British Journal of Pharmacology (1998) 125, 969–978
http://www.stockton-press.co.uk/bjp

Effects of propafenone and 5-hydroxy-propafenone on hKv1.5 channels

[1,2]Laura Franqueza, [1]Carmen Valenzuela, [1]Eva Delpón, [1]Mónica Longobardo, [1]Ricardo Caballero & [1]Juan Tamargo

[1]Institute of Pharmacology and Toxicology, CSIC/UCM, School of Medicine, Universidad Complutense, 28040 Madrid, Spain

1 The goal of this study was to analyse the effects of propafenone and its major metabolite, 5-hydroxy-propafenone, on a human cardiac K^+ channel (hKv1.5) stably expressed in Ltk^- cells and using the whole-cell configuration of the patch-clamp technique.

2 Propafenone and 5-hydroxy-propafenone inhibited in a concentration-dependent manner the hKv1.5 current with K_D values of 4.4 ± 0.3 μM and 9.2 ± 1.6 μM, respectively.

3 Block induced by both drugs was voltage-dependent consistent with a value of electrical distance (referenced to the cytoplasmic side) of 0.17 ± 0.55 ($n = 10$) and 0.16 ± 0.81 ($n = 16$).

4 The apparent association (k) and dissociation (l) rate constants for propafenone were $(8.9 \pm 0.9) \times 10^6$ M^{-1} s^{-1} and 39.5 ± 4.2 s^{-1}, respectively. For 5-hydroxy-propafenone these values averaged $(2.3 \pm 0.3) \times 10^6$ M^{-1} s^{-1} and 21.4 ± 3.1 s^{-1}, respectively.

5 Both drugs reduced the tail current amplitude recorded at -40 mV after 250 ms depolarizing pulses to $+60$ mV, and slowed the deactivation time course resulting in a 'crossover' phenomenon when the tail currents recorded under control conditions and in the presence of each drug were superimposed.

6 Both compounds induced a small but statistically significant use-dependent block when trains of depolarizations at frequencies between 0.5 and 3 Hz were applied.

7 These results indicate that propafenone and its metabolite block hKv1.5 channels in a concentration-, voltage-, time- and use-dependent manner and the concentrations needed to observe these effects are in the therapeutical range.

Keywords: K^+ channels; propafenone; 5-hydroxy-propafenone

Introduction

Propafenone is a class Ic antiarrhythmic agent used to maintain sinus rhythm in patients with supraventricular tachycardias and in the treatment of ventricular tachycardia (Funck-Brentano *et al*., 1990; Bryson *et al*., 1993; Kishore & Camm, 1995; UK Propafenone PSVT Study Group, 1995). In addition to its blocking properties of cardiac Na^+ channels, propafenone also blocks L-type Ca^{2+} current (I_{Ca}) (Delgado *et al*., 1993; Fei *et al*., 1993) and transient outward (I_{TO}), rapid and slow delayed rectifiers (I_{Kr} and I_{Ks}, respectively) and inward rectifier (I_{Kl}) potassium currents (Duan *et al*., 1993; Slawsky & Castle, 1994; Delpón *et al*., 1995). Furthermore, propafenone also exhibits β-adrenergic receptor blocking effects (Dukes & Vaughan Williams, 1984; McLeod *et al*., 1984). Propafenone undergoes extensive first-pass hepatic metabolism to its major metabolite 5-hydroxy-propafenone (Hege *et al*., 1984). During chronic therapy, this metabolite can accumulate to plasma concentrations similar to those of propafenone (Kates *et al*., 1985). 5-hydroxy-propafenone is more potent than propafenone to block Na^+ channels and, as the parent compound, it has been classified as a class Ic antiarrhythmic agent (Valenzuela *et al*., 1987, 1988; Thompson *et al*., 1988). However, 5-hydroxy-propafenone is much less potent to block I_{Ca} and β-receptors than propafenone (von Philipsborn *et al*., 1984; Delgado *et al*., 1987; Valenzuela *et al*., 1987; Malfatto *et al*., 1988; Lee *et al*., 1990; Bryson *et al*., 1993).

Propafenone has been widely used in establishing and maintaining sinus rhythm (Porterfield & Porterfield, 1989; Gentili *et al*., 1992; Kingma & Suttorp, 1992; Cobbe, 1994; Chimienti *et al*., 1996) and to prevent recurrences in patients with atrial fibrillation (U.K. Propafenone PSVT Study Group, 1995). Both propafenone (Ledda *et al*., 1981; Dukes & Vaughan Williams, 1984; Delgado *et al*., 1985) and 5-hydroxy-metabolite (Delgado *et al*., 1987) has been reported to prolong the action potential duration in guinea-pig atrial fibers. A similar effect has been observed with propafenone in rabbit sino-atrial node cells (Satoh & Hashimoto, 1984). Inhibition of I_{Na} and I_{Ca} would be expected to accelerate repolarization, so that the prolongation of the action potential suggests that both propafenone and its metabolite may interact with K^+ currents involved in atrial repolarization. Unfortunately the effects of 5-hydroxy-propafenone on cardiac K^+ currents are presently unknown.

It has been proposed that Kv1.5 channels are the cloned counterpart of the native ultrarapid delayed rectifier outward K^+ current (I_{Kur}) in human atria (Wang *et al*., 1993). Because of its rapid activation and relative lack of inactivation, hKv1.5 channels can contribute to the repolarization of the human atrial action potential (Wang *et al*., 1993; Li *et al*., 1996). However, and despite the effectiveness of propafenone on the treatment of supraventricular arrhythmias, the effects of propafenone and 5-hydroxy-propafenone on hKv1.5 current have not been studied yet. Therefore, in the present study we have examined the effects of propafenone and 5-hydroxy-propafenone on hKv1.5 channels cloned from human ventricle and stably expressed in a Ltk^- cell line. The results obtained in this study indicate that propafenone and, to a lesser extent, 5-hydroxy-propafenone block hKv1.5 channels in a concentration-, voltage-, time- and use-dependent manner.

[2] Author for correspondence.

Methods

Transfection and cell culture

The method used to establish hKv1.5 expression in a clonal mouse *Ltk*$^-$-cell line is the same as that previously described (Snyders *et al*., 1992, 1993). The expression vector contains a dexamethasone-inducible murine mammary-tumor virus promoter that controls transcription of the inserted cDNA and a gene that confers neomycin resistance driven by the SV40 early promoter. The cells used for the experiments reported in the present study displayed hKv1.5-specific mRNA expression after dexamethasone induction as evidenced by Northern blot analysis (Tamkun *et al*., 1991). Transfected cells were cultured in DMEM supplemented with 10% horse serum and 0.25 mg ml^{-1} G418 (a neomycin analog) under a 5% CO_2 atmosphere. The cultures were passed every 3–5 days by use of a brief trypsin treatment. Before experiments, subconfluent cultures were incubated with 2 μM dexamethasone for 24 h to induce expression of hKv1.5 channels. The cells were removed from the dish with a rubber policeman, a procedure that left the vast majority of the cells intact. The cell suspension was stored at room temperature (20–22°C) and used within 12 h for all the experiments reported.

Electrophysiological recording

Experiments were performed in a small volume (0.5 ml) bath mounted on the stage of an inverted microscope (Nikon model TMS, Garden City, NY, U.S.A.) perfused continuously at a flow rate of 0.5–1.0 ml min^{-1}. hKv1.5 currents were recorded at room temperature (20–22°C) using the whole-cell voltage-clamp configuration of the patch-clamp technique (Hamill *et al*., 1981) with an Axopatch 200A patch-clamp amplifier (Axon Instruments, Foster City, CA, U.S.A.). Currents were filtered at 2 kHz (four-pole Bessel filter), sampled at 4 kHz, and stored on the hard disk of a Hewlett-Packard Vectra 486/50U computer for subsequent analysis. Data acquisition and command potentials were controlled by the PCLAMP 6.0.1 software (Axon Instruments). Micropipettes were pulled from borosilicate glass capillary tubes (Narishige, GD-1, Tokyo, Japan) on a programmable horizontal puller (Sutter Instrument Co., San Rafael, CA, U.S.A.) and heat-polished with a microforge (Narishige). When filled with the intracellular solution and immersed into the bath (external solution), the pipette tip resistance ranged between 1–3 MΩ. The micropipettes were gently lowered onto the cells to obtain a gigaohm seal (16±6 GΩ) after applying suction. After seal formation, cells were lifted from the bottom of the perfusion bath and the membrane patch was ruptured with brief additional suction. The capacitive transients elicited by symmetrical 10 mV steps from −80 mV were recorded at 50 kHz (filtered at 10 kHz) for subsequent calculation of capacitative surface area, access resistance, and input impedance. Thereafter, capacitance and series resistence compensation were optimized, and 80% compensation of the effective access resistance was usually obtained.

Solutions

The intracellular pipette filling solution contained (in mM): K-aspartate 80, KCl 50, phosphocreatine 3, KH_2PO_4 10, MgATP 3, HEPES-K 10, EGTA 5 and was adjusted to pH 7.25 with KOH. The bath solution contained (in mM): NaCl 130, KCl 4, $CaCl_2$ 1.8, $MgCl_2$ 1, HEPES-Na 10, and glucose 10, and was adjusted to pH 7.35 with NaOH. Propafenone and 5-OH-propafenone (a gift from Knoll AG, Ludwigshafen, Germany) were dissolved in distilled deionized water to yield stock solutions of 1 mM from which further dilutions were made to obtain the desired final concentration.

Pulse protocol and analysis

After control data were obtained, bath perfusion was switched to drug-containing solution. Drug infusion or removal was monitored with test pulses from −80 to +60 mV, applied every 30 s until steady state was obtained (within 10 min). The holding potential was maintained at −80 mV. The 'steady-state' I-V relationships were obtained by measuring the current at the end of the 250 ms depolarizing steps. Between −80 and −40 mV, only passive linear leak was observed and least-squares fits to these data were used for passive leak correction. Deactivating 'tail' currents were recorded at −40 mV. The activation curve was obtained from the tail current amplitude immediately after the capacitive transient. Measurements were done using the CLAMPFIT program of PCLAMP 6.0.1 and by a custom-made analysis program. Activation curves were fitted with a Boltzmann equation:

$$y = 1/[1 + \exp(-)(E - E_h)/s], \quad (1)$$

in which s represents the slope factor, E the membrane potential and E_h the voltage at which 50% of the channels are open. In order to describe the time course of currents during depolarizing pulses and tail currents upon repolarization, an exponential analysis was used as an operational approach, fitting these processes to an equation of the form:

$$y = \mathrm{C} + \mathrm{A_1}\exp(-\mathrm{t}/\tau_1) + \mathrm{A_2}\exp(-\mathrm{t}/\tau_2) + \ldots + \mathrm{A_n}\exp(-\mathrm{t}/\tau_\mathrm{n})$$

where τ_1, τ_2 and τ_n are the system time constants, A_1, A_2 and A_n are the amplitudes of each component of the exponential, and C is the baseline value. The curve-fitting procedure used a non-linear least-squares (Gauss-Newton) algorithm; results were displayed in linear and semilogarithmic format, together with the difference plot. Goodness of fit was judged by the χ^2 criterion and by inspection for systematic non-random trends in the difference plot. The activation kinetics of hKv1.5 have been described as a sigmoidal process (Snyders *et al*., 1993). However, in the present study and in order to describe the dominant time constant of this process and the effects of propafenone and 5-hydroxy-propafenone on it, the latter part of the current was fitted to a single exponential, following a procedure previously described and used for the same purpose (Snyders *et al*., 1993; Delpón *et al*., 1996; Valenzuela *et al*., 1996).

A first-order blocking scheme was used to describe drug-channel interaction. The apparent affinity constant, K_D, and Hill coefficient, n_H, were obtained from fitting the fractional block, f, at various drug concentrations [D]:

$$f = 1/[1 + (K_D/[D])^{n_H}], \quad (2)$$

and apparent rate constants for binding (k) and unbinding (l) were obtained from solving:

$$k \times [D] + l = 1/\tau_B \quad (3a)$$

$$l/k = K_D \quad (3b)$$

Voltage dependence of block was determined as follows: leak-corrected current in the presence of drug was normalized to matching control to yield the fractional block at each voltage ($f = 1 - I_{drug}/I_{control}$). The voltage dependence of block was fitted to:

$$f = [D]/([D] + K_D^* \times \exp(-\delta zFE/RT)), \quad (4)$$

where z, F, R and T have their usual meaning, δ represents the fractional electrical distance, i.e., the fraction of the transmembrane electrical field sensed by a single charge at the receptor site and K_D^* represents the apparent dissociation constant at the reference potential (0 mV).

Statistical methods

Results are expressed as means ± s.e.mean. Direct comparisons between mean values in control conditions and in the presence of drug for a single variable were performed by paired Student's t-test. Student's t-test was also used to compare two regression lines. Differences were considered significant if $P < 0.05$.

Results

Voltage- and concentration-dependent block by propafenone and 5-hydroxy-propafenone

Figure 1 shows superimposed tracings of potassium current through hKv1.5 channels expressed in mouse Ltk^- cells in control conditions and in the presence of propafenone (a) and 5-hydroxy-propafenone (b). The cell was held at −80 mV and 250 ms depolarizing pulses from −60 to +60 mV in 20 mV steps were applied every 10 s. Outward currents were followed by decaying outward tail currents upon repolarization to −40 mV. Under control conditions (Figure 1, upper panels), depolarizations positive to −40 mV elicited outward currents that progressively increased with further depolarizations. The activation proceeded with a sigmoidal time course, the rate of activation being faster at more depolarized levels. The activation time constant was 1.2 ± 0.06 ms at +60 mV ($n = 25$). At +60 mV, after the current reached the maximum peak, it declined slowly during the maintained depolarization (slow and partial inactivation). Outward tail currents exhibited a dominant time constant of deactivation of 33.7 ± 1.6 ms ($n = 28$), as it has been previously described (Snyders *et al.*, 1992, 1993).

Middle panels of Figure 1 illustrate the differential effectiveness of propafenone and 5-hydroxy-propafenone at an identical concentration (10 μM). Both drugs not only reduced the current amplitude but also altered the time course of the current during depolarization, modifying the initial activation time course of the current. Propafenone and 5-hydroxy-propafenone reduced the peak outward current elicited by pulses to +60 mV by 59.2 ± 2% ($n = 4$) and 37.6 ± 4.1% ($n = 8$) ($P < 0.01$), respectively. Moreover, both drugs increased the rate of decay of the current during the application of the depolarizing pulse, which reached a steady-state level within 250 ms. Thus, at the end of 250 ms step to +60 mV, propafenone and 5-hydroxy-propafenone reduced the hKv1.5 current by 75.9 ± 1.4% ($n = 4$) and 47.1 ± 2.9% ($n = 9$) ($P < 0.01$) i.e., propafenone displayed a higher potency than its metabolite to inhibit the hKv1.5 current. After 15 min of perfusion with drug-free solution, the currents were restored to 87 ± 2% ($n = 8$) and 92 ± 2% ($n = 4$) of the control values, respectively; indicating that the effects of both drugs were reversible upon washout.

Bottom panels of Figure 1 show the effects of 10 μM propafenone and 5-hydroxy-propafenone on the steady state current-voltage (I-V) relationship for the hKv1.5 channel constructed by plotting the current amplitudes after 250 ms

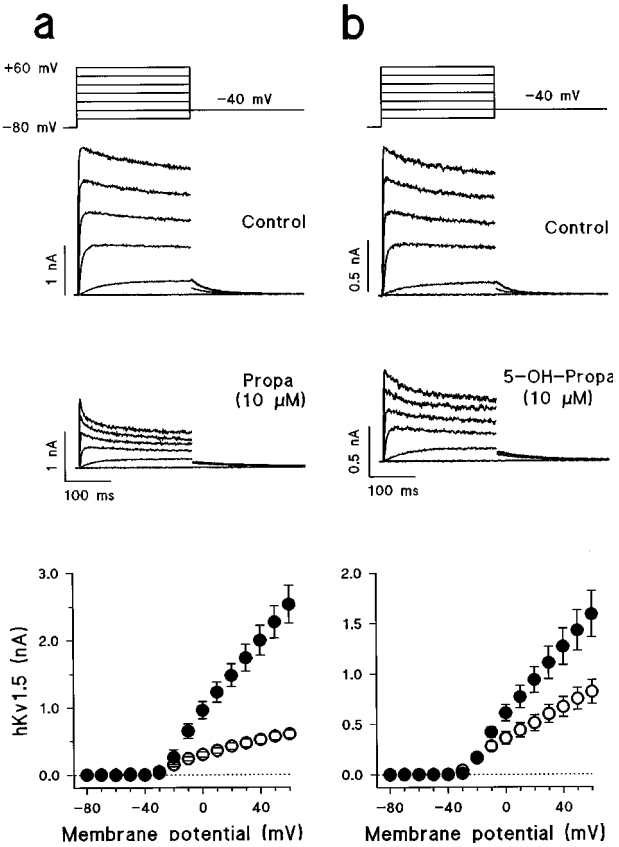

Figure 1 Effects of propafenone (a) and 5-hydroxy-propafenone (b) on hKv1.5 channels expressed in a Ltk^- cell line. Currents are shown for 250 ms depolarizing pulses from −80 mV to voltages between −60 and +60 mV in steps of 20 mV. Upper panels: traces obtained under control conditions. Middle panels: traces recorded in the presence of 10 μM propafenone or 10 μM 5-hydroxy-propafenone. Cell capacitance, 25 pF (a) and 23 pF (b). Data filtered at 2 kHz (four-pole Bessel) and digitized at 10 kHz. Bottom panels: effects of 10 μM propafenone (a) and 10 μM 5-hydroxy-propafenone (b) on the steady-state current-voltage (I-V) relationships (250 ms isochronal). Data represent the means ± s.e.mean of four (propafenone) or eight (5-hydroxy-propafenone) experiments. (●): Data obtained under control conditions, (○): data obtained in the presence of propafenone (a) or 5-hydroxy-propafenone (b).

depolarization as a function of the test pulse voltage. The control I-V relationship was almost linear for depolarizations positive to +10 mV. The sigmoidicity observed between −30 and +10 mV reflected the voltage-dependence of channel opening (Snyders *et al.*, 1993). Propafenone and 5-hydroxy-propafenone induced a voltage-dependent inhibition of the hKv1.5 current, i.e., drug induced block was higher after very positive potentials (>0 mV) than at more negative ones (between −30 and 0 mV). To quantify this voltage-dependence of hKv1.5 block, the relative current $I_{Drug}/I_{Control}$ was plotted as a function of membrane potential (Figure 2a). The dotted line represents the activation curve obtained under control conditions. The current begins to activate at −30 mV and the conductance of the channel is fully saturated at 0 mV. The midpoint and the slope factor from Boltzmann equation (eq. 1, see Methods) yielded values of −15.4 ± 1.2 mV ($n = 18$) and 4.4 ± 0.2 mV ($n = 18$), respectively. In the presence of either propafenone or 5-hydroxy-propafenone the blockade increased steeply between −30 mV and 0 mV, which corresponds to the voltage range of channel opening (Snyders *et al.*, 1993). These results suggested that both drugs bind preferentially to the open state of the hKv1.5 channels.

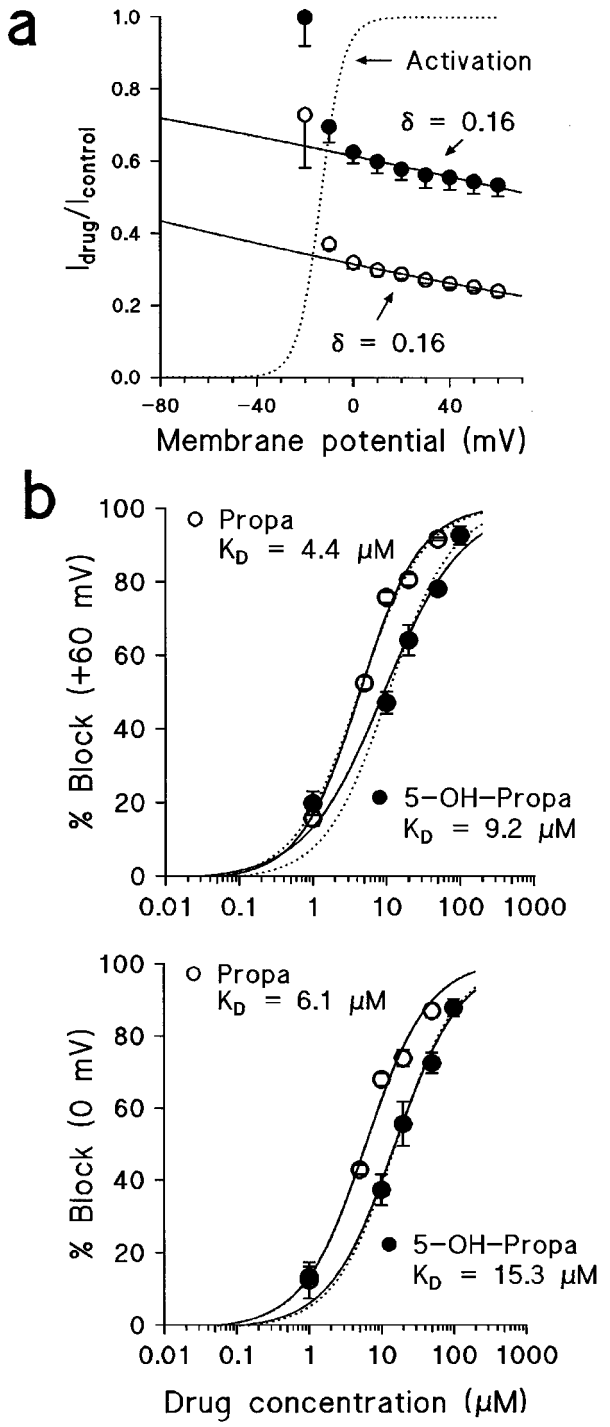

Figure 2 (a) Voltage-dependence of hKv1.5 block. Relative current expressed as $I_{Drug}/I_{Control}$ from data obtained in the absence and in the presence of propafenone (○) and 5-hydroxy-propafenone (●). Individual data points are means±s.e.mean of four (propafenone) or six (5-hydroxy-propafenone) experiments. The dotted line shows the activation curve of the hKv1.5 channel. Block steeply increased between −30 and 0 mV. For membrane potentials positive to 0 mV, a continued but shallower voltage dependence was observed for both compounds. This voltage-dependence was fitted (continuous line) to equation 4 (see Methods) and yielded δ values of 0.16 for propafenone and 5-hydroxy-propafenone. (b) Concentration-dependence of propafenone (○) and 5-hydroxy-propafenone (●)-induced block of hKv1.5. Percentage of block at the end of 250 ms depolarizations to +60 mV (top panel) and to 0 mV (bottom panel) was used as index of block. Individual data points are means±s.e.mean of 3–9 experiments. In both panels, the continuous line represents the fit of the experimental data to the equation 2 (see Methods). The dashed line represents the fit for a Hill coefficient (n_H) of 1.

Between 0 mV and +60 mV, block continued to increase but with a shallower voltage dependence. It is unlikely that the shallow voltage dependence of block observed at membrane potentials positive to 0 mV was due to channel gating, since hKv1.5 activation had reached saturation over this voltage range (Snyders *et al*., 1992, 1993). Propafenone and 5-hydroxy-propafenone are weak bases with pK_a values of 9.0 and 9.3, respectively (Valenzuela *et al*., 1988). Therefore, at the intracellular pH (7.25) both drugs are predominantly present in the charged form. Thus this shallow component could be due to the influence of the transmembrane electrical field on the interaction between the charged form of both drugs and the channel receptor. If these compounds reach the receptor from the inside, block is expected to increase in a voltage-dependent manner and described by a Boltzmann relationship based on the Woodhull model (eq. 4 in Methods) (Woodhull, 1973). The parameter δ in this equation represents the fractional electrical distance, i.e., the fraction of the membrane electrical field sensed by a single charge at the receptor site. The solid lines in Figure 2a represent the fits of this Boltzmann equation to the data points positive to 0 mV. Using this analysis, we obtained similar δ values of 0.17±0.55 ($n=10$) and 0.16±0.81 ($n=16$; $P>0.05$) for propafenone and 5-hydroxy-propafenone, respectively. Thus, the block mechanism induced by both drugs seems to be similar, but the effects occurred at lower concentrations in presence of propafenone compared to 5-hydroxy-propafenone.

Figure 2b shows the concentration dependence of propafenone and 5-hydroxy-propafenone block of hKv1.5 in a range of concentrations between 1–100 μM. The inhibition of the current was determined from the reduction in current amplitudes after 250 ms depolarizing pulses from −80 mV to +60 mV (upper panel) and from −80 mV to 0 mV (bottom panel). At concentrations tested >1 μM, the degree of hKv1.5 block induced by propafenone was significantly greater than that induced by 5-hydroxy-propafenone ($P<0.05$). Thus, in the presence of 20 μM propafenone and 5-hydroxy-propafenone, hKv1.5 inhibition averaged 80.7±1.5% ($n=4$) and 64.2±4.2% ($n=4$) ($P<0.05$) at +60 mV and 73.8±2.3% ($n=4$) and 55.6±6.2% ($n=4$) ($P<0.05$) at 0 mV, respectively. A nonlinear least-squares fit of the concentration-response equation (eq. 2, see Methods) to the individual data points yielded an apparent K_D of 4.4±0.3 μM and 9.2±1.6 μM ($P<0.05$) at +60 mV for propafenone and 5-hydroxy-propafenone, respectively, whereas at 0 mV the K_D values were 6.1±0.5 μM and 15.3±2.0 μM ($P<0.05$), respectively. The Hill coefficients obtained by this fitting procedure in the presence of propafenone and 5-hydroxy-propafenone were 1.10±0.08 and 0.81±0.10 at +60 mV, while at 0 mV, the corresponding values were 0.99±0.08 and 0.94±0.12, respectively. Similar K_D values were obtained for propafenone and 5-hydroxy-propafenone when the Hill coefficient was constrained to unity at +60 mV (4.3±0.3 μM and 10.7±1.5 μM, respectively) and 0 mV (6.1±0.4 μM and 16.0±1.7 μM, respectively), suggesting that binding of one drug molecule/channel is sufficient to block potassium permeation.

Time course of channel block and its concentration-dependence

Figure 3 shows superimposed recordings of 250 ms depolarizations from −80 to +60 mV under control conditions and in the presence of 10 μM propafenone and 20 μM 5-hydroxy-propafenone, respectively. In the presence of propafenone and 5-hydroxy-propafenone, the activation kinetics of hKv1.5 current was modified and the time to peak current was

decreased in a concentration-dependent manner. The activation time constant of the currents elicited by test pulses to +60 mV was significantly accelerated at concentrations >1 μM (1.4±0.1 *vs* 0.6±0.08 ms, in control conditions and in the presence of 10 μM propafenone, respectively, $P<0.01$, $n=4$; 1.1±0.07 *vs* 0.7±0.04 ms, in control conditions and in the presence of 10 μM 5-hydroxy-propafenone, respectively, $P<0.01$, $n=6$) (Figure 3). In the presence of either drug the inactivation time course displayed an additional rapid exponential component superimposed on the slow inactivation. The time constant (τ_b) of this rapid component was concentration-dependent and it was only visible at high concentrations. Therefore, it was considered to represent the time constant of development of block. In the presence of propafenone and 5-hydroxy-propafenone, the τ values of the fast component were 6.6±0.2 ms ($n=4$) and 19.6±3.1 ms ($n=5$, $P<0.01$), respectively. Thus, the decay of the current was faster in the presence of propafenone than in the presence of 5-hydroxy-propafenone. The apparent rate constants for binding (k) and unbinding (l) were obtained from solving the equations (3a) and (3b) (see Methods). In the presence of propafenone k and l values were $(8.9\pm0.9)\times10^6$ M^{-1} s^{-1} and 39.5±4.2 s^{-1}, respectively, while in the presence of 5-hydroxy-propafenone these values were $(2.3\pm0.3)\times10^6$ M^{-1} s^{-1} and 21.4±3.1 s^{-1}, respectively. These results indicate that both the apparent rate constants for binding and unbinding were faster in the presence of propafenone than in the presence of 5-hydroxy-propafenone ($P<0.01$) and that these differences in the kinetics of block can explain the different potency to block hKv1.5 channels.

The time dependent effects induced by propafenone and 5-hydroxy-propafenone were also studied on the deactivation process of hKv1.5 channels, which represents the transition from the open to the closed state of the channel (O→C). Figure 4 shows the superposition of the tail currents obtained at −40 mV after 250 ms depolarization to +60 mV under control conditions and in the presence of 5 μM propafenone

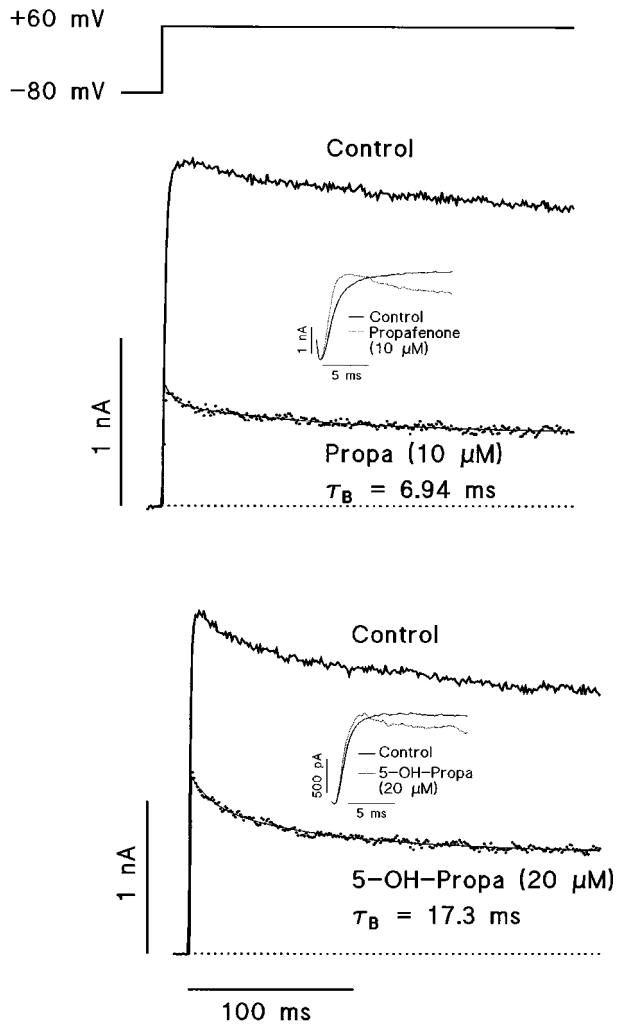

Figure 3 Time-dependent block of hKv1.5 channels induced by propafenone and 5-hydroxy-propafenone. The figure shows current traces obtained by applying 250 ms depolarizing pulses from −80 mV to +60 mV in control conditions and in the presence of 10 μM propafenone (upper panel) or 20 μM 5-hydroxy-propafenone (lower panel) in two different cells. In the presence of either propafenone or 5-hydroxy-propafenone the current exhibited a fast decline during the application of the depolarizing step which was superimposed to the slow inactivation of the current. The decay of the current in the presence of either drug was fitted (continuous line) by a biexponential function from which the fast time constant was taken as an index of the time course of development of block (τ_B). Insets: First 15 ms of activation under control conditions and in the presence of propafenone or 5-hydroxy-propafenone. In both cases, hKv1.5 current obtained in the presence of drug was normalized to match maximum control values. Dotted line represents the zero current level.

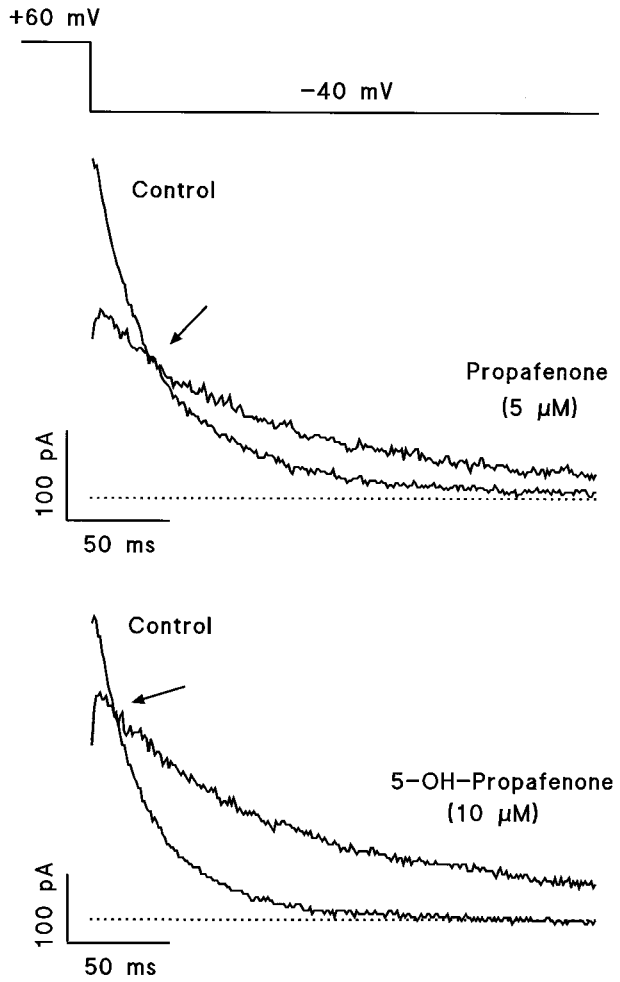

Figure 4 Tail current crossover in the presence of 5 μM propafenone (upper panel) and 10 μM 5-hydroxy-propafenone (bottom panel). Tail currents were elicited on return to −40 mV after 250 ms duration depolarizing pulses from a holding potential of −80 mV to +60 mV in the absence and in the presence of either drug. The arrows indicate the 'crossover' of the trace recorded in the presence of propafenone and 5-hydroxy-propafenone with those recorded under control conditions. The dotted line represents the zero current level.

and 10 μM 5-hydroxy-propafenone. In control conditions, the channel deactivated with a time constant of 32.6 ± 1.5 ($n=13$). After exposure to either drug, the initial amplitude of the tail currents was reduced and displayed an initial rising phase, indicating the dissociation of the drug (D) from the open state of the channel (OD$\rightarrow$O). The subsequent decline of the tail current was slower than in control conditions, which resulted in a 'crossover' phenomenon. This slower time course of deactivation can be attributed to the drug-induced block of the available open channels at -40 mV (OD$\rightleftarrows$O$\rightarrow$C). In the presence of propafenone and 5-hydroxy-propafenone the time constant of deactivation was significantly increased to 68.9 ± 4.2 ms ($n=4$, $P<0.01$) and 95.1 ± 5.6 ($n=9$, $P<0.01$), respectively. These results indicated that drug unbinding was required before channels can close and supported an open channel interaction between both drugs and the open state of hKv1.5 channels.

Use-dependent block of hKv1.5 by propafenone and 5-hydroxy-propafenone

Although 5 μM propafenone and 10 μM 5-hydroxy-propafenone induced approximately 50% steady-state block, this amount of block may not be attained during a single action potential. Therefore, we tested whether propafenone- and 5-hydroxy-propafenone-induced block displayed use-dependence. Trains of 15 depolarizing pulses of 250 ms duration from -80 to $+60$ mV were applied at four different stimulation frequencies (0.5, 1, 2 and 3 Hz), separated one from another by a rest period of 1 min. Top panels in Figures 5 and 6 show original current records obtained after applying a pulse train protocol at a frequency of 1 Hz in the absence and in the presence of either 5 μM propafenone or 10 μM 5-hydroxy-propafenone, respectively. Under control conditions, the outward K^+ current displayed a slight decline ($9.5 \pm 1.5\%$; $n=10$, at 1 Hz). In the presence of propafenone and 5-hydroxy-propafenone, the size of the current decayed progressively until it reached a steady-state block, in a manner similar to control conditions. Left bottom panels in Figures 5 and 6 show the effects of propafenone and 5-hydroxy-propafenone, on the maximal peak currents. Following the perfusion with propafenone and 5-hydroxy-propafenone, the peak current amplitude elicited by the first depolarizing pulse in each train preceded by a rest period was reduced (i.e. 'tonic block') by $36.6 \pm 1.5\%$ ($n=5$) and $40.2 \pm 6.9\%$ ($n=4$) ($P>0.05$), respectively. In order to describe the use-dependent effects of the drugs without the complications of the decline of the current under control conditions, right bottom panels of these figures represent the relative current ($I_{Drug}/I_{Control}$) elicited during the application of these pulse protocols as a function of the number of pulses in the train (relative use-dependent block). As it is shown in the presence of either drug, the degree of frequency-dependent hKv1.5 block increased with the driven rate, this increase being more marked at fast (1, 2 and 3 Hz) than at slow (0.5 Hz) stimulation frequencies. Table 1 summarizes the relative use-dependent block of hKv1.5 channels induced by propafenone and 5-hydroxy-propafenone at the end of the pulse trains.

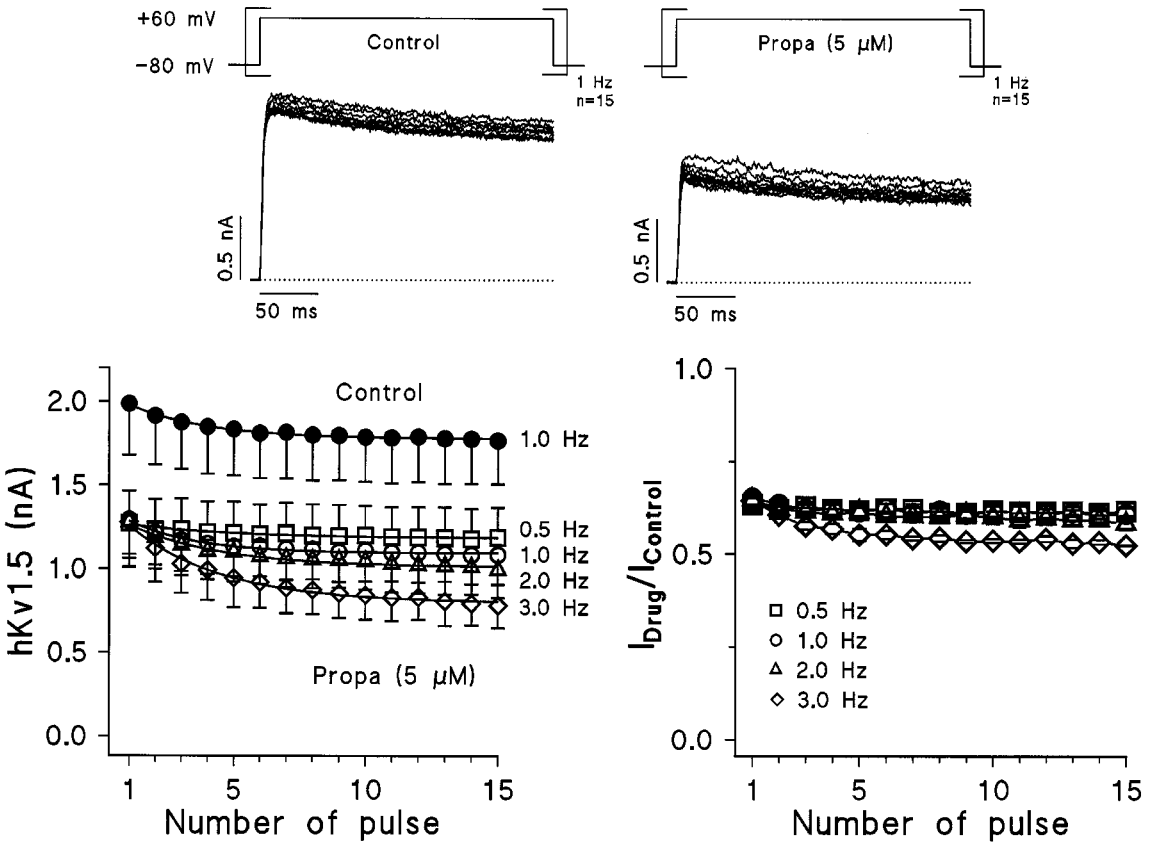

Figure 5 Use-dependent block of hKv1.5 current induced by propafenone. Upper panels: original records obtained in the absence and in the presence of 5 μM propafenone when applying 15 depolarizing pulses (250 ms in duration) from -80 mV to $+60$ mV at a frequency of 1 Hz. The dotted line represents the zero current level. Left bottom panel: peak outward current amplitude at 1 Hz in control conditions (●) and at 0.5 (□), 1 (○), 2 (Δ) and 3 Hz (◇) in the presence of 5 μM propafenone expressed as a function of pulses in the train. Right bottom panel: relative current ($I_{Drug}/I_{Control}$) plotted as a function of the number of pulses in the train. Individual data points represent the means$\pm$s.e.mean of five experiments.

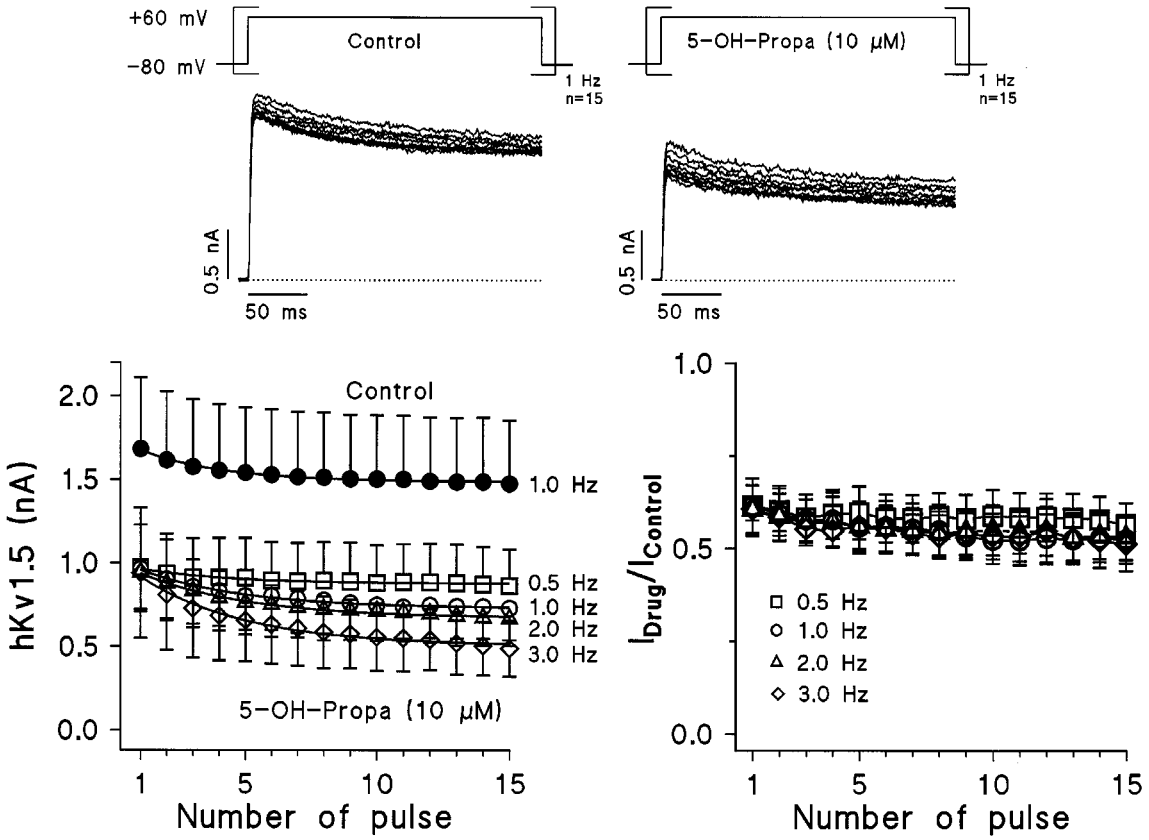

Figure 6 Use-dependent block of hKv1.5 current induced by 5-hydroxy-propafenone. Upper panels: original records obtained in the absence and in the presence of 10 μM 5-hydroxy-propafenone when applying 15 depolarizing pulses (250 ms in duration) from -80 mV to $+60$ mV at a frequency of 1 Hz. The dotted line represents the zero current level. Left bottom panel: peak outward current amplitude at 1 Hz in control conditions (●) and at 0.5 (□), 1 (○), 2 (Δ) and 3 Hz (◇) in the presence of 10 μM 5-hydroxy-propafenone expressed as a function of pulses in the train. Right bottom panel: relative current ($I_{Drug}/I_{Control}$) plotted as a function of the number of pulses in the train. Individual data points represent the means$\pm$s.e.mean of five experiments.

Table 1 Relative use-dependent block induced by 5 μM propafenone and 10 μM 5-hydroxy-propafenone on hKv1.5 currents (%)

Frequency of stimulation (Hz)	*Use-dependent block of hKv1.5* (%) *Propafenone* (5 μM)	*5-hydroxy-propafenone* (10 μM)
0.5	2.0$\pm$0.6*	6.4$\pm$1.4*
1	4.4$\pm$1.1*	7.0$\pm$1.7**
2	6.1$\pm$0.9**	9.6$\pm$2.1**
3	12.3$\pm$2.4**	15.0$\pm$1.5**

Values are means$\pm$s.e.mean ($n=5$). *$P<0.05$; **$P<0.01$ *versus* control decay of the current during the application of train pulses.

Discussion

In the present study we have analysed the interactions between propafenone, a class Ic antiarrhythmic agent widely used in the treatment of cardiac arrhythmias, and its main and more potent metabolite, 5-hydroxy-propafenone, with hKv1.5 channels stably expressed in *Ltk*$^-$ cells. This model provides a good tool for studying drug effects on a unique human cardiac K^+ channel subtype, avoiding contamination of other voltage-gated channels (Snyders *et al.*, 1993). The main conclusions of the present paper are that: (1) propafenone and 5-hydroxy-propafenone interact with hKv1.5 channels in a time-, voltage- and state-dependent manner and (2) propafenone is 2 fold more potent than 5-hydroxy-propafenone.

The current generated by hKv1.5 channels is similar in voltage dependence, kinetics and pharmacological sensitivity to the very rapidly activating delayed rectifier K^+ current recorded in human atrial myocytes (I_{Kur}) (Wang *et al.*, 1993), dog ventricle (Jeck & Boyden, 1992) and rat atria (Boyle & Nerbonne, 1991). In fact, the hKv1.5 channel protein has been located in human atrial and ventricular myocardium explanted from newborn and adult patients (Mays *et al.*, 1995). However, electrophysiological studies (Konarzewska *et al.*, 1995; Li *et al.*, 1996) have shown the absence of hKv1.5-like current in human ventricular myocytes. All these results suggest that I_{Kur} is the native counterpart to hKv1.5 channels in human atria (Tamkun *et al.*, 1991; Snyders *et al.*, 1993; Wang *et al.*, 1993; Deal *et al.*, 1996) and therefore, it can contribute to the repolarization process of the human atrial action potential (Wang *et al.*, 1993; Deal *et al.*, 1996). Block of cardiac K^+ channels has been considered to be the mechanism by which class III antiarrhythmic drugs slow repolarization and prolong the action potential duration (Colatsky *et al.*, 1990; Hondeghem & Snyders, 1990; Roden, 1993). Indeed, selective block of the of the hKv1.5-like current in human atrial myocytes results in significant prolongation of the action potential duration (Wang *et al.*, 1993) and, therefore, it represents a potential molecular target for class III antiarrhythmic drugs (Wang *et al.*, 1993; Snyders & Yeola, 1995).

Propafenone and 5-hydroxy-propafenone block the open state of hKv1.5 channels

Propafenone and 5-hydroxy-propafenone accelerated the time course of activation and induced an initial fast decline of the hKv1.5 current during depolarizations (Figures 1 and 3) which superimposed on the slow inactivation process which characterizes this current at positive potentials, thus suggesting that both drugs bind to the open state of hKv1.5 channels. Moreover, the interaction of propafenone and 5-hydroxy-propafenone with the hKv1.5 channels was voltage-dependent (Figure 2a), reaching a higher degree of block at more positive than at more negative membrane potentials. These results are consistent with an open channel block mechanism, since the probability of opening increases at more positive membrane potentials. The similar δ values obtained for both drugs support the notion that they bind to the same receptor site in the ion channel pore. Moreover, these δ values are very similar to those previously described for other hKv1.5 blocking agents, i.e., local anaesthetics, antiarrhythmic drugs and antihistamines (Snyders *et al*., 1992; Rampe *et al*., 1993a,b; Valenzuela *et al*., 1995, 1996, 1997; Yang *et al*., 1995; Delpón *et al*., 1996; Caballero *et al*., 1997) and for internal tetraethyl ammonium block in *Shaker* K^+ channels (Yellen *et al*., 1991; Choi *et al*., 1993), which probably suggests that all these compounds share the same receptor site in hKv1.5 channels. Another finding supporting the open state block induced by these drugs is the 'crossover' observed between the deactivating tail currents recorded at -40 mV under control conditions and in the presence of either propafenone or 5-hydroxy-propafenone (Figure 4).

We also studied the effects of different frequencies on the degree of block induced by propafenone and 5-hydroxy-propafenone by applying trains of depolarizations at different frequencies (from 0.5–3 Hz). Our results demonstrate that propafenone and 5-hydroxy-propafenone decreased ($\approx$37 and 40%) the peak current elicited by the first depolarization of each train of stimuli (i.e., 'tonic block'). Tonic block has been 'classically' considered a measure of rested-state block. However, tonic block reflects the sum of the degree of block induced before the test pulse (rested state block) and that occurred during the application of the test pulse (open or slow inactivated channel block). On the other hand, use-dependent block requires a slow association rate constant, since if it is too fast, most block will occur during the first pulse of the train and it will appear as tonic block. This is indeed what we observed in the presence of either propafenone or 5-hydroxy-propafenone. In both cases we observed a very low degree of use-dependent block. Thus, the high degree of 'tonic block' (which mostly represents open channel block) and the low degree of use-dependent block induced by both drugs are likely due to the fast association rate constant that propafenone and 5-hydroxy-propafenone exhibit.

Differences in potency between propafenone and 5-hydroxy-propafenone

The present results indicate that propafenone and 5-hydroxy-propafenone produced a concentration-dependent and reversible block of hKv1.5 channels. The K_D values obtained in the present study with propafenone at $+60$ mV (4.4 μM) were similar to those previously reported for quinidine ($K_D = 6.2$ μM) (Snyders *et al*., 1992) and zatebradine ($K_D = 1.8$ μM) (Valenzuela *et al*., 1996). From the analysis of the concentration-dependence of block, it was evident that the potency of propafenone to block hKv1.5 channels was 2 fold higher than the potency of its metabolite 5-hydroxy-propafenone at both 0 mV and $+60$ mV. Differences in potency between both drugs could be attributed to differences in the association (k) or in the dissociation (l) rate constants of each drug. Our results indicate that differences between k values for each compound are more marked than the differences between l values. Thus, the different potency to block hKv1.5 channels can be explained by the faster association rate constant observed for the parent compound than for its metabolite. These kinetic differences in the k values may be due to the higher molecular weight of 5-hydroxy-propafenone and therefore, to a higher steric hindrance to access to the binding receptor site.

Clinical implications

The mean effective therapeutic plasma concentrations of propafenone ranged from 3.14–1812 ng ml^{-1} (equivalent to 0.9–5.3 μM) (Harron & Brogden, 1987; Siddoway *et al*., 1987) and since it is about 90% protein bound (Gillis & Kates, 1986; Funck-Brentano *et al*., 1990), *in vitro* concentrations of 0.2–0.6 μM probably correspond in action to clinically effective free drug concentrations. On the other hand, plasma concentrations of 5-hydroxy-propafenone ranged from 30–513 ng ml^{-1} (equivalent to 0.08–1.44 μM) (Siddoway *et al*., 1987; Funck-Brentano *et al*., 1990; Lee *et al*., 1990). In the present study, the K_D values for propafenone and its main metabolite at $+60$ mV were 4.4 and 9.2 μM, respectively. Thus, these results would suggest that both drugs block hKv1.5 channels only at concentrations much higher than the therapeutic plasma levels. However, it has been demonstrated that propafenone and 5-hydroxy-propafenone accumulates in myocardium at concentrations 10- and 20 fold higher than those observed in plasma, respectively (Latini *et al*., 1989). These data suggested that propafenone may reach cardiac concentrations ranging between 2–6 μM, which correspond to the range at which propafenone can block cardiac ion channels.

This is the first study in which the effects on hKv1.5 induced by propafenone and 5-hydroxy-propafenone were studied. The present results demonstrate that both the parent and the hydroxy-metabolite inhibit the hKv1.5 current in a concentration-, voltage- and time-dependent manner. The concentrations needed to observe these effects are in the therapeutical range and therefore, block of hKv1.5 channels can underlie the antiarrhythmic effect of propafenone. Moreover, the efficacy of 5-hydroxy-propafenone to block these channels at therapeutic concentrations could be also contribute to the previously reported efficacy of propafenone in the treatment of supraventricular tachycardias.

The authors want to express their thanks to Drs Michael M. Tamkun and Dirk J. Snyders for providing us with the cell line transfected with the gene encoding the expression of hKv1.5 channels. We also thank Guadalupe Pablo and Rubén Vara for their excellent technical assistance. This study was supported by FIS 95/0318(C.V.) and CICYT SAF96-0042(J.T.) and SAF98-0058(C.V.) Grants.

References

BOYLE, W.A. & NERBONNE, J.M. (1991). A novel type of depolarization-activated K^+ current in adult rat atrial myocytes. *Am. J. Physiol.,* **260,** H1236 – H1247.

BRYSON, H., PALMER, K., LANGTRY, H. & FITTON, A. (1993). Propafenone. A reappraisal of its pharmacology, pharmacology, pharmacokinetics and therapeutic use in cardiac arrhythmias. *Drugs,* **45,** 85 – 130.

CABALLERO, R., DELPÓN, E., VALENZUELA, C., LONGOBARDO, M., FRANQUEZA, L. & TAMARGO, J. (1997). Effect of descarboethoxyloratadine, the major metabolite of loratadine, on the human cardiac potassium channel Kv1.5. *Br. J. Pharmacol.,* **122,** 796 – 798.

CHIMIENTI, M., CULLEN, M.T., JR. & CASADEI, G. (1996). Safety of long-term flecainide and propafenone in the management of patients with symptomatic paroxysmal atrial fibrillation: report from the flecainide and propafenone italian study investigators. *Am. J. Cardiol.,* **77,** 60A – 75A.

CHOI, K.L., MOSSMAN, C., AUBE, J. & YELLEN, G. (1993). The internal quaternary ammonium receptor site of *Shaker* potassium channels. *Neuron,* **10,** 533 – 541.

COBBE, S.M. (1994). Drug therapy of supraventricular tachyarrhythmias – based on efficacy or futility? *Eur. Heart J.,* **15**(suppl A), 22 – 26.

COLATSKY, T.J., FOLLMER, C.H. & STARMER, C.F. (1990). Channel specificity in antiarrhythmic drug action mechanism of potassium channel block and its role in suppressing and aggravating cardiac arrhythmias. *Circulation,* **82,** 2235 – 2242.

DEAL, K., ENGLAND, S. & TAMKUN, M. (1996). Molecular physiology of cardiac potassium channels. *Physiol. Rev.,* **76,** 49 – 67.

DELGADO, C., TAMARGO, J. & TEJERINA, T. (1985). Electrophysiological effects of propafenone in untreated and propafenone-pretreated guinea-pig atrial and ventricular muscle fibres. *Br. J. Pharmacol.,* **86,** 765 – 775.

DELGADO, C., TAMARGO, J., TEJERINA, T. & VALENZUELA, C. (1987). Effects of 5-hydroxy-propafenone in guinea-pig atrial fibres. *Br. J. Pharmacol.,* **90,** 575 – 582.

DELGADO, C., TAMARGO, J., HENZEL, D. & LORENTE, P. (1993). Effects of propafenone on calcium current in guinea-pig ventricular myocytes. *Br. J. Pharmacol.,* **108,** 721 – 727.

DELPÓN, E., VALENZUELA, C., PÉREZ, O., CASIS, O. & TAMARGO, J. (1995). Propafenone preferentially blocks the rapidly activating component of delayed rectifier K^+ current in guinea-pig ventricular myocytes. Voltage-independent and time-dependent block of the slowly activating component. *Circ. Res.,* **76,** 223 – 235.

DELPÓN, E., VALENZUELA, C., PÉREZ, O., FRANQUEZA, L., GAY, P., SNYDERS, D.J. & TAMARGO, J. (1996). Mechanisms of block of a human cloned potassium channel by the enantiomers of a new bradycardic agent: S-16257-2 and S-16260-2. *Br. J. Pharmacol.,* **117,** 1293 – 1301.

DUAN, D., FERMINI, B. & NATTEL, S. (1993). Potassium channel blocking properties of propafenone in rabbit atrial myocytes. *J. Pharmacol. Exp. Ther.,* **264,** 1113 – 1123.

DUKES, I.D. & VAUGHAN WILLIAMS, E.M. (1984). The multiple modes of action of propafenone. *Eur. Heart J.,* **5,** 115 – 125.

FEI, L., GILL, J.S., MCKENNA, W.J. & CAMM, A.J. (1993). Effects of propafenone on calcium currents in single ventricular myocytes of guinea-pig. *Br. J. Pharmacol.,* **109,** 178 – 182.

FUNCK-BRENTANO, C., KROEMER, H.K., LEE, J.T. & RODEN, D.M. (1990). Propafenone. *N. Engl. J. Med.,* **322,** 518 – 525.

GENTILI, C., GIORDANO, F., ALOIS, A., MASSA, E. & BIANCONI, L. (1992). Efficacy of intravenous propafenone in acute atrial fibrillation complicating open-heart surgery. *Am. Heart J.,* **123,** 1225 – 1228.

GILLIS, A.M. & KATES, R.E. (1986). Myocardial uptake kinetics and pharmacodynamics of propafenone in the isolated perfused rabbit heart. *J. Pharmacol. Exp. Ther.,* **237,** 708 – 712.

HAMILL, O.P., MARTY, A., NEHER, E., SAKMANN, B. & SIGWORTH, F.J. (1981). Improved patch clamp techniques for high-resolution current recording from cells and cell-free membrane patches. *Pflügers. Arch.,* **391,** 85 – 100.

HARRON, D.W.G. & BROGDEN, R.N. (1987). Propafenone. A review of its phamacodynamic and pharmacokinetic properties and therapeutic use in the treatment of arrhythmias. *Drugs,* **34,** 617 – 647.

HEGE, H.G., HOLLMANN, M., KAUMEIER, S. & LIETZ, H. (1984). The metabolic fate of 2H-labelled propafenone in man. *Eur. J. Drug Metab. Pharmacokin.,* **9,** 41 – 55.

HONDEGHEM, L.M. & SNYDERS, D.J. (1990). Class III Antiarrhythmic agents have a lot of potential, but a long ways to go: reduced effectiveness and dangers of reverse use-dependence. *Circulation,* **81,** 686 – 690.

JECK, C.D. & BOYDEN, P.A. (1992). Age-related appearance of outward currents may contribute to developmental differences in ventricular repolarization. *Circ. Res.,* **71,** 1390 – 1403.

KATES, R.E., YEE, Y.G. & WINKLE, R.A. (1985). Metabolite cumulation during chronic propafenone dosing in arrhythmia. *Clin. Pharmacol. Ther.,* **37,** 610 – 614.

KINGMA, J.H. & SUTTORP, M.J. (1992). Acute pharmacologic conversion of atrial fibrillation and flutter: the role of flecainide, propafenone and verapamil. *Am. J. Cardiol.,* **70,** 56A – 61A.

KISHORE, A. & CAMM, A.J. (1995). Guidelines for the use of propafenone in treating supraventricular arrhythmias. *Drugs,* **50,** 250 – 262.

KONARZEWSKA, H., PEETERS, G.A. & SANGUINETTI, M.C. (1995). Repolarizing K^+ currents in nonfailing human hearts. similarities between right septal subendocardial and left subepicardial ventricular myocytes. *Circulation,* **92,** 1179 – 1187.

LATINI, R., BARBIERI, E., CASTELLO, C., MARCHI, S., SICA, A., GEROSA, G., ROSSI, R. & ZARDINI, P. (1989). Propafenone and 5-hydroxypropafenone concentrations in the right atrium of patients undergoing heart surgery. *Am. Heart J.,* **117,** 497 – 498.

LEDDA, F., MANTELLI, L., MANZINI, S., AMERINI, S. & MUGELLI, A. (1981). Electrophysiological and antiarrhythmic properties of propafenon in isolated cardiac preparations. *J. Cardiovasc. Pharmacol.,* **3,** 1162 – 1173.

LEE, J.T., KROEMER, H.K., SILBERSTEIN, D.J., FUNCK-BRENTANO, C., LINEBERRY, M.D., WOOD, A.J., RODEN, D.M. & WOOSLEY, R.L. (1990). The role of genetically determined polymorphic drug metabolism in the beta-blockade produced by propafenone. *N. Engl. J. Med.,* **322,** 1764 – 1768.

LI, G.R., FENG, J., WANG, Z., FERMINI, B. & NATTEL, S. (1996). Adrenergic modulation of ultrarapid delayed rectifier K^+ current in human atrial myocytes. *Circ. Res.,* **78,** 903 – 915.

MALFATTO, G., ZAZA, A., FORSTER, M., SODOWICK, B., DANILO, P., JR. & ROSEN, M.R. (1988). Electrophysiologic, inotropic and antiarrhythmic effects of propafenone, 5-hydroxypropafenone and n-depropylpropafenone. *J. Pharmacol. Exp. Ther.,* **246,** 419 – 426.

MAYS, D.J., FOOSE, J.M., PHILIPSON, L.H. & TAMKUN, M.M. (1995). Localization of the Kv1.5 K^+ channel protein in explanted cardiac tissue. *J. Clin. Invest.,* **96,** 282 – 292.

MCLEOD, A.A., STILES, G.L. & SHAND, D.G. (1984). Demonstration of beta adrenoceptor blockade by propafenone hydrochloride: clinical pharmacologic, radioligand binding and adenylate cyclase activation studies. *J. Pharmacol. Exp. Ther.,* **228,** 461 – 466.

PORTERFIELD, J.G. & PORTERFIELD, L.M. (1989). Therapeutic efficacy and safety of oral propafenone for atrial fibrillation. *Am. J. Cardiol.,* **63,** 114 – 116.

RAMPE, D., WIBLE, B., BROWN, A.M. & DAGE, R.C. (1993a). Effects of terfenadine and its metabolites on a delayed rectifier K^+ channel cloned from human heart. *Mol. Pharmacol.,* **44,** 1240 – 1245.

RAMPE, D., WIBLE, B., FEDIDA, D., DAGE, R.C. & BROWN, A.M. (1993b). Verapamil blocks a rapidly activating delayed K^+ channel cloned from human heart. *Mol Pharmacol.,* **4,** 642 – 648.

RODEN, D.M. (1993). Current status of class III antiarrhythmic drug therapy. *Am. J. Cardiol.,* **72,** 44B – 49B.

SATOH, H. & HASHIMOTO, K. (1984). Effect of propafenone on the membrane currents of rabbit sino-atrial node cells. *Eur. J. Pharmacol.,* **99,** 185 – 191.

SIDDOWAY, L.A., THOMPSON, K.A., MCALLISTER, C.B., WANG, T., WILKINSON, G.R., RODEN, D.M. & WOOSLEY, R.L. (1987). Polymorphism of propafenone metabolism and disposition in man: clinical and pharmacokinetic consequences. *Circulation,* **75,** 785 – 791.

SLAWSKY, M.T. & CASTLE, N.A. (1994). K^+ channel blocking actions of flecainide compared with those of propafenone and quinidine in adult rat ventricular myocytes. *J. Pharmacol. Exp. Ther.,* **269,** 66 – 74.

SNYDERS, D.J., KNOTH, K.M., ROBERDS, S.L. & TAMKUN, M.M. (1992). Time-, voltage-, and state-dependent block by quinidine of a cloned human cardiac potassium channel. *Mol. Pharmacol.,* **41,** 322 – 330.

SNYDERS, D.J., TAMKUN, M.M. & BENNETT, P.B. (1993). A rapidly activating and slowly inactivating potassium channel cloned from human heart. Functional analysis after stable mammalian cell culture expression. *J. Gen. Physiol.,* **101,** 513 – 543.

SNYDERS, D.J. & YEOLA, S.W. (1995). Determinants of antiarrhythmic drug action. Electrostatic and hydrophobic components of block of the human cardiac hKv1.5 channel. *Circ. Res.,* **77,** 575 – 583.

TAMKUN, M.M., KNOTH, K.M., WALBRIDGE, J.A., KROEMER, H., RODEN, D.M. & GLOVER, D.M. (1991). Molecular cloning and characterization of two voltage-gated K^+ channel cDNAs from human ventricle. *FASEB. J.,* **5,** 331 – 337.

THOMPSON, K.A., IANSMITH, D.H., SIDDOWAY, L.A., WOOSLEY, R.L. & RODEN, D.M. (1988). Potent electrophysiologic effects of the major metabolites of propafenone in canine purkinje fibers. *J. Pharmacol. Exp. Ther.,* **244,** 950 – 955.

UK PROPAFENONE PSVT STUDY GROUP. (1995). A randomized, placebo-controlled trial of propafenone in the prophylaxis of paroxysmal supraventricular tachycardia and paroxysmal atrial fibrillation. *Circulation,* **92,** 2550 – 2557.

VALENZUELA, C., DELGADO, C. & TAMARGO, J. (1987). Electrophysiological effects of 5-hydroxypropafenone on guinea-pig ventricular muscle fibers. *J. Cardiovasc. Pharmacol.,* **10,** 523 – 529.

VALENZUELA, C., DELPÓN, E. & TAMARGO, J. (1988). Tonic and phasic V_{max} block induced by 5-hydroxypropafenone in guinea-pig ventricular muscles. *J. Cardiovasc. Pharmacol.,* **12,** 423 – 431.

VALENZUELA, C., DELPÓN, E., TAMKUN, M.M., TAMARGO, J. & SNYDERS, D.J. (1995). Stereoselective block of a human cardiac potassium channel (Kv1.5) by bupivacaine enantiomers. *Biophys. J.,* **69,** 418 – 427.

VALENZUELA, C., DELPÓN, E., FRANQUEZA, L., GAY, P., PÉREZ, O., TAMARGO, J. & SNYDERS, D.J. (1996). Class III antiarrhythmic effects of zatebradine. Time-, state-, use-, and voltage-dependent block of hKv1.5 channels. *Circulation,* **94,** 562 – 570.

VALENZUELA, C., DELPÓN, E., FRANQUEZA, L., GAY, P., SNYDERS, D.J. & TAMARGO, J. (1997). Effects of ropivacaine on a potassium channel (hKv1.5) cloned from human ventricle. *Anesthesiology,* **86,** 718 – 728.

VON PHILIPSBORN, G., GRIES, J., HOFMANN, H.P., KREISKOTT, H., KRETZSCHMAR, R., MULLER, C.D., RASCHACK, M. & TESCHENDORF, H.J. (1984). Pharmacological studies on propafenone and its main metabolite 5-hydroxypropafenone. *Arzn. Forsch.,* **34,** 1489 – 1497.

WANG, Z., FERMINI, B. & NATTEL, S. (1993). Sustained depolarization-induced outward current in human atrial myocytes. Evidence for a novel delayed rectifier K^+ current similar to Kv1.5 cloned channel currents. *Circ. Res.,* **73,** 1061 – 1076.

WOODHULL, A.M. (1973). Ionic blockade of sodium channels in nerve. *J. Gen. Physiol.,* **61,** 687 – 708.

YANG, T., PRAKASH, C., RODEN, D.M. & SNYDERS, D.J. (1995). Mechanism of block of a human cardiac potassium channel by terfenadine racemate and its enantiomers. *Br. J. Pharmacol.,* **115,** 267 – 274.

YELLEN, G., JURMAN, M.E., ABRAMSON, T. & MACKINNON, R. (1991). Mutations affecting internal TEA blockade identify the probable pore-forming region of a K^+ channel. *Science,* **251,** 939 – 942.

(Received March 10, 1998
Revised June 15, 1998
Accepted July 22, 1998)