



# Effects of propafenone on K currents in human atrial myocytes

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**1** The class Ic anti-arrhythmic agent, flecainide is known to inhibit the transient outward K current (Ito) selectively in human atrium. We studied the effects of propafenone, another class Ic anti-arrhythmic agent, on K currents in human atrial myocytes using a whole-cell voltage-clamp method.

**2** Propafenone inhibited both Ito and the sustained or ultra-rapid delayed rectifier K current (Isus or Ikur) evoked by depolarization pulses. The concentration for half-maximal inhibition (IC<sub>50</sub>) was 4.9 μM for Ito and 8.6 μM for Isus. Propafenone blocked Ito and Isus in a voltage- and use-independent fashion and accelerated the inactivation time constant of Ito [from 28.3 to 6.7 ms at 10 μM propafenone].

**3** The steady-state inactivation curve for Ito was unaffected by propafenone. Propafenone did not affect the initial current at depolarizing potentials, but it did produce a block that increased as a function of time after depolarization (time constant of 3.4 ms). This suggests that propafenone preferentially blocked Ito in the open state.

**4** Propafenone had no significant effect on the rate at which Ito recovered from inactivation at –80 mV suggesting that propafenone dissociates rapidly from the channel.

**5** The steady-state activation curve for Isus was not affected by propafenone. Propafenone slowed the time course of the onset of the Isus tail current. This suggests that propafenone blocked Isus in the open state.

**6** The present results suggest that, unlike flecainide, propafenone blocks both Ito and Isus in human atrial myocytes in the open state at clinically relevant concentrations.

**Keywords:** Human atrial cells; K<sup>+</sup> channels, anti-arrhythmic drugs; propafenone; whole-cell voltage-clamp method

**Abbreviations:** Isus or Ikur, sustained or ultra-rapid delayed rectifier K current; Ito, transient outward K current

## Introduction

Wang *et al.* (1993b) reported that the transient outward current (Ito) and the sustained or ultra-rapid delayed rectifier K current (Isus or Ikur) contribute significantly to action potential duration in the human atrium. In fact, the activation and inactivation of Ito are both rapid, and thus it contributes to the phase 1 repolarization of the atrial action potential. On the other hand, Ikur or Isus activates very rapidly but inactivates slowly, and it contributes to the whole repolarization phase of the atrial action potential.

The class Ic antiarrhythmic agents flecainide and propafenone are widely used for the conversion and treatment of atrial tachyarrhythmia (Harrison, 1985). According to Kosmala *et al.* (1994), Botto *et al.* (1994), Capucci *et al.* (1994) and Chimienti *et al.* (1996), these two drugs are equally effective at converting atrial fibrillation. Further, Stroobandt *et al.* (1997) found that low-dose propafenone was well tolerated and that it was effective in maintaining sinus rhythm in atrial fibrillation (af) patients for 6 months after pharmacological or electrical restoration of sinus rhythm. As mentioned above, these anti-arrhythmic drugs have been shown to be clinically useful in cases of atrial tachyarrhythmia; however, relatively few studies have evaluated the effects of these agents on membrane currents in the human atrium. Wang *et al.* (1995) have demonstrated that flecainide at clinically relevant concentrations inhibits Ito without affecting Isus in the human atrium, but the effects of propafenone on K currents in human atrial cells have not been properly evaluated. Although flecainide and propafenone both belong to the same class of drugs, we

cannot take for granted that they have the same effect on voltage-gated K channels in the human atrium. The present study was designed to study the effects of propafenone on K currents in human atrial cells using the whole-cell patch-clamp method.

## Methods

### Preparation of single cells

The cell-isolation procedure for human atrial myocytes was derived from a technique described previously (Escande *et al.*, 1987; Sakai *et al.*, 1995; Kajimoto *et al.*, 1997). Small segments of myocardium sampled from right atrial appendages were obtained at the time of open-heart surgery, in accordance with institutional guidelines governing research on human subjects. Each patient had a normal right atrial pressure and a normal sinus rhythm and was between 1 and 56 years of age. The clinical diagnosis was congenital heart disease in 35 patients and valvular disease in five patients. In brief, each segment of human myocardium was cut into pieces and incubated in warm Tyrode's solution for 10 min. The strips were then cut into even smaller pieces and left in nominally Ca<sup>2+</sup>-free Tyrode's solution for 17 min. They were then transferred to nominally Ca<sup>2+</sup>-free Tyrode's solution containing 0.8 mg ml<sup>-1</sup> collagenase (Yakult, Tokyo, Japan) with 1 mg ml<sup>-1</sup> protease (Type XX VII, Sigma Chemical Co, St. Louis, MO, U.S.A.) for 70–80 min at 37°C. The collagenase was then washed out by rinsing with high-K<sup>+</sup>, low Cl<sup>-</sup> solution (Isenberg & Klöckner, 1982) and the digested tissue was stored in the same solution.

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### Electrical recordings

The whole-cell voltage-clamp method used was one described previously (Hamill *et al.*, 1981; Hagiwara *et al.*, 1992a,b; Matsuda *et al.*, 1996; Sakai *et al.*, 1996; Kajimoto *et al.*, 1997; Shoda *et al.*, 1997). Patch pipettes were pulled from 1.25 mm borosilicate capillaries (D941-8.5-85 Clinitubes; Radiometer A/S, Copenhagen, Denmark). The resistance of each electrode, when filled with the pipette solution, was within the range 2–3 M $\Omega$ . The amplifier (TM-1000, ACT ME Laboratory, Tokyo, Japan) employed a 100 M $\Omega$  feedback resistor, and series resistance was partially compensated. The current-voltage (I-V) signals were stored on a video recorder (S-6000, Victor, Tokyo, Japan) *via* a PCM converter system (RP-880, NF Electronic Instruments, Tokyo, Japan) being used for computer analysis (PC 9801 RA, NEC, Tokyo, Japan). The current signals were fed from the video recorder to the computer *via* a 2.5 kHz, 8 pole Bessel-type low-pass filter. No leakage correction was applied. From a holding potential of  $-40$  mV, ramp pulses of amplitude  $\pm 90$  mV (0.72 V/sec) were applied to enable measurement of cell membrane capacitance (Hagiwara *et al.*, 1992a). The value so obtained was  $72.6 \pm 4.4$  pF ( $n=33$ ). In accordance with the previous reports (Wang *et al.*, 1993b, 1995), we used a double-pulse protocol to separate Ito and Isus (see legend to Figure 2), however, the Isus derived by the second pulse in double-pulse protocol did not differ from that at the end of the first 200 ms pulse. Therefore, we measured Isus as the amplitude of the current at the end of the first or second pulse relative to the zero current level. We have measured the amplitude of Ito as the difference between the peak current

and the sustained current at the end of the pulse to minimize the contamination of Isus. Experiments were performed at room temperature.

### Solutions

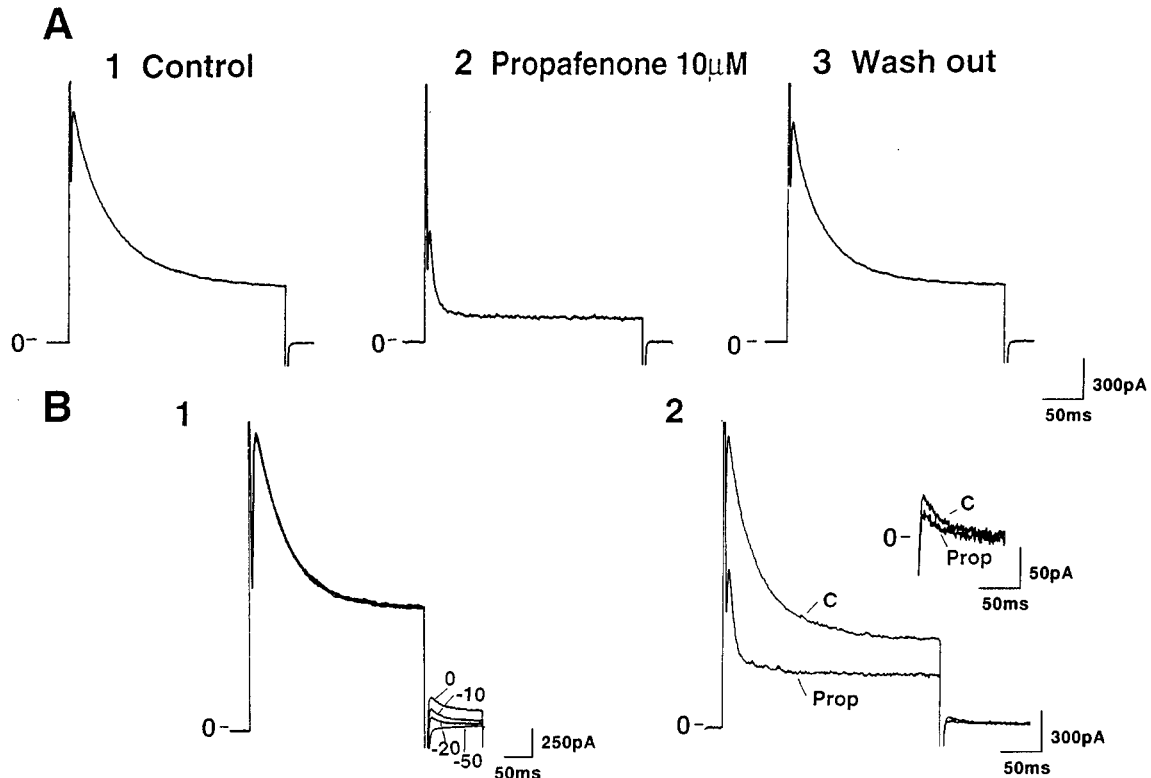
The normal Tyrode's solution contained (in mM): NaCl 136.9, KCl 5.4, CaCl<sub>2</sub> 1.8, NaH<sub>2</sub>PO<sub>4</sub> 0.33, glucose 5 and HEPES 5 (pH adjusted to 7.4 with NaOH). The standard pipette solution contained (in mM): KOH 110, KCl 20, EGTA 10, MgCl<sub>2</sub> 2, K<sub>2</sub>-ATP 5, K<sub>2</sub>-creatine phosphate 5 and HEPES 5 (pH=7.4 with aspartic acid of approximately 60 mM). The standard external solution contained (in mM): N-methyl-D-glucamine (NMG) 150, KCl 5.4 and HEPES pH=7.4 with HCl) 5; CdCl<sub>2</sub> 0.2 and BaCl<sub>2</sub> 1 were added to eliminate contamination by Ca currents, inward rectifying K currents and delayed rectifying K currents (Wang *et al.*, 1995).

### Drugs

Propafenone HCl was kindly provided by Yamanouchi Pharmaceuticals (Tokyo, Japan) and dissolved in dimethyl sulphoxide (DMSO) as a 250 mM stock solution. The final concentration of DMSO did not exceed 0.1%; this concentration was found to have no effect on the control membrane currents.

### Statistical Analysis

Paired and unpaired Student's *t*-tests were used to evaluate the statistical significance of differences between means. Values of



**Figure 1** Effects of propafenone on K currents in a human atrial cell. (A) Current traces elicited by a 300 ms depolarization pulse from a holding potential of  $-50$  mV to  $+60$  mV exhibited Ito followed by a sustained outward current. Current traces were obtained under control conditions (A1), in the presence of  $10 \mu\text{M}$  propafenone (A2) and after a 6-min wash-out (A3). (B) The tail current of the sustained outward current. Tail currents were obtained at  $-50$ ,  $-20$ ,  $-10$  and  $0$  mV after 300 ms depolarizing pulses from a holding potential of  $-50$  mV to  $+60$  mV (B1). Ten  $\mu\text{M}$  propafenone inhibited both the sustained current and the tail current obtained at  $-20$  mV (B2). Inset shows (at a greater gain) the change in the tail current determined in the same cell.

$P < 0.05$  were considered to indicate statistical significance. All statistical data are given as mean  $\pm$  s.e.mean.

## Results

### Effects of propafenone on Ito and Isus

To evaluate the effects of propafenone on the transient outward current (Ito) and the sustained delayed rectifier K current (Isus) in human atrial cells, we used an external solution containing NMG,  $\text{Cd}^{2+}$  and  $\text{Ba}^{2+}$  in order to block the Na current, Ca current and other K currents (IK1 and IK; Wang *et al.*, 1995). Figure 1 illustrates the effects of propafenone on K currents in a human atrial cell. The current traces elicited by a 300 ms depolarization pulse from a holding potential of  $-50$  mV to  $+60$  mV exhibited Ito followed by a sustained outward current (A1). Ten  $\mu\text{M}$  propafenone inhibited both Ito and the sustained outward current (by 52.3 and 51.8%, respectively, in the cell illustrated) (A2). Inhibition of these currents by propafenone was completely reversed after a 6-min wash-out (A3).

To determine whether the sustained outward current observed in Figure 1A is indeed the Isus previously observed in the human atrium, we measured the tail currents of this sustained outward current. Figure 1B1 shows the tail currents obtained at  $-50$ ,  $-20$ ,  $-10$  and  $0$  mV. The amplitude of the tail current was largest at  $0$  mV and it became progressively smaller as greater and greater negative potentials were used. Ten  $\mu\text{M}$  propafenone inhibited both the sustained current and the tail current obtained at  $-20$  mV (B2) and these currents were completely blocked by  $1$  mM 4-AP (results not

illustrated). These results indicated that the sustained outward current observed in Figure 1 is indeed the Isus previously reported in human atrial myocytes (Wang *et al.*, 1993b).

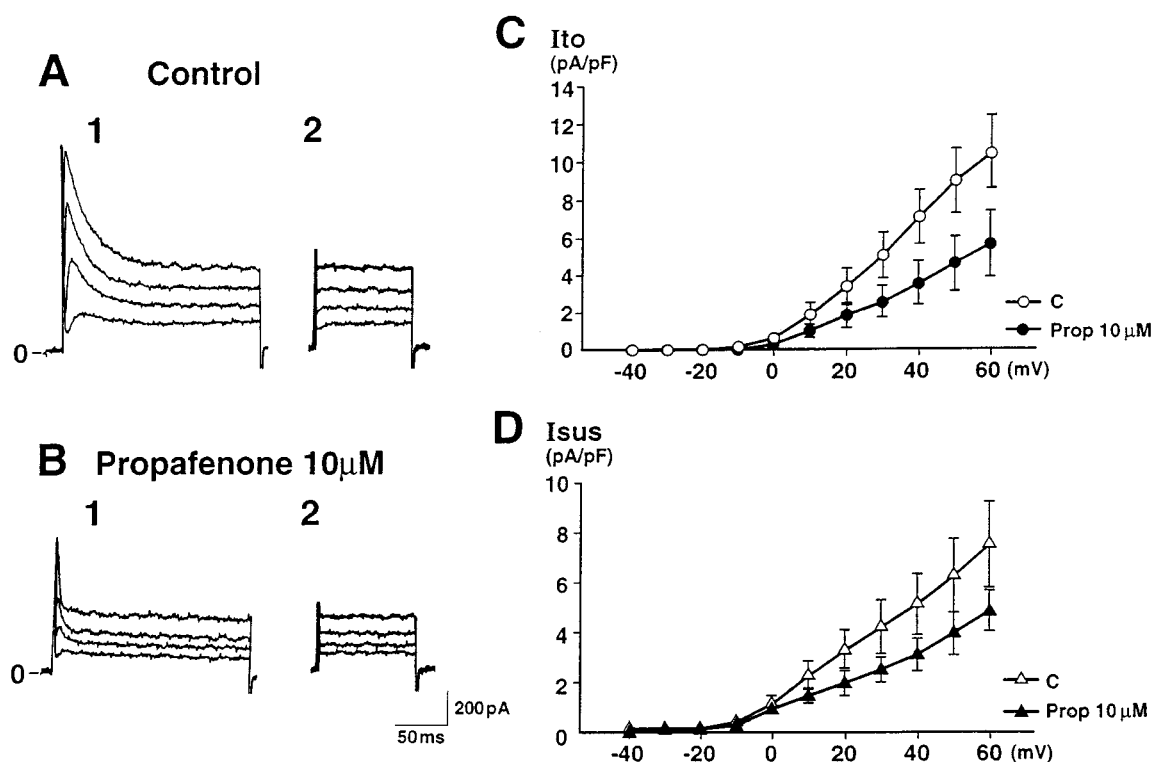
Figure 2 shows current traces obtained in the absence and presence of  $10 \mu\text{M}$  propafenone (A and B). Current traces elicited by 200 ms depolarizing pulses from a holding potential of  $-50$  mV to various depolarizing potentials (A1 and B1) exhibited Ito followed by Isus (A2 and B2). Since reactivation of Ito is known to occur at around 50–60 ms (Wang *et al.*, 1993b), we used a double-pulse protocol (see legend to Figure 2) to separate Ito and Isus. Current-voltage relationships for Ito and Isus are shown in Figure 2C and D, respectively. Ito and Isus were both reduced by  $10 \mu\text{M}$  propafenone at all membrane potentials examined suggesting that, whereas flecainide only blocks Ito (Wang *et al.*, 1995), propafenone inhibits both Ito and Isus in human atrial cells.

### Concentration-dependence of inhibition of Ito and Isus

Dose-response relationships for the inhibition of Ito and Isus by propafenone are illustrated in Figure 3. In the presence of propafenone, current amplitude was reduced and the inactivation of Ito was accelerated, both effects occurring in a dose-dependent manner. Propafenone blocked Ito and Isus with  $\text{IC}_{50}$  values of  $4.9 \mu\text{M}$  and  $8.6 \mu\text{M}$ , respectively.

### Voltage-dependence of effects of propafenone on Ito and Isus

To examine the voltage-dependence of the effects of propafenone on these K currents, the relationships between



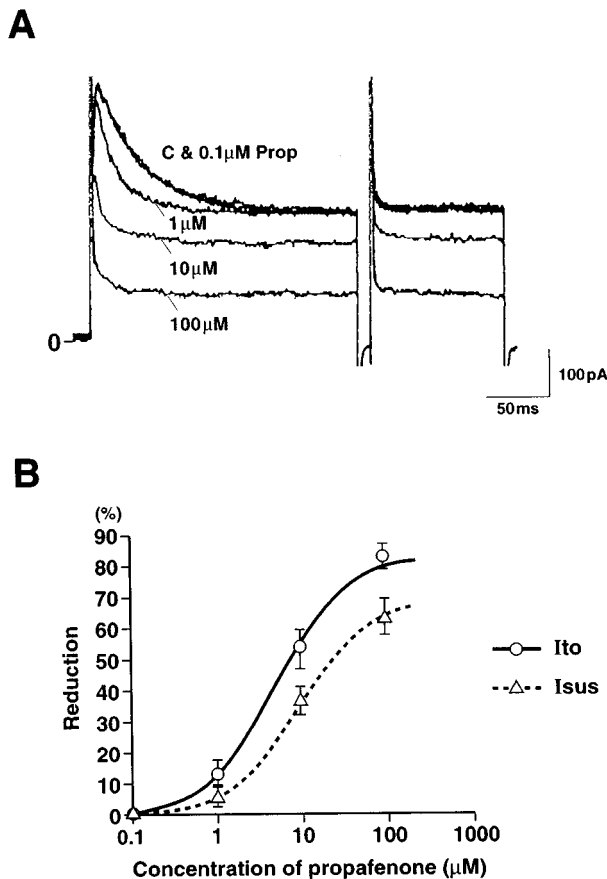
**Figure 2** Effect of propafenone on Ito and Isus in a human atrial cell. Current traces were obtained in the absence (A) or presence (B) of  $10 \mu\text{M}$  propafenone. Ito was elicited by 200 ms depolarizing pulses from a holding potential of  $-50$  mV to  $0$ ,  $+20$ ,  $+40$  and  $+60$  mV (A1 and B1). Isus was elicited by 100 ms depolarizing pulses from the same holding potential 10 ms after a 200 ms prepulse to  $+60$  mV had been delivered to inactivate Ito (A2 and B2). (C and D) Current-voltage (I-V) relationships for Ito and Isus ( $n=8$ ) before and after application of  $10 \mu\text{M}$  propafenone showing that both Ito and Isus were reduced by propafenone at every membrane potential examined. Currents were normalized with respect to cell capacitance. Current densities of Ito and Isus were  $10.39 \pm 1.94$  pA/pF and  $7.47 \pm 1.73$  pA/pF, respectively, at  $+60$  mV under control conditions, while the corresponding values after application of propafenone were  $5.57 \pm 1.81$  pA/pF and  $4.83 \pm 0.87$  pA/pF, respectively.

the amplitude of the test potential and the reduction in  $I_{to}$  and  $I_{sus}$  induced by  $10 \mu\text{M}$  propafenone were analysed (Figure 4). For a given current, the reduction induced by  $10 \mu\text{M}$  propafenone was much the same regardless of the test potential, indicating that propafenone blocked  $I_{to}$  and  $I_{sus}$  in a voltage-independent fashion.

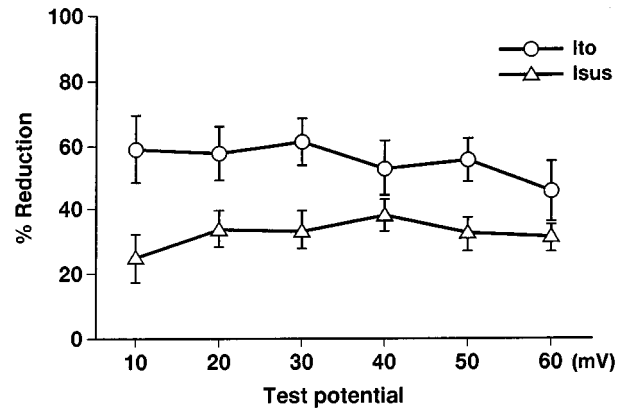
#### Use-dependence of effects of propafenone on $I_{to}$ and $I_{sus}$

The actions of anti-arrhythmic drugs are known to be state-dependent, resulting in effects that depend on both the voltage and frequency of depolarization. To determine the use-dependency of the effect of propafenone on  $I_{to}$  and  $I_{sus}$ , a train of depolarizing pulses to  $+60 \text{ mV}$  from a holding potential of  $-80 \text{ mV}$  was delivered at  $2 \text{ Hz}$  once the cells had fully equilibrated following application of  $10 \mu\text{M}$  propafenone. Under control conditions,  $I_{to}$  was not altered by repetitive pulsing at  $2 \text{ Hz}$  (Figure 5A1). Figure 5A2 shows representative current traces evoked by a  $2 \text{ Hz}$  train of constant amplitude

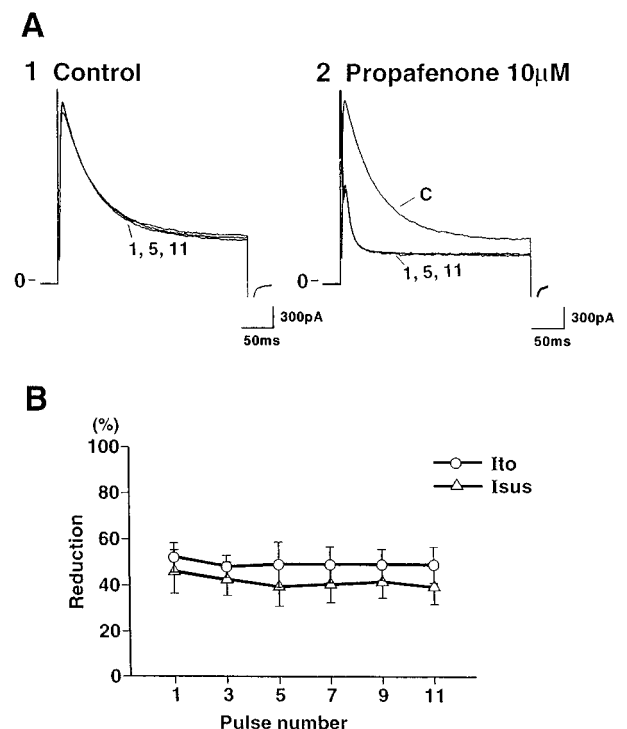
stimuli (to  $+60 \text{ mV}$ ) in the presence of  $10 \mu\text{M}$  propafenone, and in Figure 5B, the percentage inhibition of each current is plotted against pulse number. The currents did not alter in the course of a train of stimuli. Similarly, the currents did not alter in the course of a  $1 \text{ Hz}$  train of stimuli from  $-80 \text{ mV}$  (holding potential) to  $+60 \text{ mV}$  or  $+30 \text{ mV}$  (results not illustrated). These results indicated that propafenone blocked  $I_{to}$  and  $I_{sus}$



**Figure 3** Concentration-dependence of inhibitory effect of propafenone on  $I_{to}$  and  $I_{sus}$ . (A) Tracings showing the effects of 0.1, 1, 10 and  $100 \mu\text{M}$  propafenone.  $I_{to}$  was elicited by  $200 \text{ ms}$  depolarizing pulses from a holding potential of  $-50 \text{ mV}$  to  $+60 \text{ mV}$ .  $I_{sus}$  was elicited as in Figure 2. (B) Dose-response relationships for the effects of propafenone on  $I_{to}$  and  $I_{sus}$ . Data points show the percentage reduction in  $I_{to}$  and  $I_{sus}$  induced by propafenone.  $I_{to}$  and  $I_{sus}$  were not affected by  $0.1 \mu\text{M}$  propafenone. Mean reduction in  $I_{to}$  was by  $14.2 \pm 3.8$ ,  $53.7 \pm 6.1$  and  $83.1 \pm 4.1\%$  ( $n=14$ ) of control and that in  $I_{sus}$  was by  $7.3 \pm 2.7$ ,  $38.0 \pm 4.4$  and  $63.8 \pm 5.7\%$  ( $n=12$ ) of control at 1, 10 and  $100 \mu\text{M}$  propafenone, respectively. Lines were derived by a nonlinear least-squares fit of the values for the mean percentage reduction to the Michaelis equation: percentage reduction =  $E_{\text{max}}(\text{propafenone})/[(\text{propafenone}) + IC_{50}] \times 100$ . Ordinate shows percentage reduction in  $I_{to}$  and  $I_{sus}$ . The values of  $E_{\text{max}}$  and  $IC_{50}$  obtained from these curves were  $83.3\%$  and  $4.9 \mu\text{M}$ , respectively (for  $I_{to}$ ) and  $70.1\%$  and  $8.6 \mu\text{M}$ , respectively (for  $I_{sus}$ ).



**Figure 4** Dependence of drug effects of propafenone on test potential. Currents were elicited using the same experimental protocol as in Figure 2. The values for the mean percentage reductions in  $I_{to}$  and  $I_{sus}$  induced by  $10 \mu\text{M}$  propafenone are plotted against the corresponding test potential. No significant voltage-dependence was observed for the blocks induced by propafenone ( $n=4$  for  $I_{to}$ ,  $n=6$  for  $I_{sus}$ ).



**Figure 5** Use-dependence of effects of propafenone on  $I_{to}$  and  $I_{sus}$ . A train of  $300 \text{ ms}$  depolarizing pulses was applied at  $2 \text{ Hz}$  from a holding potential of  $-80 \text{ mV}$  to  $+60 \text{ mV}$  under control conditions (A1) and 2 min after superfusion of the cell with  $10 \mu\text{M}$  propafenone (A2). Current traces were obtained from the control, 1st, 5th and 11th pulses. (B) The mean percentage reduction in  $I_{to}$  and  $I_{sus}$  induced by  $10 \mu\text{M}$  propafenone when a train of  $2 \text{ Hz}$  stimuli was used ( $n=3$ ) is plotted against the appropriate pulse number. No significant interaction was observed between pulse number and drug effect.

each in a use-independent manner, and it produced tonic block of these currents.

#### *Changes in the kinetic properties of I<sub>to</sub> induced by propafenone*

To evaluate whether propafenone affects the kinetic properties of I<sub>to</sub>, we also analysed the time course of inactivation and the steady-state inactivation curve in both the absence and presence of 10 μM propafenone (Figures 6 and 7). After exposure to 10 μM propafenone, the inactivation of I<sub>to</sub> was clearly accelerated. Under control conditions, the inactivation of I<sub>to</sub> was well fitted by a single exponential relation with a time constant of 28.3 ± 2.2 ms at +60 mV ( $\tau(C)$ ,  $n = 10$ ). After addition of 10 μM propafenone,  $\tau$  (Prop) was 6.7 ± 0.6 ms ( $n = 10$ ). In fact, the time course of inactivation was accelerated by propafenone in a dose-dependent manner, the time constants being 23.1 ± 2.8 ( $n = 6$ ) and 6.6 ± 0.8 ms ( $n = 5$ ), respectively, with 1 and 100 μM propafenone.

Next, we examined the effect of propafenone on the steady-state inactivation curve for I<sub>to</sub> (Figure 7). The membrane potential was held at various levels between -80 mV and +20 mV and depolarizing test pulses to +60 mV were applied. The peak amplitudes of I<sub>to</sub> were normalized, plotted against the membrane potential, and fitted to a Boltzmann equation as follows:

$$y^\infty = \{1 + \exp[(V_m - V_{0.5}/s)]^{-1}$$

where  $y^\infty$  is the inactivation parameter,  $V_m$  is the membrane potential,  $V_{0.5}$  is the potential required to give a half value, and  $s$  is the slope factor. The slope factor under control conditions was 8.2 mV and  $V_{0.5}$  was -25.3 mV. The corresponding values were 8.5 mV and -24.9 mV, respectively, in the presence of 10 μM propafenone. These results indicated that propafenone did not modify the voltage-dependence of the steady-state inactivation of I<sub>to</sub>.

The voltage-dependent activation of I<sub>to</sub> was also analysed by examining the tail currents obtained on repolarization to -20 mV after a 5 ms pulse to test potentials between -40 and +80 mV. These data showed that the slope factor under control conditions was 8.6 mV and that  $V_{0.5}$  was 13.5 mV. The corresponding values were 9.1 mV and 13.9 mV, respectively, in the presence of 10 μM propafenone (results not illustrated), suggesting that propafenone did not alter the voltage-dependence of the steady-state activation of I<sub>to</sub>.

#### *Changes in the kinetic properties of I<sub>sus</sub> induced by propafenone*

We also examined the effect of propafenone on the activation kinetics for I<sub>sus</sub> (Figure 8). The steady-state activation curve of I<sub>sus</sub> was constructed from the tail current measurements at -20 mV (see legend to Figure 8). These data were well described by a single Boltzmann equation. The slope factor under control conditions was 6.1 mV and  $V_{0.5}$  was 4.8 mV. The corresponding values were 6.1 mV and 3.1 mV, respectively, in the presence of 10 μM propafenone, indicating that propafenone did not modify the voltage-dependence of the steady-state activation of I<sub>sus</sub>.

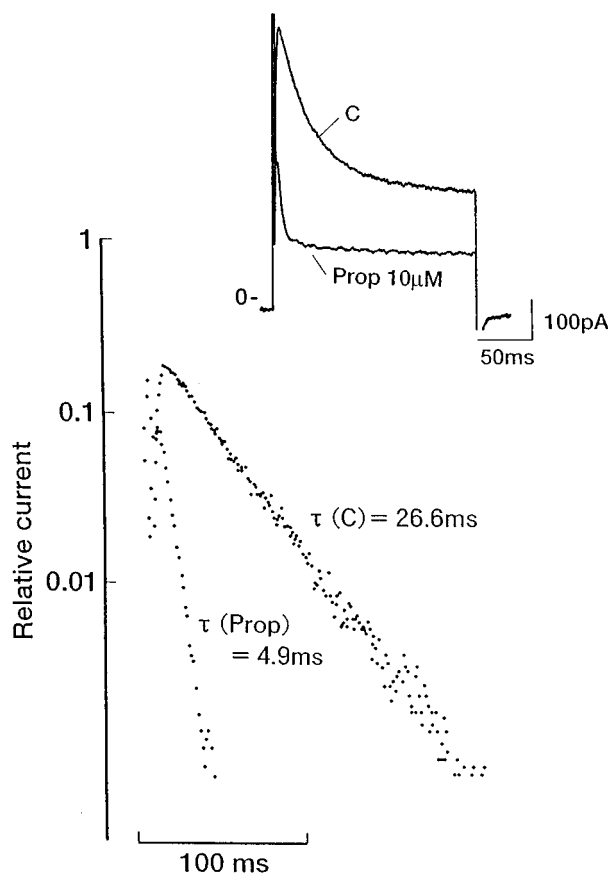
#### *Time-dependence of inhibition of I<sub>to</sub> by propafenone*

An acceleration of the inactivation of I<sub>to</sub> is known to result from a rapid blocking of open channels (Dukes *et al.*, 1990). To determine whether this occurred with propafenone, we plotted the degree of propafenone-induced I<sub>to</sub> blockade against the

time elapsed after the onset of the depolarizing clamp pulse (Figure 9). In the presence of propafenone, the initial current at depolarizing potentials did not differ from that obtained under control conditions. However, as shown in Figure 9, the current gradually declined with time after depolarization, indicating that propafenone blocked I<sub>to</sub> in an open state. The time constants obtained for the onset of the block were averaged for three experiments giving a value of 3.4 ± 0.4 ms at 10 μM propafenone. This value was of the same order as that obtained with 5 μM flecainide (6.3 ± 1.2 ms), an agent known to reduce I<sub>to</sub> by producing a rapid open-channel block in the human atrium (Wang *et al.*, 1995). In addition, these values were of the same order as the inactivation time constant (6.7 ± 0.6 ms) obtained with 10 μM propafenone (Figure 6), supporting the conclusion that the acceleration of the inactivation of I<sub>to</sub> induced by propafenone was due to an open-channel block.

#### *Effects of propafenone on the reactivation of I<sub>to</sub>*

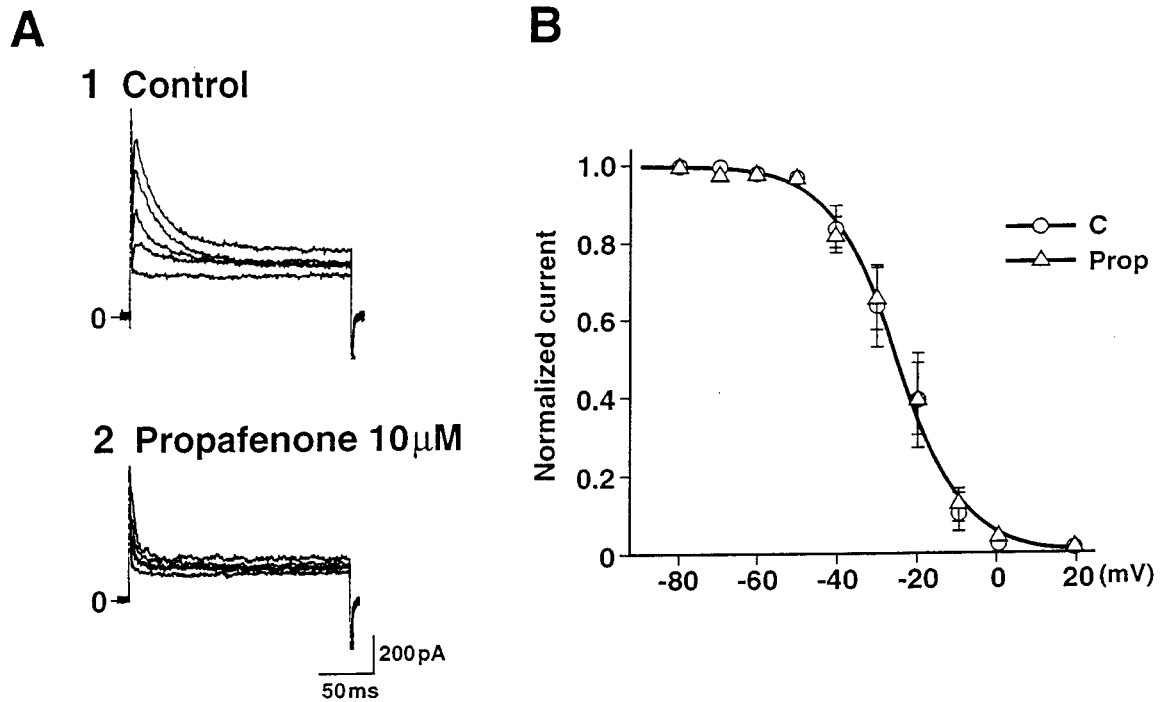
The recovery of I<sub>to</sub> from inactivation in the presence of drug reflects the dissociation rate constant of the drug from the I<sub>to</sub> channel. A rapid dissociation of propafenone from its binding site was suggested by the observed absence of a use-dependent block of I<sub>to</sub> (Figure 5). To elucidate the time-dependence of the cessation of the propafenone block that occurred on returning to the holding potential, we measured the reactivation of I<sub>to</sub> both in the absence and presence of propafenone.



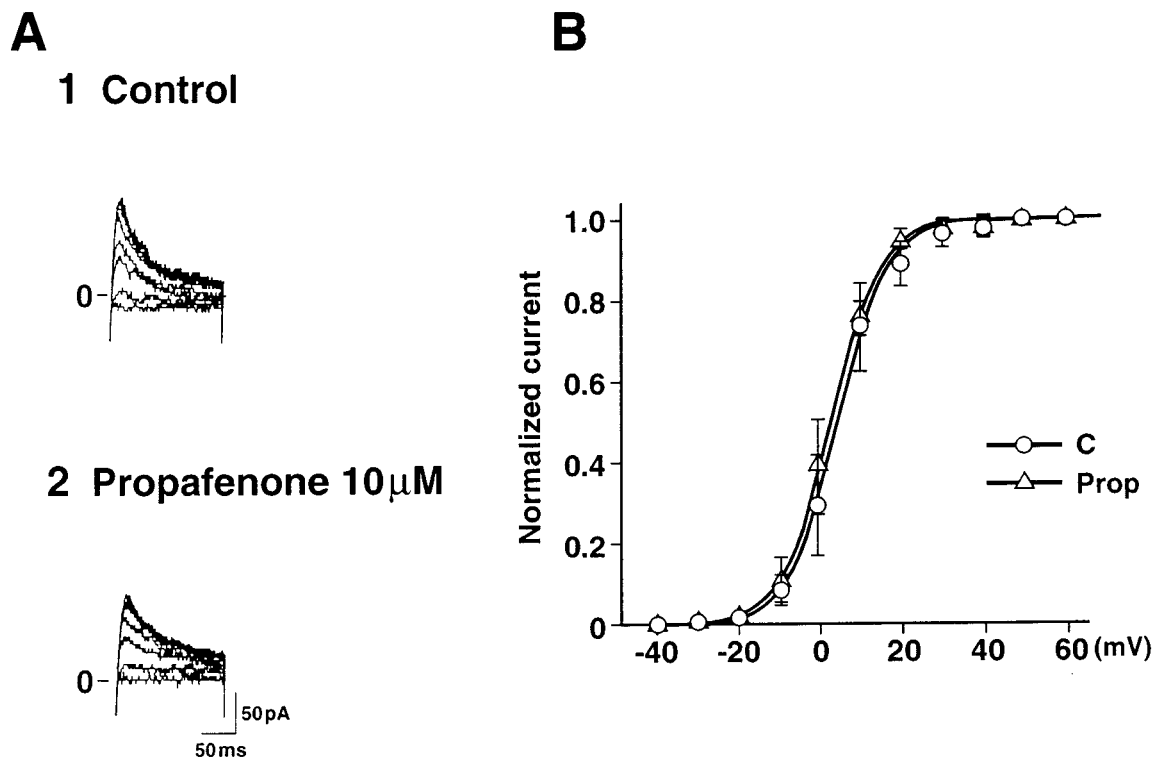
**Figure 6** Change induced by propafenone in the inactivation time course of I<sub>to</sub>. The graph shows semilogarithmic plots of I<sub>to</sub>. The inactivation of I<sub>to</sub> was well fitted by a single exponential relation with a time constant of 26.6 ms ( $\tau(C)$ ) under control conditions. After the addition of 10 μM propafenone,  $\tau$  (Prop) was 4.9 ms. Inset shows current traces before and after addition of 10 μM propafenone. Basic experimental protocol was as in Figure 3.

Figure 10A shows current traces obtained by applying 200 ms (P1) and 100 ms (P2) depolarizing pulses from a holding potential of  $-80$  mV to  $+60$  mV when the P1-P2 interval was

varied between 10 and 300 ms. The curves derived from such data revealed no significant difference in the reactivation of  $I_{to}$  whether  $10 \mu\text{M}$  propafenone was or was not present



**Figure 7** Effects of propafenone on the voltage-dependence of the steady-state inactivation curve for  $I_{to}$ . Steady-state inactivation curves were obtained in both the absence ( $n=14$ ) and presence of  $10 \mu\text{M}$  propafenone ( $n=9$ ). (A) Superimposed current tracings elicited by depolarizing pulses to  $+60$  mV from various holding potentials between  $-80$  mV and  $+20$  mV before (A1) and after (A2) application of  $10 \mu\text{M}$  propafenone. (B) Steady-state inactivation curves for  $I_{to}$  before and after addition of  $10 \mu\text{M}$  propafenone.



**Figure 8** Effects of propafenone on the voltage-dependence of the steady-state activation curve for  $I_{sus}$ . (A) Tail currents were obtained at  $-20$  mV after a 300 ms depolarization pulse to between 0 and  $+60$  mV from a holding potential of  $-50$  mV before (A1) and after (A2) application of  $10 \mu\text{M}$  propafenone. (B) Steady-state activation curves for  $I_{sus}$  before ( $n=6$ ) and after addition of  $10 \mu\text{M}$  propafenone ( $n=6$ ).

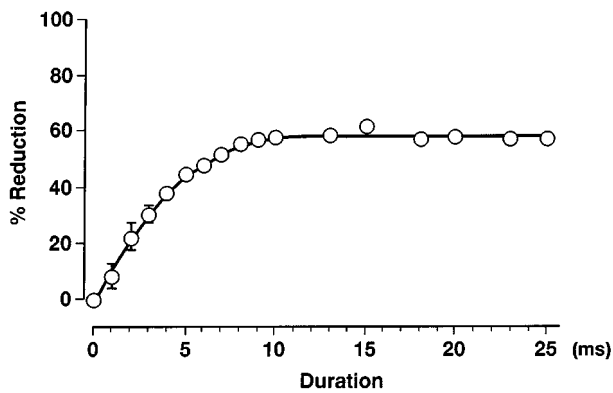
( $35.3 \pm 6.8$  ms and  $26.4 \pm 5.6$  ms,  $n=6$ , C & Prop, Figure 8B). Similarly, the reactivation of Ito was not significantly altered by propafenone at a holding potential of  $-50$  mV ( $54.6 \pm 8.4$  ms and  $68.4 \pm 16.7$  ms,  $n=7$ , C & Prop). This result, suggesting that propafenone dissociates rapidly from

the Ito channel, is consistent with the lack of use-dependency reported above.

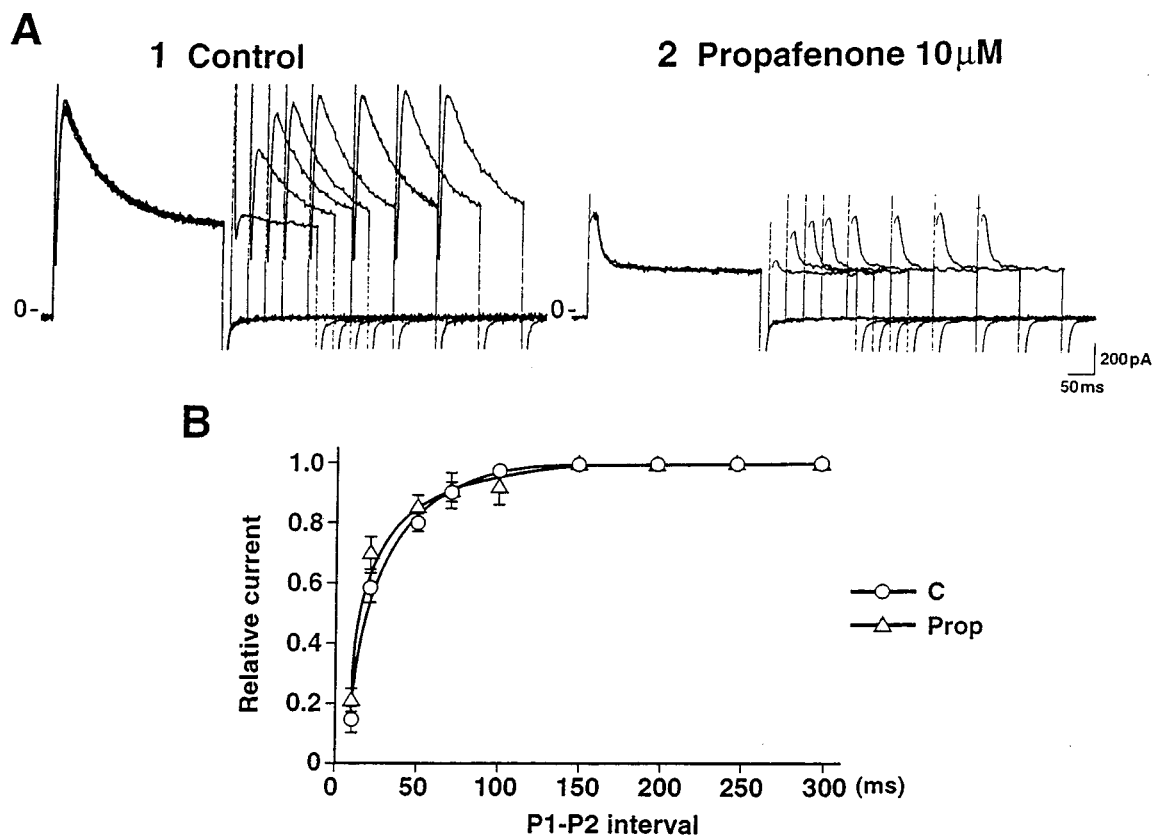
## Discussion

As mentioned in Introduction, Ito and Isus both make contributions to the repolarization phase of the action potential, and hence to action potential duration, in human atrial cells. It is known that a low dose of 4-aminopyridine (4-AP) blocks Isus selectively in human atrial cells. In fact, Wang *et al.* (1993b) reported that  $50 \mu\text{M}$  4-AP prolongs action potential duration ( $\text{APD}_{90}$ ) by approximately 66% in human atrial myocytes. This implies that Isus plays an important role in the repolarization phase of the atrial action potential. In addition, the recovery from inactivation of human Ito (time constant  $35.3 \pm 6.8$  ms, Figure 10) is much faster than that previously observed in the rabbit atrium (time constant  $650.6 \pm 159.0$  ms; Fermini *et al.*, 1992). Thus, the contribution made by Ito to the action potential may be important in the human atrium even in atrial tachyarrhythmias.

The efficacy of anti-arrhythmic drugs in the treatment of atrial fibrillation (af) is related to their ability to prolong atrial refractoriness (Rensma *et al.*, 1988; Kirchhof *et al.*, 1991; Wang *et al.*, 1992, 1993a), generally by prolonging the duration of the atrial action potential (Wang *et al.*, 1990). In actual fact, inhibiting K channels in atrial cells may be an important way of treating supraventricular arrhythmia. However, the effects of anti-arrhythmic drugs on K channels



**Figure 9** Time-dependence of inhibition of Ito by propafenone. Propafenone-induced Ito blockade is plotted against the time elapsed after the onset of the depolarizing pulse. The time constant for the onset of the block was averaged for three experiments: the value so obtained was  $3.4 \pm 0.4$  ms at  $10 \mu\text{M}$  propafenone. The curve was derived by a least-squares fit to the equation:  $B = B_{\text{max}} [1 - \exp(-kt)]$ , where  $B$  is the current block (percentage reduction in Ito),  $B_{\text{max}}$  is the maximum reduction produced by  $10 \mu\text{M}$  propafenone (found to be 58%),  $k$  is the rate constant and  $t$  is pulse duration.



**Figure 10** Effects of propafenone on reactivation of Ito. (A) Current traces before (1) and after (2) application of  $10 \mu\text{M}$  propafenone obtained using 200 ms (P1) and 100 ms (P2) depolarizing pulses to  $+60$  mV from a holding potential of  $-80$  mV. The P1-P2 interval was varied between 10 and 300 ms. (B) The normalized currents ( $\text{P2/P1}$ ) obtained in the absence or presence of  $10 \mu\text{M}$  propafenone were plotted as a function of the P1-P2 interval ( $n=6$ ). This process is well described by a single exponential with a time constant of  $35.3 \pm 6.8$  ms (control) or  $26.4 \pm 5.6$  ms (Prop). No significant change in the reactivation of Ito was seen following the application of  $10 \mu\text{M}$  propafenone.

have not yet been thoroughly evaluated in the human atrium. It was reported by Wang *et al.* (1995) that flecainide blocks Ito without affecting Isus in human atrial cells, and propafenone, another class Ic anti-arrhythmic agent, is known to be effective at converting atrial fibrillation (Capucci, 1994; Weiner, 1994) and maintaining a normal sinus rhythm (Chimienti *et al.*, 1996). However, the effects of propafenone on K currents in human atrial cells have not previously been evaluated. For this reason, we decided to investigate the effects of propafenone on K channels in human atrial cells in the present experiments.

#### *Mechanism underlying the block of Ito by propafenone in human atrial myocytes*

Propafenone inhibited Ito with an  $IC_{50}$  value of  $4.9 \mu M$  (Figure 3) and its effect on Ito showed no significant voltage- or use-dependence (Figures 4 and 5). Propafenone accelerated the inactivation time constant for Ito in a dose-dependent manner (Figures 3 and 6) and, while it did not affect the initial current at depolarizing potentials, it did produce a block that increased as a function of time after depolarization (with a time constant of  $3.4 \pm 0.4$  ms) (Figure 9). These results indicated that channel opening was required for the blocking action of propafenone on Ito. A shift of the steady-state inactivation curve was not observed in the present experiment, suggesting a lack of drug effect on the inactivation gating of Ito (Figure 7). The reactivation time constant of Ito was not modulated by propafenone, indicating that the dissociation of the drug from the Ito channel might be rapid, a conclusion consistent with the lack of a use-dependent effect of this drug on Ito (Figure 10).

In other mammalian cardiac species, propafenone is known to inhibit Ito channels. For example, it has been reported that propafenone inhibits Ito in the rabbit atrium and rat ventricle (Duan *et al.*, 1992; Slawsky & Castle, 1993) with  $IC_{50}$  values of  $5.9 \mu M$  and  $3.3 \mu M$ , respectively. In the present experiment, propafenone inhibited Ito with an  $IC_{50}$  of  $4.9 \mu M$ , a value similar to that obtained in these other species. The following two pieces of evidence obtained in rabbit atrial and rat ventricular myocytes (Duan *et al.*, 1992; Slawsky & Castle, 1993) suggest that propafenone inhibits Ito through a preferential interaction with the open state of the channel. First, there appears to be no inhibition of Ito at the onset of an activating depolarization; however, during continued depolarization, inhibition develops in an exponential manner at a rate and magnitude that are both concentration-dependent. Second, although there is an increase in the rate constant of apparent Ito inactivation in cells exposed to the drug, there is a minimal shift in the voltage-dependence of the steady-state inactivation. These results, together with our present data, strongly suggest that propafenone inhibits the Ito channel through a preferential interaction with the open state in mammalian cardiac cells.

#### *Blocking mechanisms underlying the state-dependent Ito block: comparison between propafenone and other drugs*

Quinidine blocks Ito in human atrial cells (Wang *et al.*, 1995) in a manner similar to that observed with propafenone in the present experiment: viz. quinidine accelerates the inactivation time course, produces no inhibition at the onset of depolarization and does not affect the steady-state inactivation curve. These results indicate that quinidine, too, preferentially blocks Ito in an open state. However, the profiles of the blocks produced by these two drugs differ in certain important respects. First, quinidine blocks Ito in a voltage-dependent

manner, suggesting that the block would be enhanced at more positive potentials. Second, the time constant of the onset of the block during continued depolarization is  $14.5 \pm 4.2$  ms at  $16^\circ C$  and it is  $5.5 \pm 1.1$  ms even at  $36^\circ C$ , values slower than that obtained here with propafenone ( $3.4 \pm 0.4$  ms at room temperature). Third, quinidine slows the reactivation of Ito, the time constant increasing from a control value of 20 ms to 41 ms. This result indicates a slow removal of quinidine from the channel on returning to the holding potential, a characteristic that would result in the appearance of a use-dependent block. To judge from these results, although propafenone and quinidine both block Ito in an open state, the time course of the development of the inhibition and the dissociation from the Ito channel were both more rapid for propafenone than for quinidine.

An analysis of its effect on Ito in human atrial cells shows that flecainide exhibits a kinetic profile similar to that of propafenone. Flecainide shows no use-dependency and produces no obvious change in the reactivation time constant (Wang *et al.*, 1995). These results indicate that flecainide dissociates rapidly from the Ito channel, as suggested here for propafenone. However, flecainide shifts the steady-state inactivation curve in the hyperpolarizing direction, suggesting that it inhibits Ito through a preferential interaction with the inactivated state of the channel (Wang *et al.*, 1995). Thus, we conclude that although propafenone and flecainide belong to the same class of antiarrhythmic agents (class Ic), they inhibit Ito by acting on different gating states of the channel. The former drug interacts preferentially with the open state, while the latter interacts with the inactivated state of the Ito channel.

#### *Effect of propafenone on Isus*

Wang *et al.* (1993b) reported that Isus is highly  $K^+$  selective in human atrial myocytes. In the present experiment, the reversal potential of the sustained outward current was more positive (Figure 1B1) than the theoretical equilibrium potential for  $K^+$  (estimated  $E_K = -86$  mV). One possibility of this discrepancy is that the amplitude of the tail current was too small to measure and the inward capacitive current overlapped this component, therefore, we could not detect an outward tail current at  $-50$  mV. Another possibility is that this current was not carried only by the  $K^+$  ion. However, 1 mM of 4-AP completely blocked both the sustained current and the tail current (result not illustrated). These results indicated that the sustained outward current observed in the present experiment is indeed the Isus previously reported in human atrial myocytes (Wang *et al.*, 1993b).

Propafenone blocked Isus with an  $IC_{50}$  value of  $8.6 \mu M$ , and it blocked Isus in a voltage- and use-independent manner (Figures 3, 4, and 5). Quinidine has been reported to block Isus with an  $IC_{50}$  of around  $5 \mu M$  in human atrial cells (Wang *et al.*, 1995) but it blocks Isus in a voltage-dependent manner, suggesting an open-channel block. In the present experiment, propafenone blocked Isus in a voltage-independent manner, implying that the blocking profiles of the two drugs may be different. A quinidine-induced change in the tail current of a cloned human cardiac potassium channel (HK2), which is believed to be the equivalent of the native Isus, was clearly demonstrated by Snyders *et al.* (1993). Quinidine blocks HK2 in a voltage-dependent manner and induces the tail 'crossover' phenomenon in which a rising phase appears in the tail current and there is a retardation of the tail current's decline (Snyders *et al.*, 1991; Delpon *et al.*, 1997). Since blocked channels do not conduct, a conversion to open channels would result initially in a rising phase in the tail current; subsequently, the



tail current would display a slower decline if some of the open channels became blocked again, rather than closing irreversibly. These results indicate that quinidine preferentially blocks the Isus channel in the open state. In our preliminary experiments on human atrial cells, propafenone slowed the time course of the onset of the Isus tail current relative to control (Figure 1B2, inset). The retardation of Isus tail current was much the same regardless of the changes in depolarizing potentials or pulse durations, indicating that this phenomenon was not due to depletion nor accumulation of  $K^+$  ions. The retardation of Isus tail current also occurred when quinidine was applied to the HK2 channel, suggesting that propafenone blocked Isus in the open state. The mechanism by which propafenone blocks Isus needs to be evaluated by analysing the onset of its blocking action and the change it induces in the time course of the tail current. In the present experiments, we were not able to analyse the onset of the block of Isus produced by propafenone because it was very difficult to isolate Isus from Ito. For this reason, future studies should be performed using a cloned K channel.

#### *Anti-arrhythmic effect of propafenone*

Propafenone is clinically effective for the treatment of atrial fibrillation (af). In fact, with this drug the reported conversion rate for paroxysmal or chronic af ranges from 29 to 87% (Kosmala *et al.*, 1994; Weiner *et al.*, 1994; Aliot & Denjoy, 1996; Stroobandt *et al.*, 1997), the average being 66.2%. Maintenance of a normal sinus rhythm was achieved in 30 to 75% of cases (Antmann *et al.*, 1988; Porterfield and Porterfield 1988; Pritchett *et al.*, 1991; Reimold *et al.*, 1993; Cobbe & Rae, 1995; Chimienti *et al.*, 1996; Crijns *et al.*, 1996; Stroobandt *et al.*, 1997), the average being 52.9%. This suggests that

propafenone should be an effective anti-arrhythmic agent for use against atrial tachyarrhythmias.

Recently, the ionic changes occurring during atrial fibrillation were described by Yue *et al.* (1997) in the canine atrium. Rapid atrial pacing decreases the action potential duration (APD) and its adaptation to changes in rate. The APD changes are accompanied by decreases in Ca current and Ito, without alterations in the other ionic currents flowing during the action potential plateau. The authors suggested that in the canine model these effects would produce changes in atrial refractoriness associated with an enhanced ability to maintain af. If this is also true for patients with atrial fibrillation, both the Ito and the Ca current would be already suppressed in such patients. Thus, inhibition of other outward currents, such as Isus, should be effective in prolonging the atrial APD. At the time of the conversion to sinus rhythm from atrial fibrillation, the plasma level of propafenone was  $1.26 \pm 0.71 \text{ mg l}^{-1}$  (around  $3.3 \text{ } \mu\text{M}$ ) following its intravenous administration (Suttorp *et al.*, 1990). If we take into account the 70 to 90% plasma protein binding of propafenone (Gillis and Kates, 1986), the estimated free concentration of this drug would have been within the range 0.33 to  $1 \text{ } \mu\text{M}$ . Therefore, the present experiments demonstrate that propafenone blocks both Ito and Isus at clinically relevant concentrations. This therefore suggests that propafenone may be effective in improving atrial refractoriness by inhibiting Isus in patients with atrial tachyarrhythmias.

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