

Original Article

# Comparison of the effects of antiarrhythmic drugs flecainide and verapamil on fKv1.4ΔN channel currents in *Xenopus* oocytes

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**Aim:** To study the effects of Na<sup>+</sup> channel blocker flecainide and L-type Ca<sup>2+</sup> channel antagonist verapamil on the voltage-gated fKv1.4ΔN channel, an N-terminal-deleted mutant of the ferret Kv1.4 K<sup>+</sup> channel.

**Methods:** fKv1.4ΔN channels were stably expressed in *Xenopus* oocytes. The K<sup>+</sup> currents were recorded using a two-electrode voltage-clamp technique. The drugs were administered through superfusion.

**Results:** fKv1.4ΔN currents displayed slow inactivation, with a half-inactivation potential of -41.74 mV and a slow recovery from inactivation ( $\tau=1.90$  s at -90 mV). Flecainide and verapamil blocked the currents with IC<sub>50</sub> values of 512.29±56.92 and 260.71±18.50 μmol/L, respectively. The blocking action of the drugs showed opposite voltage-dependence: it was enhanced with depolarization for flecainide, and was attenuated with depolarization for verapamil. Both the drugs exerted state-dependent blockade on fKv1.4ΔN currents, but verapamil showed a stronger use-dependent blockage compared with flecainide. Flecainide accelerated the C-type inactivation rate without affecting the recovery kinetics and the steady-state activation. Verapamil also accelerated the inactivation kinetics of the currents, but unlike flecainide, it affected both the recovery and the steady-state activation, causing slower recovery of fKv1.4ΔN channel and a depolarizing shift of the steady-state activation curve.

**Conclusion:** The results demonstrate that widely used antiarrhythmic drugs flecainide and verapamil substantially inhibit fKv1.4ΔN channels expressed in *Xenopus* oocytes by binding to the open state of the channels. Therefore, caution should be taken when these drugs are administered in combination with K<sup>+</sup> channel blockers to treat arrhythmia.

**Keywords:** Kv1.4; fKv1.4ΔN channel; *Xenopus* oocytes; antiarrhythmic drugs; flecainide; verapamil; activation; C-type inactivation; Na<sup>+</sup> channel blockers; Ca<sup>2+</sup> channel antagonists

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## Introduction

Kv1.4 voltage-gated potassium channels are expressed in the subendocardium of human and ferret heart and in the rat ventricular septum<sup>[1–3]</sup>. This channel conducts transient outward potassium currents ( $I_{to}$ ). Kv1.4 expression in the mammalian endocardium is upregulated during hypertrophy and heart failure<sup>[4–7]</sup>, and this channel plays an important role in the repolarization of cardiac myocytes. The inactivation of Kv1.4 occurs through two distinct mechanisms: fast N-type inactivation and slow C-type inactivation<sup>[8]</sup>. The slow C-type inactivation involves the conformational changes of the external side of the pore<sup>[9,10]</sup>. In *Xenopus* oocytes, C-type inactivation controls the recovery of Kv1.4 channels<sup>[11]</sup>. Because the recovery

rate of Kv1.4 determines the availability of the channels for repolarization of the subsequent action potential, it is important to investigate the effects of cardiac medicines on C-type inactivation of Kv1.4 channels.

From a basic biophysical perspective, the slow inactivation of the Kv1.4 channels resembles the inactivation of sodium and calcium channels<sup>[12]</sup>. We assumed, therefore, that some calcium and sodium channel antagonists could affect Kv1.4 inactivation. In our previous study, we investigated the effects of the L-type calcium channel antagonist diltiazem and the sodium channel antagonist propafenone on Kv1.4 channels. We discovered that both diltiazem and propafenone accelerated Kv1.4 channel inactivation, providing evidence that C-type inactivation maybe a common mechanism underlying channel inactivation for sodium and calcium channels<sup>[13,14]</sup>.

The sodium channel antagonist flecainide and the L-type calcium channel antagonist verapamil have been used

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widely for the treatment of atrial fibrillation, supraventricular arrhythmias, and long QT syndrome<sup>[15–18]</sup>. In addition to sodium channels and L-type calcium channels, these antagonists also block several voltage-gated potassium channels. Flecainide may inhibit the wild-type Kv4.2 channel (Kv4.2WT) currents in a concentration-dependent manner, and it has been shown to induce a voltage-dependent block of hERG channels<sup>[19, 20]</sup>. In addition, verapamil has been reported to produce a potent use- and frequency-dependent block of hERG channels<sup>[21]</sup> and to inhibit the hKv1.5 channel in low micromolar concentrations<sup>[22]</sup>. Flecainide and verapamil have been shown recently to inhibit the conductance of Kv1.4 channels expressed in *Xenopus* oocytes<sup>[13, 23]</sup>. The detailed characteristics of this effect, which may lead to a better understanding of their antiarrhythmic mechanism, have not been studied.

In the present study, we studied the inhibitory effects of flecainide and verapamil on Kv1.4ΔN, an N-terminal deletion construct of Kv1.4 that lacks rapid N-type inactivation but exhibits robust C-type inactivation<sup>[24]</sup>, to provide a more detailed understanding of their mechanisms of action.

## Materials and methods

### Molecular biology

fKv1.4ΔN cDNA (GenBank accession No. U06156) was a gift from Prof Randall L RASMUSSEN (University at Buffalo, SUNY, USA). The constructs and sequences have been described previously<sup>[11, 12, 24]</sup>. 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. Transcribed fKv1.4ΔN cRNA was prepared *in vitro* using an 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 a tricaine solution (1.5 g/L, Sigma) for 30 min, and the oocytes were removed surgically through a lateral incision in the lower abdomen, as described previously<sup>[14]</sup>. The oocytes were digested by placing the ovarian lobes in a collagenase-containing, Ca<sup>2+</sup>-free oocyte Ringer's (OR<sub>2</sub>) solution (mmol/L): 82.5 NaCl, 2 KCl, 1 MgCl<sub>2</sub>, 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 approximately 1 h and washed several times with Ca<sup>2+</sup>-free OR<sub>2</sub> solution as described previously<sup>[14]</sup>. Finally, defolliculated (stage IV) oocytes were selected and placed in ND96 solution (mmol/L): 96 NaCl, 2 KCl, 1 MgCl<sub>2</sub>, 1.8 CaCl<sub>2</sub>, and 5 HEPES at pH 7.4. Each oocyte was injected with approximately 25 to 50 nL of fKv1.4ΔN cRNA using a microinjector (WPI, Sarasota, FL, USA) and incubated at 18 °C in ND96 solution with 100 IU/mL penicillin for a minimum of 16 h.

## Electrophysiology

The two-electrode voltage clamp technique was used for the 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 they were filtered at 2.5 kHz. The recordings were made in 2 mmol/L K<sup>+</sup><sub>o</sub>. Flecainide and verapamil were dissolved in distilled water to give stock solutions of 100 mmol/L. All drugs were purchased from Sigma Chemical Co (St Louis, MO, USA). The drugs were superfused for 10 min before testing to allow equilibration with the oocytes. After this wash-on period, a series of 500-ms depolarizing steps (from -90 to +50 mV, 1 min) were applied to ensure steady-state block before beginning the experimental protocols<sup>[12, 14]</sup>.

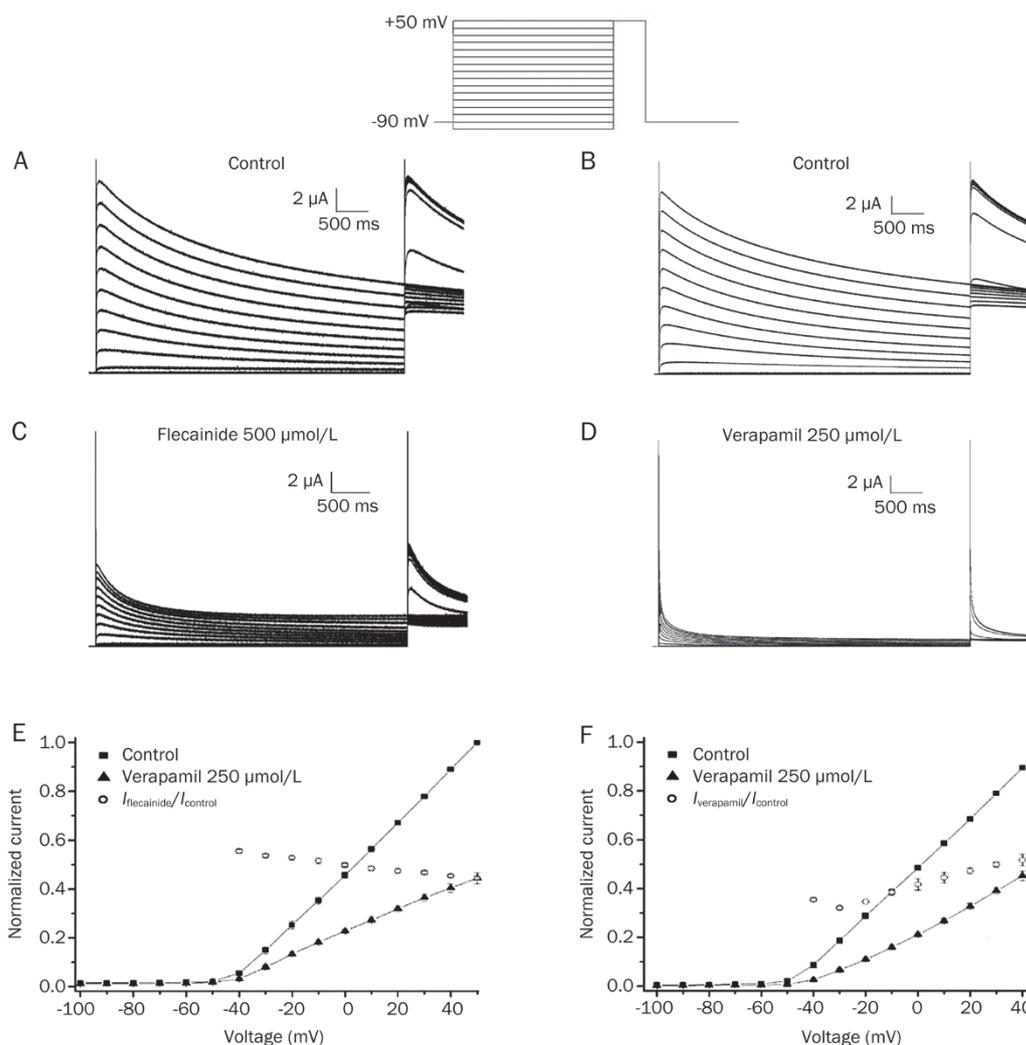
## Data analysis

The data were digitized and analyzed 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). Assuming a 1:1 binding stoichiometry between the drug and the receptor, concentration-response curves were generated using the Hill equation:  $f = K_D / (K_D + D)$ , where  $f$  is the fractional current,  $K_D$  is the apparent dissociation constant, and  $D$  is the drug concentration<sup>[12]</sup>. Inactivation time constants were obtained using a single or double exponential decay model fitted to the raw current tracings. The peak current activation was fitted to 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 activation, and  $k$  is the slope factor. The steady-state inactivation was fitted to the Boltzmann equation:  $I / I_{\max} = (1 - \alpha) / \{1 + \exp[(V - V_{1/2}) / k]\} + \alpha$ , where  $I_{\max}$  is the maximal peak current,  $\alpha$  is the non-inactivating component of the curves,  $V$  represents the test potential,  $V_{1/2}$  is the mid-point of inactivation, and  $k$  is the slope factor. The apparent rate constants for association ( $k_{+1}$ ) and dissociation ( $k_{-1}$ ) were obtained from the equation:  $1 / \tau_{\text{block}} = k_{+1}[D] + k_{-1}$ , where  $D$  is the drug concentration<sup>[23, 25]</sup>. The time course of recovery from inactivation was fitted to a single exponential equation:  $f = 1 - A \cdot \exp(-t / \tau)$ , where  $t$  is the duration (in seconds),  $\tau$  is the time constant, and  $A$  is the amplitude of the current. A paired Student's  $t$ -test was used for statistical analysis of the data. The results are expressed as the mean ± SEM, and values of  $P < 0.05$  were considered significant.

## Results

### Inhibition of fKv1.4ΔN currents by flecainide and verapamil

Figure 1 shows superimposed traces of currents from fKv1.4ΔN channels expressed in *Xenopus* oocytes under control conditions (A and B) and in the presence of flecainide (C) or verapamil (D). The cells were held at -90 mV, and the fKv1.4ΔN currents were examined with a series of 5-s step stimuli from -100 to +50 mV at 10-mV increments, followed by



**Figure 1.** Effects of flecainide and verapamil on the fKv1.4ΔN channel expressed in *Xenopus* oocytes. Representative curves were shown for 5 s depolarizing steps from -90 mV to voltages between -100 and +50 mV in steps of 10 mV. (A and B) Traces recorded under control conditions. (C and D) Current traces obtained in the presence of 500 μmol/L flecainide (C) or 250 μmol/L verapamil (D). (E and F) Effects of 500 μmol/L flecainide (E) or 250 μmol/L verapamil (F) on the peak current-voltage ( $I$ - $V$ ) relationships. Currents were normalized to the peak current at +50 mV under control conditions. The  $I_{drug}/I_{control}$  ratio was plotted as a function of the membrane potential. Data are shown as mean±SEM ( $n=5$ ).

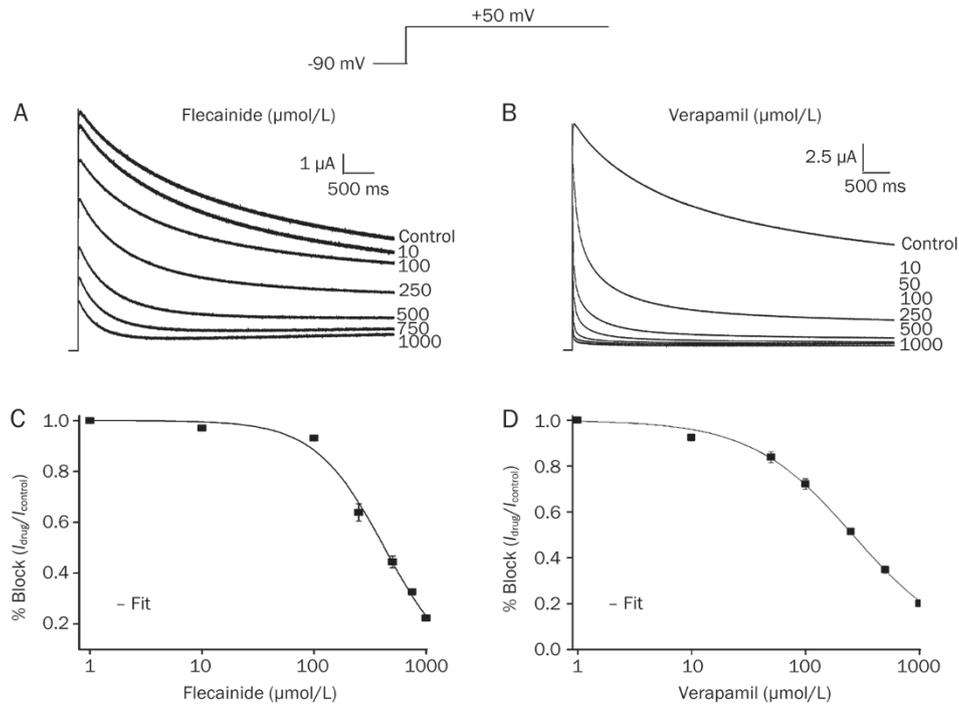
a step clamping to +50 mV. Under control conditions, positive depolarization to -30 mV elicited outward currents that declined slowly during the maintained depolarization (slow C-type inactivation). Figures 1C and 1D show the effects of flecainide (500 μmol/L) and verapamil (250 μmol/L). Both drugs not only reduced the current amplitude but also accelerated the inactivation. Flecainide and verapamil reduced the amplitude of the peak current at +50 mV to  $44.5\pm 2.3\%$  and  $53.5\pm 6.2\%$  ( $n=5$ ) of control, respectively. Flecainide and verapamil showed similar efficacy in inhibiting fKv1.4ΔN currents, and the inhibitory effects were completely abrogated by a 10-min washout of the drug (data not shown).

The effects of 500 μmol/L flecainide and 250 μmol/L verapamil on the peak current-voltage ( $I$ - $V$ ) relationships for the fKv1.4ΔN channel are shown in Figures 1E and 1F. The  $I$ - $V$  relationships were constructed by plotting the normalized

currents as a function of the membrane potential. Flecainide and verapamil induced a voltage-dependent inhibition of the fKv1.4ΔN currents. To quantify the voltage dependency of the fKv1.4ΔN block, the relative current ( $I_{drug}/I_{control}$ ) was plotted as a function of the membrane potential. The current began to activate at the activation threshold (between -40 and -20 mV), and both drugs decreased the peak current at positive potentials to the level of the activation threshold. The blockage by flecainide and verapamil showed differential voltage-dependence, with a voltage-dependent enhancement for flecainide and a voltage-dependent attenuation for verapamil.

#### Flecainide and verapamil inhibit fKv1.4ΔN currents in a concentration-dependent manner

Figure 2 shows representative fKv1.4ΔN currents superim-



**Figure 2.** Concentration-response relationships for the inhibition of the fKv1.4ΔN currents by flecainide and verapamil. (A and B) Representative current traces were elicited in the absence and presence of increasing concentrations of flecainide (A) or verapamil (B). Currents were recorded by depolarizing steps to +50 mV from a holding potential of -90 mV. (C and D) The peak currents were normalized to the maximum peak current under control conditions and plotted against flecainide (C) and verapamil (D) concentrations. The curves were derived by fitting data to the Hill equation. Symbols and error bar are mean±SEM ( $n=5$ ).

posed on the variable concentrations of flecainide (A) and verapamil (B), between 0 and 1000  $\mu\text{mol/L}$ , to evaluate the concentration dependence. The fKv1.4ΔN currents were recorded by depolarizing steps 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 equation yielded  $K_D$  values of  $512.29\pm 56.92$   $\mu\text{mol/L}$  (flecainide) and  $260.71\pm 18.50$   $\mu\text{mol/L}$  (verapamil) ( $n=5$ ).

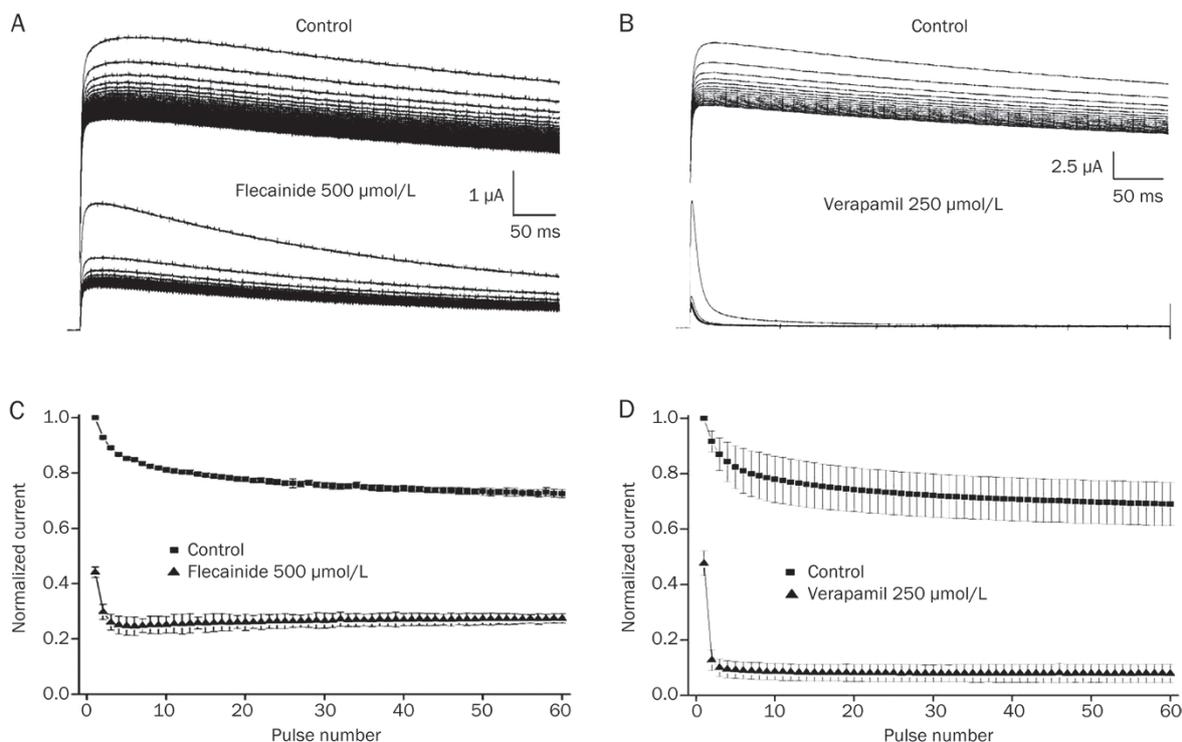
#### Use-dependent block of fKv1.4ΔN currents by flecainide and verapamil

Although 500  $\mu\text{mol/L}$  flecainide and 250  $\mu\text{mol/L}$  verapamil induced approximately 50% steady-state block, this degree of a block may not be attained during a single action potential. Therefore, we tested whether channel blockade by flecainide and verapamil displayed use-dependence. Trains of 60 depolarizing steps 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. Figures 3A and 3B show the original current records obtained after applying a step train protocol in the absence and presence of either 500  $\mu\text{mol/L}$  flecainide (A) or 250  $\mu\text{mol/L}$  verapamil (B), respectively. The peak currents were normalized to the maximum control value without drug and plotted in Figures 3C and 3D. Under control conditions, the fKv1.4ΔN currents were decreased by 20%–30%. However, the fKv1.4ΔN currents in the presence

of flecainide and verapamil showed greater decay than under control conditions until reaching a steady-state block. The peak amplitudes of the fKv1.4ΔN currents after 60 depolarizing steps were reduced by 62.1% in the presence of flecainide and by 83.4% in the presence of verapamil, suggesting a stronger use-dependent blockage by verapamil when compared with that caused by flecainide.

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

Figure 4 shows the representative currents and peak current activation relationships. The activation curves were measured in the absence (A and B) and presence of either 500  $\mu\text{mol/L}$  flecainide (C) or 250  $\mu\text{mol/L}$  verapamil (D). The steady-state activation curves were depicted by plotting the peak tail currents (obtained at -40 mV) against the depolarizing potentials (from -100 to +50 mV). Continuous lines represent the fit of the data to a Boltzmann equation. We found that flecainide did not affect the steady-state activation:  $V_{1/2\text{control}}=-22.24\pm 0.69$  mV,  $k_{\text{control}}=9.27\pm 0.61$ ;  $V_{1/2\text{flecainide}}=-25.71\pm 0.67$  mV,  $k_{\text{flecainide}}=8.78\pm 0.59$ ;  $n=5$ ,  $P>0.05$  (Figure 4E). However, verapamil shifted the fKv1.4ΔN steady-state activation curve to the right (Figure 4F), with a significant change in the  $V_{1/2}$  and the  $k$  values:  $V_{1/2\text{control}}=-22.36\pm 0.84$  mV,  $k_{\text{control}}=9.16\pm 0.74$  ( $n=5$ );  $V_{1/2\text{verapamil}}=-10.29\pm 1.05$  mV,  $k_{\text{verapamil}}=14.19\pm 0.92$  ( $n=5$ ,  $P<0.05$ ).



**Figure 3.** Use-dependent block of the fKv1.4ΔN currents by flecaïnide and verapamil. (A and B) Sixty repetitive depolarizing steps from -90 to +50 mV for 500 ms each were applied in the absence and presence of flecaïnide (A) or verapamil (B). (C and D) Each peak current was normalized to the peak current at the first step under control conditions and then plotted against the number of steps. Data are shown as mean±SEM ( $n=5$ ).

### Changes in the inactivation and recovery kinetics of fKv1.4ΔN by flecaïnide and verapamil

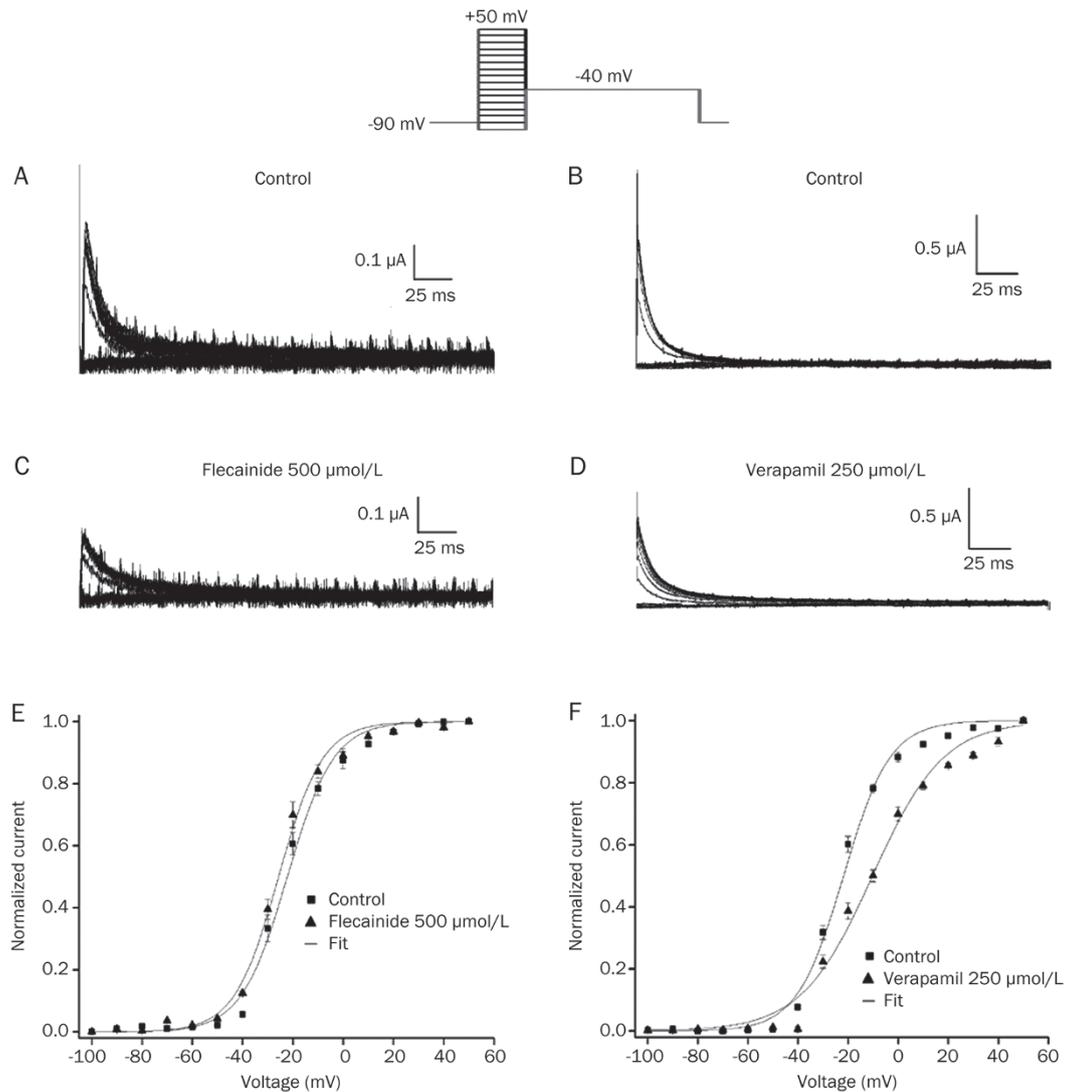
Inactivation of the fKv1.4ΔN currents was best fitted by a single exponential function with a time constant of  $2.20 \pm 0.07$  seconds at +50 mV ( $n=5$ ). In the presence of flecaïnide, the inactivation was best fitted to a bi-exponential function, as the inactivation processes were composed of fast and slow components. The slow component ( $\tau_{\text{slow}}$ ) was regarded as intrinsic C-type inactivation of the fKv1.4ΔN channel. The fast component ( $\tau_{\text{fast}}$ ) was thought to represent the time constant for the drug-induced blockade of the fKv1.4ΔN currents. After application of 500  $\mu\text{mol/L}$  flecaïnide,  $\tau_{\text{fast}}$  was  $0.36 \pm 0.04$  seconds and  $\tau_{\text{slow}}$  was  $0.79 \pm 0.05$  seconds (at +50 mV,  $n=5$ ). In the presence of verapamil, the inactivation of fKv1.4ΔN was well fitted to a bi-exponential function, with  $\tau_{\text{fast}} = 0.01 \pm 0.002$  seconds and  $\tau_{\text{slow}} = 0.27 \pm 0.04$  seconds at +50 mV ( $n=5$ ). We found that 500  $\mu\text{mol/L}$  flecaïnide and 250  $\mu\text{mol/L}$  verapamil accelerated the intrinsic C-type inactivation.

We then compared the effects of flecaïnide and verapamil on the steady inactivation curve for the fKv1.4ΔN channel (Figure 5). The membrane potential was held at various levels between -100 mV and +50 mV, and the tail currents were obtained upon depolarization to +50 mV. The peak currents were normalized, plotted against the membrane potential, and fitted by a Boltzmann equation. The steady-state inactivation relationships were normalized, as shown in Figures 5A and 5B. Although 500  $\mu\text{mol/L}$  flecaïnide produced

a slight leftward shift in the steady-state inactivation curve,  $V_{1/2}$  was not significantly changed:  $V_{1/2 \text{ control}} = -42.84 \pm 5.72$  mV;  $V_{1/2 \text{ flecaïnide}} = -42.28 \pm 4.33$  mV ( $n=5$ ,  $P > 0.05$ ). In the presence of 250  $\mu\text{mol/L}$  verapamil, the steady-state inactivation curve was shifted to the left:  $V_{1/2 \text{ control}} = -41.81 \pm 6.20$  mV;  $V_{1/2 \text{ verapamil}} = -52.12 \pm 6.35$  mV ( $n=5$ ,  $P < 0.05$ ). These results indicate that verapamil, but not flecaïnide, alters the voltage-dependency of the steady-state inactivation of the fKv1.4ΔN channel.

Figures 5C and 5D show  $\tau_{\text{fast}}$  at +50 mV plotted against the various drug concentrations ( $\tau_{\text{fast}}$  values were not shown). From this fit, in the presence of flecaïnide (C), an apparent association ( $k_{+1}$ ) of  $0.0022 \pm 0.0004$   $\mu\text{mol} \cdot \text{L}^{-1} \cdot \text{s}^{-1}$  and ( $k_{-1}$ ) of  $1.20 \pm 0.27$   $\text{s}^{-1}$  was obtained. The  $k_D$  value derived on the basis of a first-order reaction between the drug and the channel was 486  $\mu\text{mol/L}$  in the presence of flecaïnide. The  $k_D$  value of verapamil (D) was 242  $\mu\text{mol/L}$  ( $k_{+1} = 0.07 \pm 0.02$   $\mu\text{mol} \cdot \text{L}^{-1} \cdot \text{s}^{-1}$  and  $k_{-1} = 17.16 \pm 7.67$   $\text{s}^{-1}$ ).

We further compared the effects of flecaïnide and verapamil on the recovery kinetics of the fKv1.4ΔN channel. The recovery kinetics were examined using a standard gapped-step protocol with a variable inter-stimulus interval as shown at the top of Figure 6. Figures 6A and 6B show the recovery traces of two cells under control conditions, and Figures 6C and 6D show the recovery traces of those two cells in the presence of flecaïnide (6C) or verapamil (6D), respectively. The ratio of the current amplitude initiated by the second step



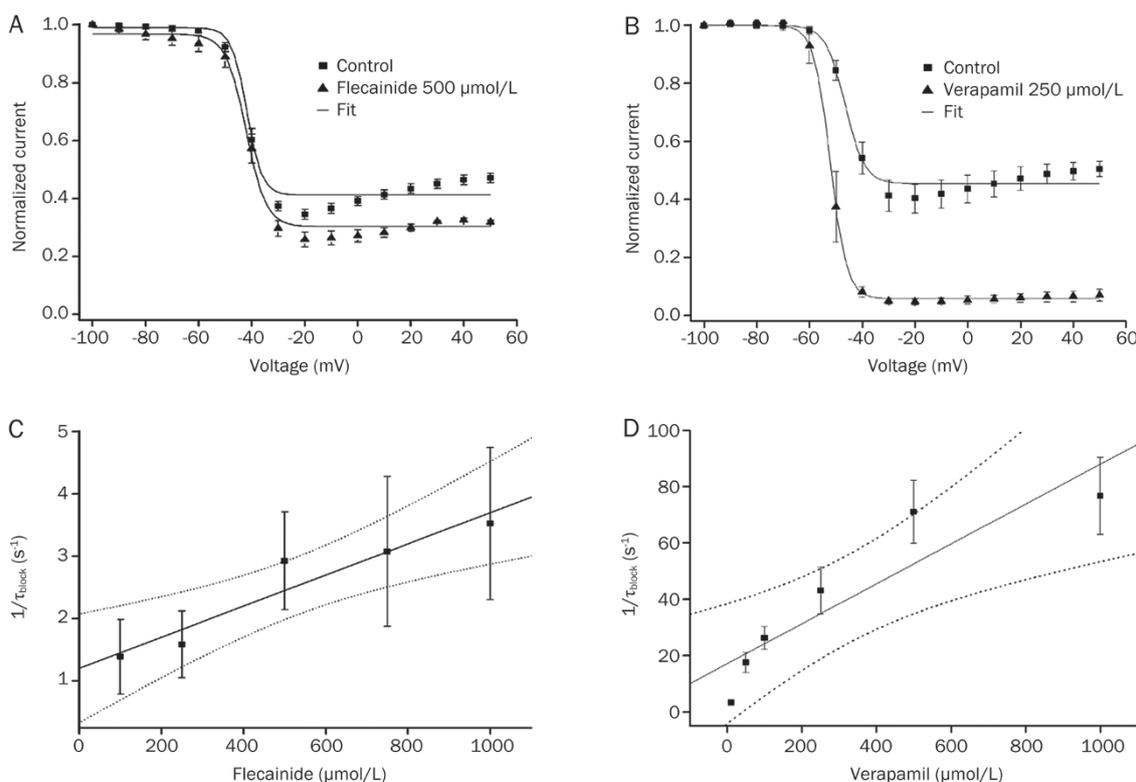
**Figure 4.** Effects of flecainide and verapamil on the activation of the fKv1.4ΔN currents. (A–D) Current traces were obtained by applying 80 ms steps to potentials ranging from -100 to +50 mV and were followed by the tail currents obtained upon repolarization to -40 mV in the absence (A and B) and presence of 500 μmol/L flecainide (C) or 250 μmol/L verapamil (D). (E and F) Peak current activation relationships in the absence and presence of 500 μmol/L flecainide (E) and 250 μmol/L verapamil (F). The steady-state activation curves were depicted by plotting the peak tail currents (obtained at -40 mV) against the depolarizing potentials (from -100 to +50 mV). Continuous lines represent the fit of the data to a Boltzmann equation:  $f = 1 / \{1 + \exp * [(V - V_{1/2}) / k]\}$ . Average data are shown as mean ± SEM ( $n = 5$ ).

pulse to that of the first step pulse reflects the percentage of channels recovered over the recovery period. Figures 6E and 6F show the recovery of two cells at control and treatment with flecainide or verapamil, respectively. The recovery time constant was obtained by a mono-exponential fit of the refraction fraction curve. The recovery time constant was  $1.90 \pm 0.09$  seconds under control conditions and  $2.38 \pm 0.18$  seconds in the presence of 500 μmol/L flecainide ( $n = 5$ ,  $P > 0.05$ ). The recovery time constant was greater in the presence of 250 μmol/L verapamil ( $2.84 \pm 0.17$  seconds) compared to a similar control value ( $1.92 \pm 0.12$  seconds;  $n = 5$ ,  $P < 0.05$ ). This result suggests that verapamil retards the fKv1.4ΔN recovery.

## Discussion

The sodium channel antagonist flecainide has distinct effects on cardiac electrophysiology and arrhythmias. Flecainide produces potent voltage- and frequency-dependent inhibition of cardiac sodium channels, attributes that have generally proved to be beneficial in the treatment of cardiac arrhythmias<sup>[26]</sup>. The effects of flecainide on other channels have also been reported. For example, flecainide inhibits several cloned potassium channels, including Kv1.1, Kv1.2, Kv1.5, Kv2.1, Kv4.2, and hERG channels<sup>[19, 20, 23]</sup>. However, blockade of these channels has generally required relatively high drug concentrations.

Limited data are available for the Kv1.4 potassium channel.



**Figure 5.** Changes in the inactivation kinetic properties of the fKv1.4ΔN currents by flecainide and verapamil. (A and B) Steady-state inactivation was studied using a two-step voltage protocol, currents were measured at +50 mV, and the 5 s pre-steps 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:  $I/I_{max} = (1 - \alpha) / [1 + \exp\{-(V - V_{1/2})/k\}] + \alpha$ . (C and D) Time constant of inhibition as a function of the drug concentration. Time constants ( $\tau_{block}$ ) were estimated from a single or double exponential fits to the tracings shown in Figure 1. The apparent rate constants for association ( $k_{+1}$ ) and dissociation ( $k_{-1}$ ) were obtained from the equation:  $1/\tau_{block} = k_{+1}[d] + k_{-1}$  ( $n=5$ ). Data are shown as mean  $\pm$  SEM ( $n=5$ ).

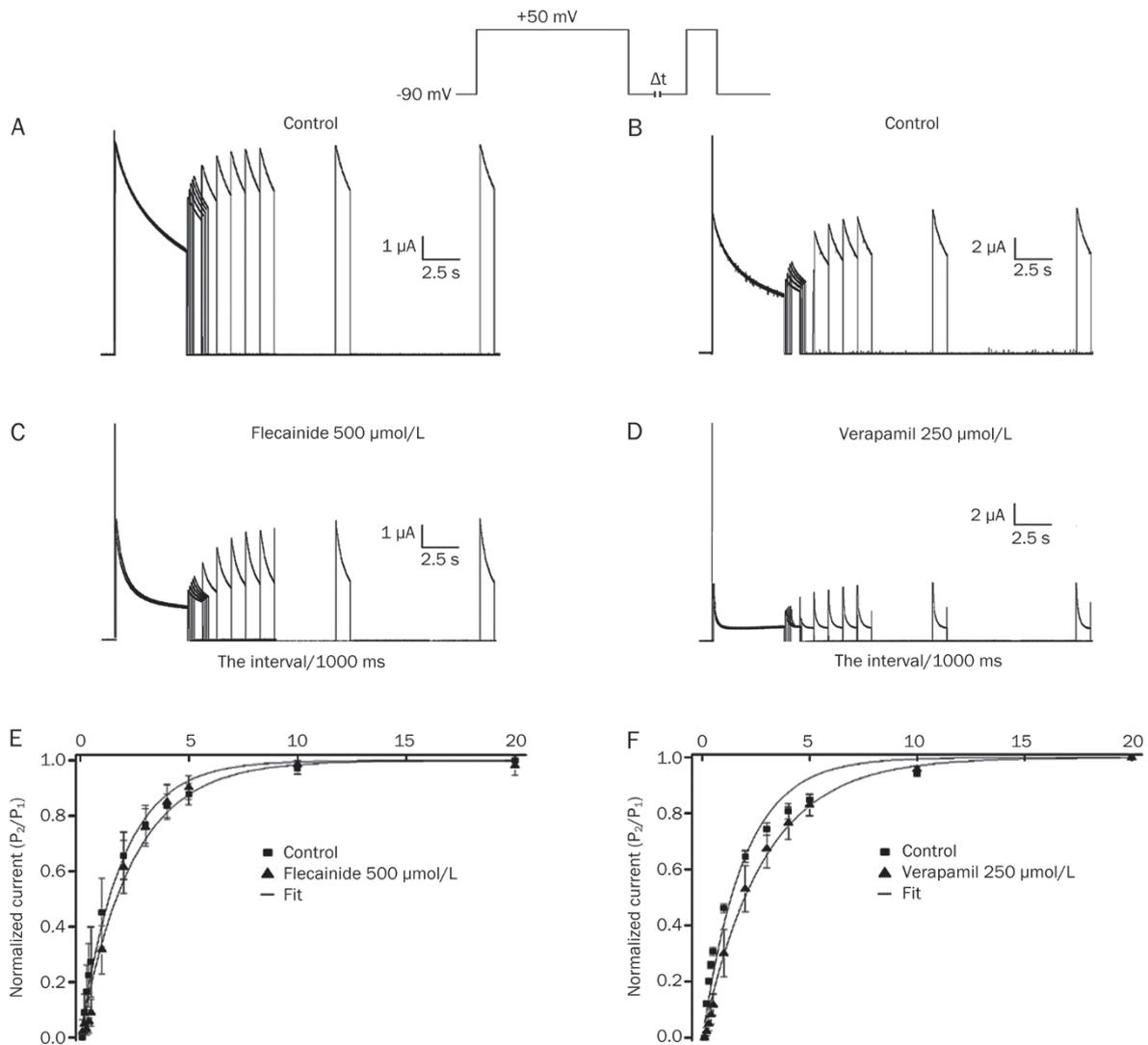
In *Xenopus* oocytes, both sodium channel antagonists and calcium channel antagonists have been reported to reduce fKv1.4 potassium channel currents. Our recent study showed that verapamil inhibited the fKv1.4 potassium channel in *Xenopus* oocytes with an  $IC_{50}$  of 263.26  $\mu\text{mol/L}$ <sup>[13]</sup>, and propafenone and diltiazem share its characteristics<sup>[14]</sup>. Flecainide was also shown to reduce Kv1.4 potassium currents<sup>[23]</sup>, but its mechanism of action was not examined. Our results provide novel information on the inhibition of fKv1.4ΔN potassium currents by flecainide. Our findings agree with previous reports, although the  $IC_{50}$  for a flecainide block (512.29  $\mu\text{mol/L}$ ) is relatively higher than that for blockade of the sodium channel<sup>[27]</sup>. However, the blockade of channels expressed in *Xenopus* oocytes by lipophilic drugs often requires much higher concentrations (five- to ten-fold) than those are needed to block the channels expressed in mammalian cells<sup>[23]</sup>. Therefore, flecainide may have a greater potency toward the fKv1.4ΔN channel than reported in this study.

Our results reveal that flecainide inhibits 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<sup>[14]</sup>. Verapamil shares these characteristics. Our results demon-

strate that flecainide and verapamil exhibit similar affinities for fKv1.4ΔN, but the drugs do not share high-affinity hERG channel blocking properties<sup>[21]</sup>.

Because flecainide and verapamil are different types of antiarrhythmic drugs, we examined the electrophysiological effects of both drugs on fKv1.4ΔN channel inactivation. Under control conditions, fKv1.4ΔN channels only show slow C-type inactivation, and the decay of the currents can be fitted with a single-exponential function. The binding of open-channel blockers introduces a blocked state. The blocked channel is rapidly inactivated, enters the blocked-inactivated state, and subsequently proceeds to the C-type inactivated state, leading to a bi-exponential decay of fKv1.4ΔN currents<sup>[12]</sup>. The fast time constant reflects the kinetics of open-channel blocking by flecainide/verapamil, and the slow time constant reflects the C-type inactivation kinetics in the presence of drugs. Both flecainide and verapamil accelerated C-type inactivation of fKv1.4ΔN.

Here, we compared the effects of flecainide and verapamil on fKv1.4ΔN inactivation. 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 flecainide and



**Figure 6.** Effects of flecainide and verapamil on the kinetics of the fKv1.4ΔN channel recovery from steady-state inactivation. The degree of recovery was measured by following a standard variable interval gapped step protocol. An initial 5 s step ( $P_1$ ) from -90 to +50 mV was followed by a second step ( $P_2$ ) to +50 mV after an interval of between 0.1 and 20 s. (A and B) Current traces were elicited in the absence of flecainide (A) or verapamil (B). (C and D) Current traces obtained in the presence of 500 μmol/L flecainide (C) or 250 μmol/L verapamil (D). (E and F) The ratio of the peak current elicited by the  $P_1$  and  $P_2$  steps ( $P_2/P_1$ ) is plotted as a function of the various interstep intervals. The continuous line represents the fit of the data to the equation:  $f=1-A*\exp(-\tau/t)$ . Data were normalized between 0 and 1 presented with intervals on a log scale. Data are shown as mean±SEM ( $n=5$ ).

shifted the steady-state 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<sup>[12, 14]</sup>. Flecainide also slightly shifted the steady-state inactivation curve to the left, but the  $V_{1/2}$  was not significantly changed. These results indicate that the voltage-dependency of the steady-state inactivation of the fKv1.4ΔN channel is altered by verapamil but not by flecainide. The lack of an effect of flecainide on the voltage dependency of steady-state inactivation suggests that flecainide is unlikely to interact with the inactivated state of the fKv1.4ΔN channel<sup>[28]</sup>. The sodium channel antagonist propafenone shares these characteristics<sup>[14]</sup>. 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 also interact simultaneously with the open and inactivated states of the fKv1.4ΔN channel<sup>[29]</sup>. Unlike verapamil, diltiazem only interacts with the fKv1.4ΔN channel in the open state and not in the inactivated state<sup>[14]</sup>. We suggest that the blockade of fKv1.4ΔN channels by verapamil is a more complex process than blockade by diltiazem and flecainide. The binding of diltiazem and flecainide to the channel affects the C-type inactivation without changing channel activation<sup>[14]</sup>, while the binding of verapamil may alter channel activation<sup>[30]</sup>.

In this study, we used fKv1.4ΔN, which lacks rapid N-type

inactivation. However, both N-type and C-type inactivation co-exist in native Kv1.4 channels<sup>[12]</sup>. The effects of flecainide and verapamil on the currents of native Kv1.4 have been studied previously<sup>[23]</sup>. The steady-state activation curves were shifted in the positive direction by verapamil but not by flecainide, similar to their effects on fKv1.4ΔN. The steady-state inactivation curves were not affected by either drug. The decay time course of native Kv1.4 was accelerated by verapamil, but it was decelerated by flecainide and quinidine. However, we found that fKv1.4ΔN decayed faster in the presence of flecainide and verapamil, and the effect of quinidine was similar to that of flecainide<sup>[12]</sup>. Although the effects of flecainide and verapamil inactivation were different for native Kv1.4 and fKv1.4ΔN, the recovery of native channels from inactivation would have to be taken into account. The slower C-type inactivation is critically important for determining the rate of recovery and is likely to be of primary physiological importance for determining the refractory period of Kv1.4 channels<sup>[11]</sup>. When a drug blocks fKv1.4ΔN, recovery is still governed by the direct C-type inactivated-to-open transition because dissociation of the drug is most likely much faster than recovery from C-type inactivation<sup>[12]</sup>. In this study, verapamil showed a greater effect on Kv1.4 than flecainide, slowing recovery of the fKv1.4ΔN channel. Flecainide did not alter channel recovery, consistent with its lack of an effect on C-type inactivation.

Using the fast time constants in the range from 10 to 1000 μmol/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 486 μmol/L in the presence of flecainide and 242 μmol/L in the presence of verapamil<sup>[14, 28, 31, 32]</sup>. The values were close to the IC<sub>50</sub> values obtained from the concentration-response curve. The similarity of the IC<sub>50</sub> values obtained by the two independent methods supports the open-channel block model used to calculate the rate constant for the fKv1.4ΔN channel<sup>[33, 34]</sup>.

Flecainide exerts effects on cardiac arrhythmias that can be distinguished from other sodium channel antagonist drugs. Although the antiarrhythmic efficacy of flecainide has usually been ascribed to its suppression of sodium channels, our findings suggest that the blockade of fKv1.4ΔN channels may also contribute to the cardiac effects of flecainide. The Kv1.4 channel, which conducts  $I_{to}$ , contributes to phase 1 and the early part of phase 2 of the cardiac action potential and plays an important role in the repolarization of cardiac myocytes<sup>[4-7]</sup>. Flecainide and verapamil share an ability to inhibit the fKv1.4ΔN currents and increase C-type inactivation.

Whether the inhibition of fKv1.4ΔN currents by flecainide or verapamil would exert a beneficial action on arrhythmias remains to be studied. On the one hand, suppression of fKv1.4ΔN channels prolongs action potential duration and increases the refractory period, and it may thereby terminate reentrant circuits<sup>[35]</sup>. Blockade of fKv1.4ΔN currents and decreasing the rate of channel recovery could attenuate the shortening of the action potential duration that results from

the inhibition of L-type calcium channels by verapamil<sup>[13]</sup>. On the other hand, Kv1.4 channels are expressed in the subendocardium of the human heart<sup>[1-3]</sup>, and the extra inhibition of Kv1.4 would increase the transmural dispersion of repolarization, which could lead to cardiac arrhythmias.

In summary, the present study provides the first indication that the widely used antiarrhythmic drugs flecainide and verapamil substantially inhibit fKv1.4ΔN potassium channels expressed in the *Xenopus* oocyte by binding to the open state of the channel. Furthermore, inhibition of Kv1.4 by flecainide and verapamil is completely independent of their inhibition of sodium channels and calcium channels. Therefore, caution should be exercised when these drugs are administered in combination with other potassium channel blockers to patients suffering from atrial fibrillation, supraventricular arrhythmias, and long QT syndrome.

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### Author contribution

Hui CHEN and Jiang-hua REN designed the research; Hui CHEN, Dong ZHANG, Lin XU, Sheng-ping CHAO, and Xue-jun JIANG performed the research; Hui CHEN and Shi-min WANG analyzed the data; and Hui CHEN wrote the paper.

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