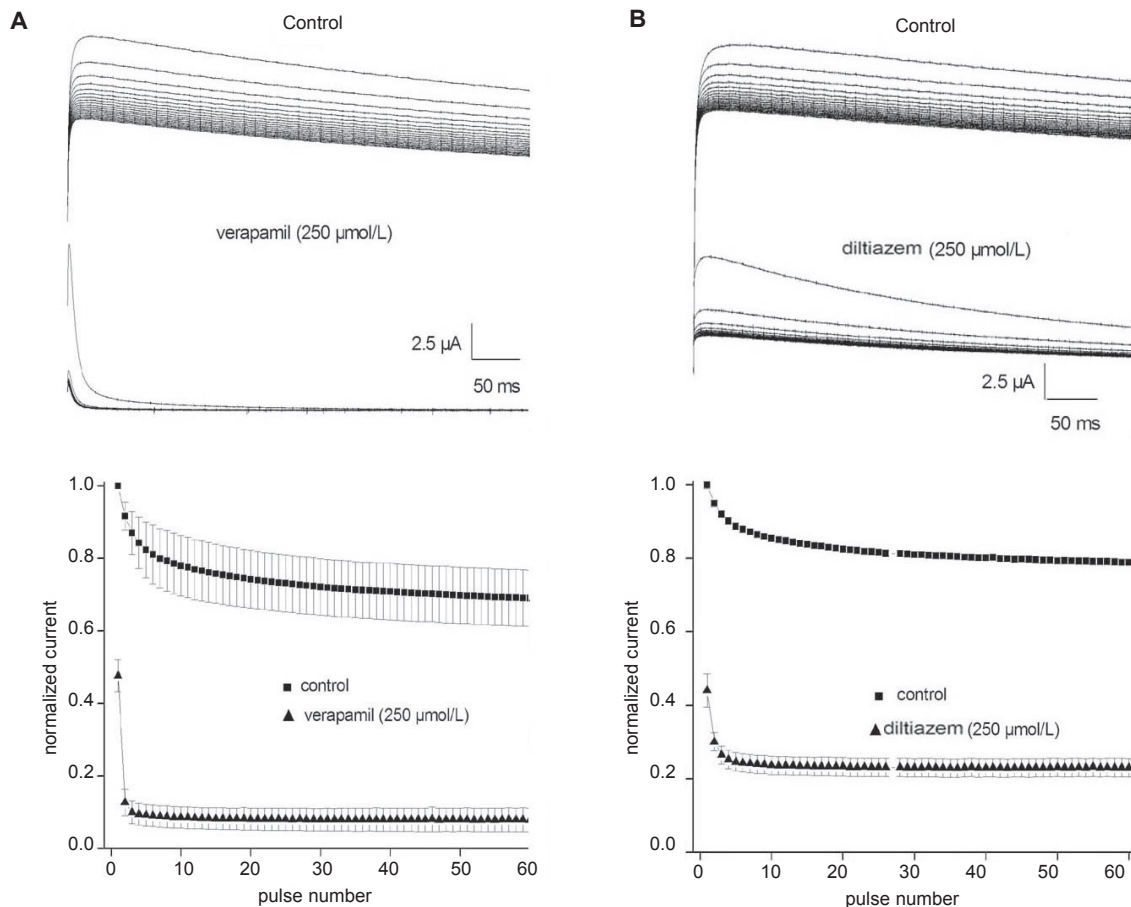


Figure 3. Use-dependent block of fKv1.4AN currents by verapamil (A) and diltiazem (B). Upper panels: Sixty repetitive depolarizing pulses from -90 to $+50$ mV for 500 ms each were applied in the absence and in the presence of verapamil (A) and diltiazem (B). Lower panels: Each peak current was normalized to the peak current at the first pulse under control conditions and then plotted against the number of pulses. Data are shown as mean \pm SEM ($n = 5$).

level of the activation threshold. The blockade remained constant at voltages above -20 mV.

Verapamil and diltiazem inhibit the fKv1.4AN currents in a concentration-dependent manner

Figure 2 shows representative fKv1.4AN currents superimposed on the variable concentrations of verapamil and diltiazem, between 0 and 1000 $\mu\text{mol/L}$, in order to evaluate concentration-dependency. The fKv1.4AN currents were recorded by depolarizing pulses to $+50$ mV from a holding potential of -90 mV. For steady-state inhibition, a nonlinear least-squares fit of concentration-response data at $+50$ mV to the Hill yielded a K_D value of 260.71 ± 18.50 $\mu\text{mol/L}$ (verapamil) and 241.04 ± 23.06 $\mu\text{mol/L}$ (diltiazem) ($n = 5$).

Use-dependent block of the fKv1.4AN currents by verapamil and diltiazem

Although 250 $\mu\text{mol/L}$ verapamil and 250 $\mu\text{mol/L}$ diltiazem induced approximately 50% steady-state block, this degree of block may not be attained during a single action potential. Therefore, we tested whether channel blockade by verapamil and diltiazem displayed use-dependence. Trains of 60 depolarizing pulses of 500 ms duration from -90 to $+50$ mV were applied at a stimulation frequency of 1 Hz, with a 1 min rest period between successive trains. The top panels in Figures 3A and B show original current records obtained after applying a pulse train protocol in the absence and presence of either 250 $\mu\text{mol/L}$ verapamil or 250 $\mu\text{mol/L}$ diltiazem, respectively. The peak currents were

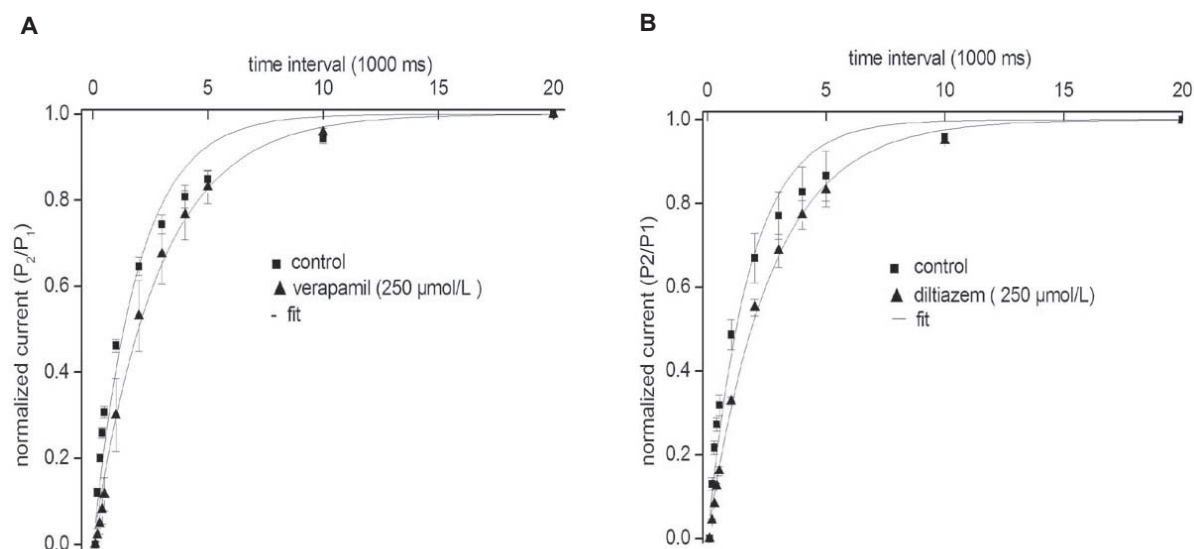


Figure 4. Effects of verapamil (A) and diltiazem (B) on the kinetics of the fKv1.4ΔN channel recovery from steady inactivation. The degree of recovery was measured by following a standard variable interval gapped pulse protocol. An initial 5-second pulse (P_1) from -90 to $+50$ mV was followed by a second pulse (P_2) to $+50$ mV after an interval of between 0.1 and 20 sec. The ratio of the peak current elicited by the P_1 and P_2 pulses (P_2/P_1) is plotted as a function of the various interpulse intervals. The continuous line represents the fit of the data to the equation: $f = 1 - A \cdot \exp(-\tau/t)$, where t is duration (in sec), τ is the time constant, A is the amplitude of the current. Data were normalized between 0 and 1 presented with intervals on a log scale. Data are shown as mean \pm SEM ($n = 5$).

normalized to the maximum control value without drug and plotted in the bottom panels (Figure 3). Under control conditions, the fKv1.4ΔN currents were decreased by 20-30%. However, fKv1.4ΔN currents in the presence of verapamil and diltiazem showed greater decay than under control conditions until reaching a steady-state block. The peak amplitudes of the fKv1.4ΔN currents after 60 depolarizing pulses were reduced by 83.4% in the presence of verapamil and by 47.8% in the presence of diltiazem. The degree of use-dependent block induced by verapamil was significantly greater than that induced by diltiazem.

Our results indicate that the recovery of the fKv1.4ΔN channel in the presence of either verapamil or diltiazem was slower than that in the absence of drugs. To investigate this result in more detail, we measured the recovery time from inactivation using a standard gapped-pulse protocol with a variable inter-stimulus interval. The ratio of the magnitude of the first to the second pulse peak current was used as an indication of the degree of the recovery from inactivation. Figure 4 shows representative

curves that were fitted with a mono-exponential function in the absence and in the presence of either 250 $\mu\text{mol/L}$ verapamil (A) or 250 $\mu\text{mol/L}$ diltiazem (B). The recovery time constant was slower in the presence of both drugs: (verapamil) 2.84 ± 0.17 sec, (control) 1.92 ± 0.12 sec ($n = 5$, $p < 0.05$); and (diltiazem) 2.66 ± 0.14 sec, (control) 1.73 ± 0.10 sec ($n = 5$, $p < 0.05$). Our findings showed that the rates of recovery of the fKv1.4ΔN channel from inhibition by verapamil and diltiazem were slower than the transition rate between the open and closed states in the absence of drugs, which may indicate that both drugs evoke use-dependent inhibition of fKv1.4ΔN currents.

Effects of verapamil and diltiazem on the activation of the fKv1.4ΔN currents

Figure 5 shows representative currents and steady-state activation relationships. The activation curves were measured in the absence and presence of either 250 $\mu\text{mol/L}$ verapamil (A) or 250 $\mu\text{mol/L}$ diltiazem (B), respectively. The peak currents were normalized to the peak current measured at $+50$ mV and were plotted as a function of the holding potential.

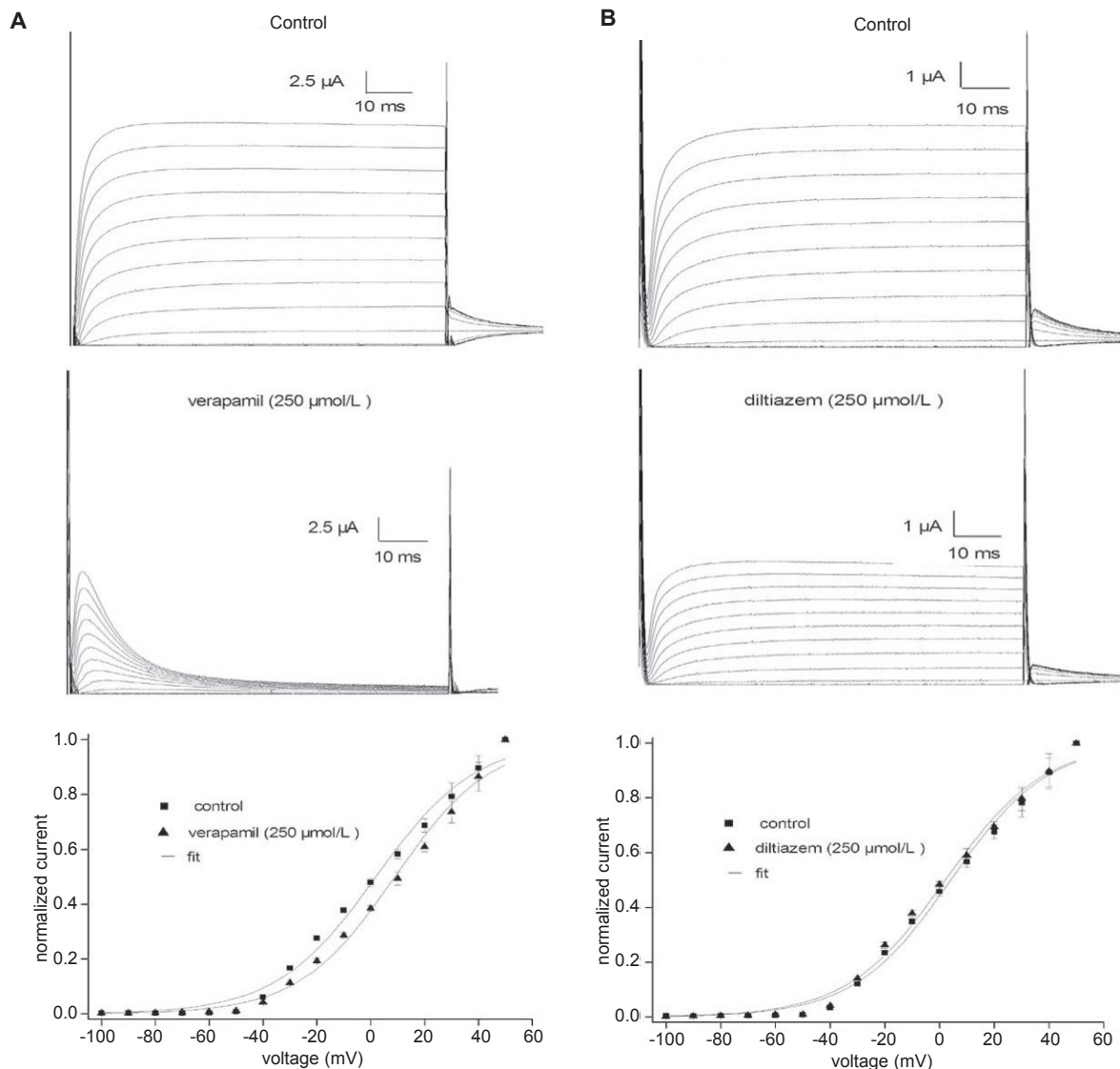


Figure 5. Effects of verapamil (A) and diltiazem (B) on the activation of the fKv1.4ΔN currents. Current traces were obtained by applying 80 ms pulses to potentials ranging from -100 to $+50$ mV and were followed by tail currents upon repolarization to -40 mV in the absence (upper panels) and in the presence of $250 \mu\text{mol/L}$ verapamil and $250 \mu\text{mol/L}$ diltiazem (middle panels). Bottom panels: steady-state activation relationships. The peak currents (measured at $+50$ mV) are plotted as a function of the holding potential. Continuous lines represent the fit of the data to a Boltzmann equation: $f = 1 / \{1 + \exp * [(V - V_{1/2}) / k]\}$, where V represents the test potential, $V_{1/2}$ is the mid-point of activation, and k is the slope factor. Average data are shown as mean \pm SEM ($n = 5$).

We found that verapamil shifted the fKv1.4ΔN steady-state activation curve to the right, with a significant change in $V_{1/2}$: $V_{1/2 \text{ Control}} = 1.94 \pm 1.10$ mV; $V_{1/2 \text{ Verapamil}} = 8.97 \pm 1.08$ mV ($n = 5$). However, diltiazem did not shift fKv1.4ΔN steady activation curve, and had no significant effect on $V_{1/2}$: $V_{1/2 \text{ Control}} = 4.03 \pm 1.04$ mV; $V_{1/2 \text{ Diltiazem}} = 2.08 \pm 1.05$ mV ($n = 5$). Thus, there was no effect of diltiazem on the steady activation of the fKv1.4ΔN channel.

Changes in the inactivation kinetics of the fKv1.4ΔN currents by verapamil and diltiazem

To evaluate whether verapamil affects the kinetic properties of the fKv1.4ΔN currents, we analyzed the time course of inactivation and the steady-state inactivation curve in the absence and presence of $250 \mu\text{mol/L}$ verapamil (Figure 1). After exposure to $250 \mu\text{mol/L}$ verapamil, inactivation of the fKv1.4ΔN channel was clearly accelerated. Under control conditions, the

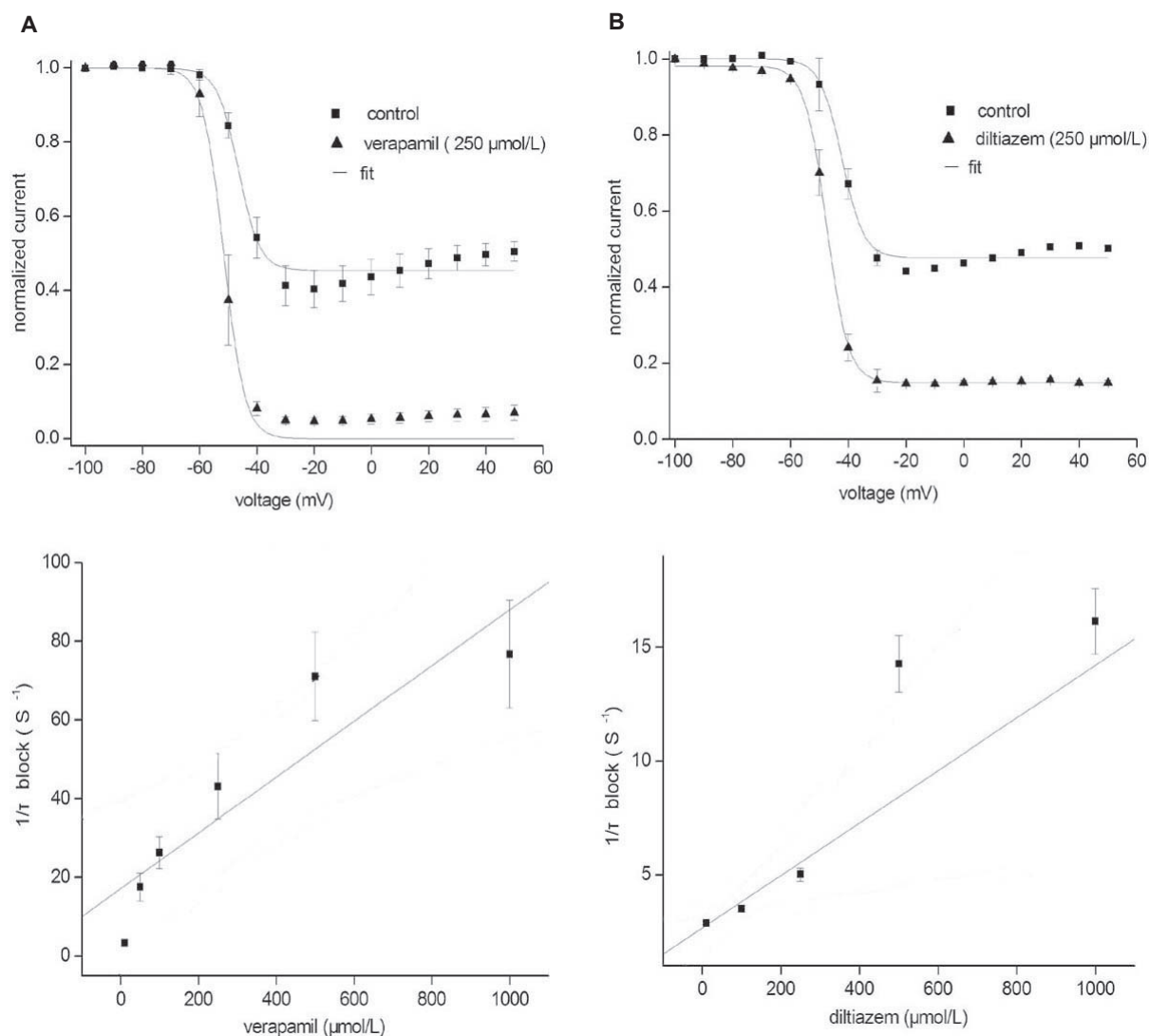


Figure 6. Changes in the inactivation kinetics of fKv1.4ΔN currents by verapamil (A) and diltiazem (B). Upper panels: Steady-state inactivation was studied using a two-pulse voltage protocol. Currents were measured at +50 mV, and the 5-sec pre-pulses to potential varied from -100 to +50 mV in steps of 10 mV. The curves for steady-state inactivation were fitted with the Boltzmann equation: $f = 1 / \{1 + \exp * [(V - V_{1/2}) / k]\}$. Where V represents the test potential, $V_{1/2}$ is the mid-point of inactivation, and k is the slope factor. Lower panels: time constant of inhibition as a function of the drug concentration. Time constants (τ_{block}) were estimated from a single or double exponential fits to the tracings shown in Figure 2. The apparent rate constants for association (k_{+1}) and dissociation (k_{-1}) were obtained from the equation: $1/\tau_{block} = k_{+1}[d] + k_{-1}$. Data are shown as mean \pm SEM (n = 5).

inactivation of fKv1.4ΔN fitted well to a single exponential function with a time constant of 2.20 ± 0.07 seconds at +50 mV (n = 5). After exposure to 250 μmol/L verapamil, the time constant was 0.27 ± 0.04 seconds at +50 mV (n = 5). Diltiazem also produced the same effect, as described previously in detail by us (9). After addition of 250 μmol/L diltiazem, the time constant was 1.78 ± 0.29 seconds at +50 mV (n = 5).

We then examined and compared the effects of verapamil and diltiazem on the steady inactivation curve for the fKv1.4ΔN channel (Figure 6). The membrane potential was held at various levels between -100 mV and +50 mV, and tail currents were obtained upon repolarization to +50 mV. The peak currents were normalized, plotted against the membrane potential, and fitted to a Boltzmann equation. The steady-state inactivation relationships were normalized, as

shown in Figure 6 (upper panels). In the presence of 250 $\mu\text{mol/L}$ verapamil, the steady inactivation curve was shifted to the left: $V_{1/2 \text{ Control}} = -41.47 \pm 4.25 \text{ mV}$; $V_{1/2 \text{ Verapamil}} = -50.83 \pm 4.58 \text{ mV}$ ($n = 5$, $p < 0.05$). Although 250 $\mu\text{mol/L}$ diltiazem also slightly shifted the steady inactivation curve to the left, $V_{1/2}$ was not obviously changed: $V_{1/2 \text{ Control}} = -38.38 \pm 0.81 \text{ mV}$; $V_{1/2 \text{ Diltiazem}} = -39.23 \pm 0.85 \text{ mV}$ ($n = 5$, $p > 0.05$). These results indicate that verapamil but not diltiazem alters the voltage-dependency of the steady-state inactivation of the fKv1.4AN channel.

Inactivation of the fKv1.4AN currents was best fitted by a single exponential function with a time constant of 2.20 ± 0.07 seconds at +50 mV ($n = 5$). In the presence of verapamil, the inactivation was best fitted to a bi-exponential function, as the inactivation processes were composed of fast and slow components. The slow component (τ_{slow}) was regarded as intrinsic C-type inactivation of the fKv1.4AN channel. The fast component (τ_{fast}) was thought to represent the time constant for drug-induced blockade of the fKv1.4AN currents. After application of 250 $\mu\text{mol/L}$ verapamil, τ_{fast} was 0.01 ± 0.002 sec and τ_{slow} was 0.27 ± 0.04 seconds (at +50 mV, $n = 5$). In the presence of diltiazem, the inactivation of fKv1.4AN was well fitted to a bi-exponential function, with $\tau_{\text{fast}} = 0.41 \pm 0.04$ sec and $\tau_{\text{slow}} = 1.78 \pm 0.29$ seconds at +50 mV ($n = 5$). We found that 250 $\mu\text{mol/L}$ verapamil and 250 $\mu\text{mol/L}$ diltiazem accelerated intrinsic C-type inactivation.

The bottom panels of Figure 6 show τ_{fast} at +50 mV plotted against the various drug concentrations (τ_{fast} values were not shown). From this fit (left panel), in the presence of verapamil, an apparent association (k_{+1}) of $0.07 \pm 0.02 \mu\text{mol/L}^{-1}\text{s}^{-1}$, and (k_{-1}) of $17.16 \pm 7.67 \text{ s}^{-1}$ were obtained. The k_{D} value derived on the basis of a first-order reaction between the drug and the channel was 242 $\mu\text{mol/L}$ in the presence of verapamil. The k_{D} value of diltiazem was 267 $\mu\text{mol/L}$, as described in our previous studies (9).

Discussion

L-type calcium channel antagonists, represented in this study by the phenylalkylamine verapamil and the benzothiazepine diltiazem, have distinct effects on cardiac electrophysiology

and arrhythmias (24). They promote both use- and voltage-dependent blockade of L-type calcium channels, and a wide range of results have been reported on the concentration dependence of the blockade (3, 25). In general, phenylalkylamines block native and cloned L-type calcium channel with IC_{50} values ranging from 250 nmol/L to 15.5 $\mu\text{mol/L}$ or higher. Benzothiazepines block L-type channels over a similar concentration range.

The effects of calcium channel antagonists on other channels have also been reported. For example, verapamil inhibits rapidly activating delayed rectifier potassium channels expressed in human embryonic kidney cells with an IC_{50} of 45 $\mu\text{mol/L}$ (4). Verapamil also produces high-affinity blockade of hERG currents, with an IC_{50} of 143.0 nmol/L, close to its IC_{50} for blockade of L-type calcium channels. The blockade of the hERG channel by verapamil was found to be use- and frequency-dependent (3). Verapamil has been shown to induce concentration-dependent block of currents at Kv1.1, Kv1.5, IKs, and hERG channels expressed in *Xenopus* oocytes with IC_{50} values of 14.0, 5.1, 161.0, and 3.8 $\mu\text{mol/L}$, respectively (26). In the human atrium, verapamil (3.2 $\mu\text{mol/L}$) was reported to reversibly suppress IK_{ur} by 50% in a concentration-dependent manner (27). However, blockade of these channels has generally required relatively high drug concentrations.

Limited data are available for the Kv1.4 potassium channel. In *Xenopus* oocytes, some calcium channel antagonists, including verapamil and diltiazem, have been reported to reduce fKv1.4 potassium channel currents. Our recent study showed that diltiazem inhibited the fKv1.4 potassium channel in *Xenopus* oocytes, (9) acting as an open channel antagonist with an IC_{50} of 241.04 $\mu\text{mol/L}$. Verapamil was also shown to reduce fKv1.4 potassium currents, (6, 8) but the drugs mechanism of action was not examined. The present observations provide novel information that verapamil inhibits fKv1.4AN potassium currents. Our findings agree with these reports, although the IC_{50} for verapamil block (260.71 $\mu\text{mol/L}$) is relatively higher than that for blockade of the calcium channel. However, the blockade of channels expressed in *Xenopus* oocytes by lipophilic drugs often requires much

higher concentrations (fivefold to tenfold) than are needed to block channels expressed in mammalian cells (6). Therefore, verapamil may have a greater potency at the fKv1.4ΔN channel than reported in this study.

Our findings show that verapamil substantially inhibits currents at fKv1.4ΔN channels expressed in *Xenopus* oocytes in a concentration-, voltage-, and use-dependent manner. The blockade significantly accelerates channel inactivation, suggesting an open channel block (9). Diltiazem shares its characteristics. Our results demonstrate that verapamil and diltiazem exhibit the same affinity for the fKv1.4ΔN channel, but the drugs do not share high-affinity hERG channel blocking properties (3).

Since verapamil and diltiazem are L-type calcium antagonists, the electrophysiological effects of both drugs on the fKv1.4ΔN channel inactivation were determined. We discovered some differences after the application of verapamil and diltiazem. The inactivation of fKv1.4ΔN currents became biexponential, with the inactivation processes exhibiting fast and slow components. The slow component was regarded as intrinsic C-type inactivation of the fKv1.4ΔN channel, and the fast component was thought to represent time constant for drug-induced blockade of the fKv1.4ΔN currents. We found that although both drugs elicited extremely fast drug-induced inactivation representing the interaction with the open state, verapamil had a more pronounced effect on C-type inactivation than diltiazem and shifted the steady inactivation curve to the left. This may be related to a mechanism in which binding of the drug to the intracellular site of the channel triggers a conformational change at the external mouth of the pore that facilitates C-type inactivation (9, 22). Diltiazem also slightly shifted the steady-state inactivation curve to the left, but the $V_{1/2}$ was not obviously changed. These results indicate the voltage-dependency of the steady-state inactivation of the fKv1.4ΔN channel is altered by verapamil, but not by diltiazem. The lack of effect of diltiazem on the voltage dependency of steady-state inactivation suggests that diltiazem is unlikely to interact with the inactivated state of the fKv1.4ΔN channel (28). In addition, diltiazem did not alter the steady-state activation

curve, suggesting that this agent interacts with the fKv1.4ΔN channel in the open state, and not the inactivated state (29). Verapamil not only changed the voltage-dependency of the steady-state inactivation of the fKv1.4ΔN channel, but also shifted the fKv1.4ΔN steady-state activation curve to the right with a significant change in $V_{1/2}$. Therefore, it is presumed that verapamil may interact simultaneously with the open and inactivated states of the fKv1.4ΔN channel (29). We suggest that the blockade of fKv1.4ΔN channels by verapamil is a more complex process than blockade by diltiazem. The binding of diltiazem to the channel affects C-type inactivation, however an interaction between verapamil binding and channel activation may exist (30).

Using the fast time constants in the range 10 to 1000 $\mu\text{mol/L}$, which represent the interaction of the drug with the open state, the constants k_{+1} and k_{-1} were obtained. The k_D values derived on the basis of a first-order reaction between the drug and channel were 242 $\mu\text{mol/L}$ in the presence of verapamil and 267 $\mu\text{mol/L}$ in the presence of diltiazem, respectively (9, 28, 31, 32). The values were very close to the IC_{50} values obtained from the concentration-response curve. The similarity of the IC_{50} values obtained by the two independent methods supports the open-channel block model used to calculate the rate constant for the fKv1.4ΔN channel (33, 34).

Verapamil exerts effects on cardiac arrhythmias that can be distinguished from those of other calcium channel antagonist drugs. Although the antiarrhythmic efficacy of verapamil has usually been ascribed to its suppression of L-type calcium channels, our findings suggest that block of the fKv1.4ΔN channel also may contribute to the cardiac effects of verapamil. As mentioned in introduction, the Kv1.4 channel, which conducts I_{to} , contributes to phase 1 and the early part of phase 2 of the cardiac action potential, playing an important role in the repolarization of cardiac myocytes (13, 16). Suppression of the fKv1.4ΔN channel prolongs action potential duration and increases the refractory period, and may thereby terminate reentrant circuits (35). Diltiazem and verapamil share an ability to increase C-type inactivation. Blockade of fKv1.4ΔN currents and decreasing

the recovery rate could attenuate the shortening of the action potential duration caused by the inhibition of L-type calcium channels by verapamil and diltiazem (9).

In the present study, we found that verapamil and diltiazem directly inhibit the fKv1.4AN potassium channel expressed in *Xenopus* oocyte by binding to the open state of the channel. Furthermore, the inhibitory effects of verapamil and diltiazem are completely independent of calcium channel inhibition. Therefore, caution should be exercised when these drugs are applied in combination with the other potassium channels blockers.

Acknowledgements

We thank Dr. Rasmusson (University at Buffalo, SUNY) for the fKv1.4AN cDNA.

References

- (1) Kumar S and Hall RJ. Drug treatment of stable angina pectoris in the elderly: defining the place of calcium channel antagonists. *Drugs Aging* (2003) 20: 805-815.
- (2) Grossman E and Messerli FH. Calcium antagonists. *Prog. Cardiovasc. Dis.* (2004) 47: 34-57.
- (3) Zhang S, Zhou Z, Gong Q, Makielski JC and January CT. Mechanism of block and identification of the verapamil binding domain to HERG potassium channels. *Circ. Res.* (1999) 84: 989-998.
- (4) Rampe D, Wible B, Fedida D, Dage RC and Brown AM. Verapamil blocks a rapidly activating delayed rectifier K⁺ channel cloned from human heart. *Mol. Pharmacol.* (1993) 44: 642-648.
- (5) Grissmer S, Nguyen AN, Aiyar J, Hanson DC, Mather RJ, Gutman GA, Karmilowicz MJ, Auperin DD and Chandy KG. Pharmacological characterization of five cloned voltage gated K⁺ channels, types Kv1.1, 1.2, 1.3, 1.5, and 3.1, stably expressed in mammalian cell lines. *Mol. Pharmacol.* (1994) 45: 1227-1234.
- (6) Rolf S, Haverkamp W, Borggreffe M, Musshoff U, Eckardt L, Mergenthaler J, Snyders DJ, Pongs O, Speckmann EJ, Breithardt G and Madeja M. Effects of antiarrhythmic drugs on cloned cardiac voltage-gated potassium channels expressed in *Xenopus* oocytes. *Naunyn Schmiedeberg Arch. Pharmacol.* (2000) 362: 22-31.
- (7) Caballero R, Gomez R, Nunez L, Moreno I, Tamargo J and Delpon E. Diltiazem inhibits hKv1.5 and Kv4.3 currents at therapeutic concentrations. *Cardiovasc. Res.* (2004) 64: 457-466.
- (8) Xu L, Huang C, Chen J, Jiang X, Li X, Bett GC, Rasmusson RL and Wang S. Effect of amiodarone on Kv1.4 channel C-type inactivation: comparison of its effects with those induced by propafenone and verapamil. *Pharmazie* (2008) 63: 475-479.
- (9) Zhang D, Wang S, Chen H, Jiang X and Chao S. Effects of diltiazem and propafenone on the inactivation and recovery kinetics of fKv1.4 channel currents expressed in *Xenopus* oocytes. *Acta Pharmacol. Sin.* (2011) 32: 465-477.
- (10) Brahmajothi MV, Campbell DL, Rasmusson RL, Morales MJ, Trimmer JS, Nerbonne JM and Strauss HC. Distinct transient outward potassium current (I_{to}) phenotypes and distribution of fast-inactivating potassium channel-subunits in ferret left ventricular myocytes. *J. Gen. Physiol.* (1999) 113: 581-600.
- (11) Kaab S, Kartmann H, Andress J, Hinterseer M, Barth A and Nabauer M. Presence of Kv4.3 and Kv1.4 potassium channel mRNA in human left ventricular myocardium: transmural mRNA-gradients and correlation with current properties. *Circ. Res.* (1999) 96 suppl: 1867.
- (12) Wickenden AD, Jegla TJ, Kaprielian R and Backx PH. Regional contributions of Kv1.4, Kv4.2 and Kv4.3 to transient outward K⁺ current in rat ventricle. *Am. J. Physiol.* (1999) 276: H1599-H1607.
- (13) Gidh-Jain M, Huang B, Jain P and el-Sherif N. Differential expression of voltage-gated K⁺ channel genes in left ventricular remodeled myocardium after experimental myocardial infarction. *Circ. Res.* (1996) 79: 669675.
- (14) Kaprielian R, Wickenden AD, Kassiri Z, Kassiri Z, Parker TG, Liu PP and Backx PH. Relationship between K⁺ channel down regulation and Ca²⁺_i in rat ventricular myocytes following myocardial infarction. *J. Physiol.* (1999) 517: 229-245.
- (15) Nishiyama A, Ishii DN, Backx PH, Pulford BE, Birks BR and Tamkun MM. Altered K⁺ channel gene expression in diabetic rat ventricle: isoform switching between Kv4.2 and Kv1.4. *Am. J. Physiol. Heart Circ. Physiol.* (2001) 281: H1800-H1807.
- (16) Lee JK, Nishiyama A, Kambe F, Seo H, Takeuchi S, Kamiya K, Kodama I and Toyama J. Downregulation of voltage-gated K⁺ channels in rat heart with right ventricular hypertrophy. *Am. J. Physiol.* (1999) 277: H1725-H1731.
- (17) Hoshi T, Zagotta WN and Aldrich RW. Biophysical and molecular mechanisms of Shaker potassium channel inactivation. *Science* (1990) 250: 533-538.
- (18) Liu Y, Jurman ME and Yellen G. Dynamic rearrangement of the outer mouth of a K⁺ channel during gating. *Neuron* (1996) 16: 859-867.
- (19) Rasmusson RL, Morales MJ, Wang S, Liu S, Campbell DL, Brahmajothi MV and Strauss HC. Inactivation of voltage-gated cardiac K⁺ channels. *Circ. Res.* (1998) 82: 739-750.
- (20) Rasmusson RL, Morales MJ, Castellino RC, Zhang Y, Campbell DL and Strauss HC. C-type inactivation controls recovery in a fast inactivating cardiac K⁺ channel (Kv1.4) expressed in *Xenopus* oocytes. *J. Physiol.* (1995) 489: 709-721.
- (21) Comer MB, Campbell DL, Rasmusson RL, Lamson, DR, Morales MJ, Zhang Y and Strauss HC. Cloning

- and characterization of an Ito-like potassium channel from ferret ventricle. *Am. J. Physiol.* (1994) 267: H1383-H1395.
- (22) Wang S, Michael J, Morales, Qu Y and Strauss HC. Kv1.4 channel block by quinidine: evidence for a drug induced allosteric effect. *J. Physiol.* (2003) 546: 387-401.
- (23) Hohnloser SH, Kuck KH and Lilienthal J. Rhythm or rate control in atrial fibrillation-pharmacological intervention in atrial fibrillation (PIAF): a randomized trial. *Lancet.* (2000) 356: 1789-1794.
- (24) Singh BN and Nademanee K. Use of calcium antagonists for cardiac arrhythmias. *Am. J. Cardiol.* (1987) 59: 153B-162B.
- (25) Hosey MM and Lazdunski M. Calcium channels: molecular pharmacology, structure and regulation. *J. Membr. Biol.* (1988) 104: 81-105.
- (26) Waldegger S, Niemeyer G, Morike K, Wagner CA, Suessbrich H, Busch AE, Lang F and Eichelbaum M. Effect of verapamil enantiomers and metabolites on cardiac K⁺ channels expressed in *Xenopus* oocytes. *Cell Physiol. Biochem.* (1999) 9: 81-89.
- (27) Gao Z, Lau CP, Chiu SW and Li GR. Inhibition of ultra-rapid delayed rectifier K⁺ current by verapamil in human atrial myocytes. *J. Mol. Cell Cardiol.* (2004) 36: 257-263.
- (28) Choi BH, Choi JS, Jeong SW, Hahn SJ, Yoon SH, Jo YH and Kim MS. Direct block by bisindolylmaleimide of rat Kv1.5 expressed in Chinese hamster ovary cells. *J. Pharmacol. Exp. Ther.* (2000) 293: 634-640.
- (29) Ko EA, Park WS, Son YK, Ko JH, Choi TH, Jung DI, Park WY, Hong DY, Kim N and Han J. Calcium channel inhibitor, verapamil, inhibits the voltage-dependent K⁺ channels in rabbit coronary smooth muscle cells. *Biol. Pharm. Bull.* (2010) 33: 47-52.
- (30) Wang S, Morales MJ, Liu S, Strauss HC and Rasmusson RL. Modulation of HERG affinity for E-4031 by [K⁺]_o and C-type inactivation. *FEBS Lett.* (1997) 417: 43-47.
- (31) Snyders DJ, Tamkun MM and Bennett PB. A rapidly activating and slowly inactivating potassium channel cloned from human heart. Functional analysis after stable mammalian cell culture expression. *J. Gen. Physiol.* (1993) 101: 513-543.
- (32) Park WS, Son YK, Ko EA, Ko JH, Lee HA, Park KS and Earm YE. The protein kinase C inhibitor, bisindolylmaleimide (I), inhibits voltage-dependent K⁺ channels in coronary arterial smooth muscle cells. *Life Sci.* (2005) 77: 512-527.
- (33) Snyders J, Knoth KM, Roberds SL and Tamkun MM. Time-, voltage-, and state-dependent block by quinidine of a cloned human cardiac potassium channel. *Mol. Pharmacol.* (1992) 41: 322-330.
- (34) Slawsky MT and Castle NA. K⁺ channel blocking actions of flecainide compared with those of propafenone and quinidine in adult rat ventricular myocytes. *J. Pharmacol. Exp. Ther.* (1994) 269: 66-74.
- (35) Roden DM. Current status of class III antiarrhythmic drug therapy. *Am. J. Cardiol.* (1993) 72: 44B-49B.

This article is available online at <http://www.ijpr.ir>
