Effects of propafenone on calcium current in guinea-pig ventricular myocytes

[1]C. Delgado, J. Tamargo, *D. Henzel & *P. Lorente

Institute of Pharmacology and Toxicology (CSIC), School of Medicine, Universidad Complutense, 28040 Madrid, Spain and *U 195 INSERM, School of Medicine, BP 38 Place Henri Dunant, 63001 Clermont Ferrand, France

1   The modulation of L-type voltage-sensitive calcium channels in guinea-pig isolated ventricular myocytes by propafenone was examined by the whole cell voltage-clamp technique.

2   Propafenone, 10^{-7}–5×10^{-5} M, produced a concentration-dependent inhibition of Ca current (I_{Ca}) without any significant change in the current-voltage relation. Half-blocking concentration (IC_{50}) of propafenone for the peak I_{Ca} at $+10$ mV was 5×10^{-6} M.

3   The voltage-dependence of I_{Ca} inactivation was shifted in the hyperpolarizing direction in the presence of 5×10^{-6} M propafenone.

4   A frequency-dependent relative block by propafenone was observed after repetitive depolarizing test pulses at a frequency of 0.5 and 1 Hz. The recovery of I_{Ca} from inactivation was prolonged by propafenone and the reactivation exhibited an additional exponential component attributed to the slow release from drug block of Ca channels.

5   These results suggest that propafenone, at therapeutic concentrations exhibits Ca channel blocking properties that may be involved in its antiarrhythmic mechanism.

Keywords: Propafenone; calcium current; patch-clamp; ventricular myocytes

Introduction

Propafenone hydrochloride is an antiarrhythmic drug widely used for the management of supraventricular and ventricular arrhythmias (Harron & Brogden, 1987; Funck-Brentano *et al.*, 1990). The drug has demonstrated a high affinity for binding to the inactivated state of the Na channel with a kinetic profile similar to flecainide and encainide (Kohlhardt *et al.*, 1983; Honjo *et al.*, 1989; Valenzuela *et al.*, 1989). In addition, propafenone has special properties that set it apart from the other class I antiarrhythmic agents. Thus, propafenone exhibits a consistent β-adrenoceptor blocking action *in vitro* (Hapke & Prigge, 1976; Delgado *et al.*, 1985; Kroemer *et al.*, 1989) and *in vivo* (Müller-Pelzer *et al.*, 1983) as well as possible calcium-channel blocking properties. As a matter of fact, propafenone exerts a clear negative inotropic effect (Dukes & Vaughan Williams, 1984; Delgado *et al.*, 1985) and at therapeutic concentrations depresses the slow action potentials elicited in fibres partially depolarized with K$^+$ (Ledda *et al.*, 1981; Delgado *et al.*, 1985). Moreover, using the voltage-clamp technique some authors have reported that propafenone also reduces the slow inward Ca current in rabbit sino-atrial cells (Satoh & Hashimoto, 1984) and ventricular cardiac muscle fibres (Kohlhardt, 1977). Furthermore, experiments on rat isolated aortae have demonstrated that propafenone inhibits Ca entry through voltage- and receptor-operated channels as well as Ca release from intracellular stores, which again suggests that propafenone may exert Ca channel blocking properties in vascular smooth muscle (Carron *et al.*, 1991).

All these results suggest that propafenone inhibits the slow inward current (I_{Ca}) in cardiac cells; however, detailed studies of the inhibitory effect of propafenone on I_{Ca} have not yet been performed in cardiac cells. Therefore, the present study was undertaken to elucidate the possible Ca channel blocking effect of propafenone by the use of single cardiac cells isolated from guinea-pig ventricles.

Our results show that propafenone blocks I_{Ca} with both concentration- and frequency-dependent characteristics, and that these effects are related to the high affinity of the drug for binding to the inactivated state of the channel.

Methods

Cell isolation

Single ventricular myocytes of the guinea-pig heart were obtained by enzymatic dissociation following the procedure described by Isenberg & Klöckner (1982). Hearts were removed from previously heparinized (500 u, i.p.) guinea-pig weighing 400–600 g, and mounted in a Langendorff perfusion system for retrograde perfusion of the coronary vascular bed. The heart was perfused at 10 ml min^{-1} with the following oxygenated solutions at 37°C: (1) HEPES Tyrode solution for 4 min; (2) nominally calcium-free solution for 10 min; (3) nominally calcium-free solution containing 0.4 mg ml^{-1} collagenase (Type II, Worthington) for 10 min and (4) modified 'KB' or 'Kraftbrühe' medium (Isenberg & Klöckner, 1982) for another 10 min period. The hearts were removed from the Langendorff column, and the ventricles dissected and cut into small pieces which were placed in a beaker containing 'KB' solution and gently shaken to disperse the isolated cells. The resulting cell suspension was filtered through a 250 μm nylon mesh and centrifuged at low speed (20 g). Finally, the cell pellet was stored in 'KB' medium for 1–2 h. An aliquot of this cell suspension was placed in a recording chamber 0.5 ml in volume attached on the stage an inverted microscope (Olympus IMT-2) and after an additional period of 20 min, the bath superfusate was replaced with the Ca-containing HEPES-Tyrode solution. Experiments were performed at room temperature (22–26°C) on Ca-tolerant healthy ventricular myocytes identified by their rod-like, striated appearance.

Recording techniques

Voltage-clamp experiments were performed with the single pipette in the whole-cell configuration of the patch-clamp

[1] Author for correspondence.

technique (Hamill et al., 1981) by using an Axopatch-1D patch-clamp system (Axon Instruments). Suction pipettes were made from 1.5-1.6 mm o.d. soft glass capillary tubing with a microprocessor-based patch pipette puller (P.80/PC, Sutter Instruments). The pipettes had a tip resistance of 1.5-3 Megaohms after backfilling with the experimental internal solution. During the experiments, membrane potential and current data were displayed on a storage oscilloscope (Tecktronix 2214). Series resistance was compensated after the seal was broken to provide the fastest capacity transient possible without ringing of the amplifier. With maximal current (I) not exceeding 1 nA, the voltage drop error resulting from the residual series resistance (Rs) estimated by I.Rs (Halliwell et al., 1987), was less than 4 mV. Currents were filtered with an eight-pole low-pass Bessel filter at a cutoff frequency of 2 kHz, digitized, stored and analyzed on an compaq IBM AT 386/25 computer system using the pClamp software package version 5.1 (Axon Instruments, Burlingame, California, U.S.A.). Selected records were printed out with a laser printer (Hewlett Packard Laser Jet printer series II). The data were compensated for the liquid junction potential between internal and external solutions (about 3 mV). Each pulse protocol was applied at intervals longer than 30 s to avoid the frequency-dependent effect of propafenone.

Solutions and drugs

Composition of the HEPES-Tyrode solution was as follows (mM): NaCl 150, KCl 5.4, CaCl$_2$ 1.8, MgCl$_2$ 1.0, NaHCO$_3$ 5.8, NaH$_2$PO$_4$ 0.4, glucose 5.5 and HEPES 5.0 (pH adjusted at 7.4 with NaOH), and the solution was equilibrated with 100% O$_2$. The nominally calcium-free solution had the same composition as that of HEPES-Tyrode without CaCl$_2$. The 'KB' medium containing (mM): KCl 30, KH$_2$PO$_4$ 10, MgCl$_2$. 6H$_2$O 5, taurine 15, glutamic acid 70, ethylene glycol bis-(beta-aminoethyl ether) N,N,N',N'-tetraacetic acid (EGTA) 0.5, ATP 5 and KOH 30 (pH = 7.3). Potassium currents were eliminated by inclusion of Cs$^+$ in the pipette solution and by removal of K$^+$ in the external solution after the whole-cell configuration was achieved. In addition, 15-30 μM tetrodotoxin (TTX) was included in the external solution to block sodium current. The pipette internal solution consisted of (mM): CsCl 140, MgCl$_2$ 2, HEPES 10, CaCl$_2$ 1, EGTA 11, ATP 5 (pH adjusted to 7.2 with CsOH). By use of these solutions Ca currents were effectively isolated from Na and K currents. In some experiments neither Cs$^+$ nor TTX was used and the composition of the internal filling solution was as follows (mM): KCl 150, MgCl$_2$ 1.0, HEPES 5, EGTA 5, ATP 5 (pH 7.2 with KOH).

Propafenone hydrochloride (Knoll Ibérica) as a powder was initially dissolved in deionized water to make a stock solution (10^{-2}–10^{-3} M), and desired drug concentration was then obtained by adding a small amount of stock solution to the perfusate.

Statistics

Results are expressed as mean ± s.e.mean and Student's t test was used to determine the significance of differences between test and control values.

Results

Blocking action of propafenone on I_{Ca}

In order to determine the effects of propafenone on inward calcium current (I_{Ca}), ventricular cells were clamped at −40 mV to inactivate the sodium current and 150 ms depolarizing test pulses to +10 mV were applied at a basal rate of 0.03 Hz. The peak I_{Ca} was related to the zero current level. Figure 1a illustrates the time course of the onset of the effect of propafenone 5 × 10^{-6} M. Propafenone decreased the I_{Ca}
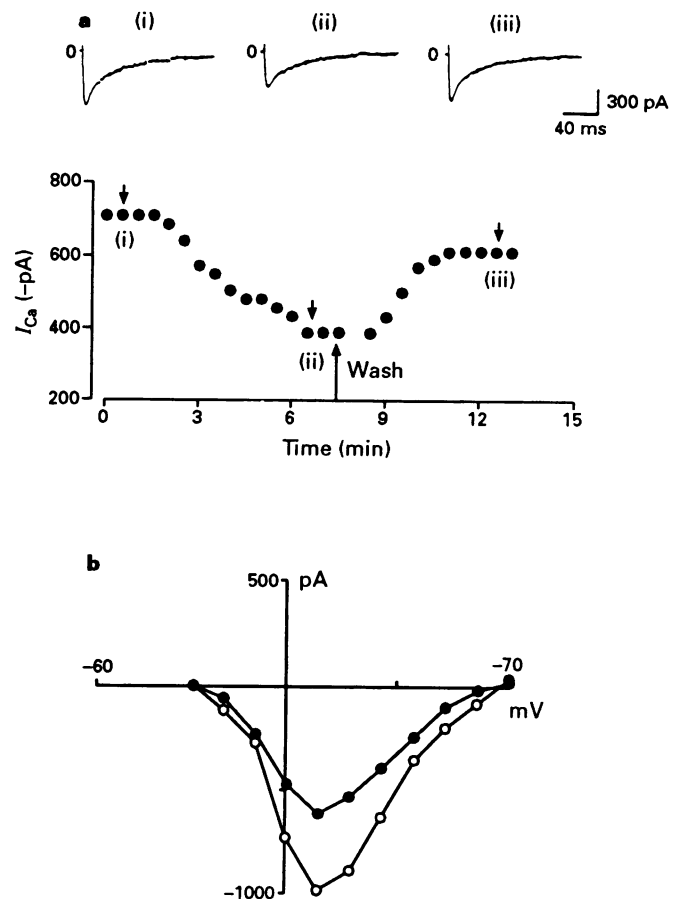

Figure 1   (a) Time course of the onset of the effects of propafenone (5 × 10^{-6} M) on the inward current (I_{Ca}) elicited by depolarization from −40 to +10 mV at 0.03 Hz. The external solution was zero K. Patch pipette internal solution contained CsCl. At the top of the figure, I_{Ca} values in control (trace i), in the presence of propafenone (trace ii) and after washing (trace iii) are shown. The time course of the drug effects is shown below after the application in (ii) and after washout in (iii). (b) I-V relationship of I_{Ca} obtained under control conditions (O) and 7 min after application of propafenone (5 × 10^{-6} M) (●). Depolarizing pulses of 150 ms in duration were applied from a holding potential of −40 mV to membrane potentials between −30 and +70 mV.

within 1-2 min after beginning of the superfusion and its effects reached a steady state after 7 min. At this time the cell was perfused with drug-free solution which was followed by a progressive recovery of current toward control, so that after 5 min the peak I_{Ca} reached approximately 80% of the control values (619 pA vs 710 pA). The differences observed at this time may occur under control conditions due to the 'run-down' of I_{Ca} which contributes in part to mask the recovery from blockade during washing.

To determine the effects of propafenone on current-voltage relationships (I-V) of I_{Ca}, 150 ms depolarizing pulses were applied at a basal rate of 0.03 Hz from a holding potential of −40 mV to membrane potentials between −30 and +70 mV. Peak amplitude of I_{Ca} was taken as the difference between the peak of the inward current and the current at the end of 150 ms. Figure 1b shows a typical I-V relationship obtained under control conditions and 7 min after application of 5 × 10^{-6} M propafenone. In the absence of propafenone, the maximum peak inward current was obtained near +10 mV and the averaged peak amplitude of I_{Ca} at this potential was 813 ± 53 pA (n = 30). Propafenone decreased the peak I_{Ca} (from 978 pA to 606 pA) but it had no effect on the threshold potential (−30 mV), the potential at which I_{Ca} was maximum and the apparent reversal potential (about

+65 mV), i.e., it had no effect on the shape of the relationship. Similar effects were obtained at all concentrations of propafenone tested.

Figure 2 shows the concentration-response plot for a wide range of propafenone concentrations. In these experiments peak I_{Ca} was obtained on depolarizations from -40 mV to $+10$ mV at 0.03 Hz and the peak I_{Ca} in control was taken as 1.0 and compared with the peak I_{Ca} measured 7 min after the beginning of drug superfusion. The average of I_{Ca} decrease produced by propafenone, 10^{-6} M, 5×10^{-6} M, 10^{-5} M and 5×10^{-5} M was $30.4 \pm 5.9\%$ $(n = 6)$, $52.8 \pm 4.1\%$ $(n = 13)$, $62.5 \pm 8.5\%$ $(n = 3)$ and $93.7 \pm 5.7\%$ $(n = 7)$ respectively. Due to the very few experimental data points obtained, we could only approximate the IC_{50} value by interpolating the regression function obtained by least square analysis on experimental data with the relative I_{Ca} value $= 0.5$. These results led to an approximate IC_{50} value of 5×10^{-6} M. At concentrations up to 10^{-6} M the washout of the cell with drug-free solution resulted in recovery of current toward control, while at 5×10^{-5} M no recovery was observed. Because of the estimated IC_{50} value, in the following experiments we selected the concentration of 5×10^{-6} M to analyze the effects of propafenone on I_{Ca}.

Tonic and frequency-dependent effects of propafenone

A standardized protocol was used to evaluate the relative contribution of tonic blockade of I_{Ca} by propafenone. I_{Ca} was elicited by 150 ms depolarizing test pulses to $+10$ mV at 0.05 Hz from a holding potential of -40 mV. After steady-state amplitude of I_{Ca} was obtained, depolarizing pulses were discontinued, drug was applied and, after a 3 min quiescent period, depolarizing test pulses were resumed. Tonic blockade was assessed as the difference in peak I_{Ca} in the control and the first pulse after drug exposure. In the experiment shown in Figure 3, propafenone produced very little inhibition of Ca current of the first clamp pulse after the quiescent period (from 560 pA to 537 pA) but blockade increased with
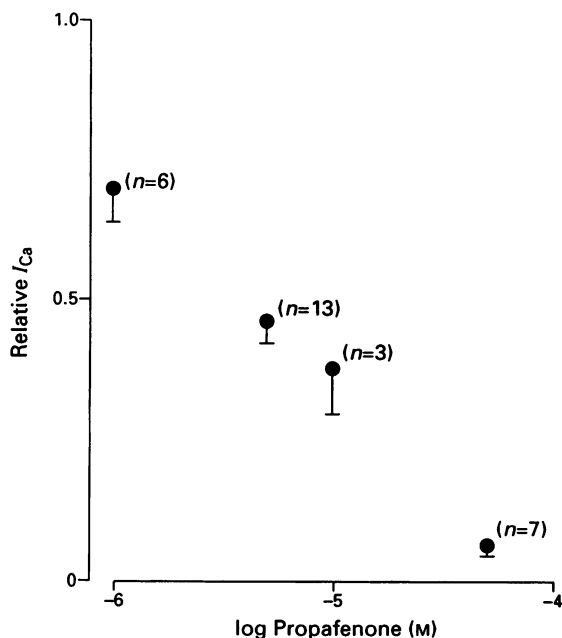
Figure 3   Relative contribution of tonic blockade of I_{Ca} by propafenone $(5 \times 10^{-6}$ M). I_{Ca} was elicited by 150 ms depolarizing test pulses to $+10$ mV at 0.05 Hz from a holding potential of -40 mV until the peak I_{Ca} reached steady-state. At that moment depolarizing pulses were discontinued and drug was applied. After 3 min of quiescence, depolarizing test pulses were resumed. Peak I_{Ca} in the control was taken as 1.0 and compared with the peak I_{Ca} recorded after 3 min of quiescence.

repeated depolarizations. In six cells the averaged tonic block induced by propafenone 5×10^{-6} M was $6.9 \pm 1.6\%$. These results suggest that propafenone shows a weak affinity for binding to the resting state of the calcium channel.

As previously described with other Ca channel blocking drugs (Ehara & Kaufmann, 1978; McDonald et al., 1980; Trautwein et al., 1981; Pelzer et al., 1982; Lee & Tsien, 1983; Uehara & Hume, 1985) the inhibitory effect on I_{Ca} induced by propafenone was greatly enhanced by repetitive membrane depolarization, which indicated a large degree of frequency-dependent block. An example of such an experiment is illustrated in Figure 4, where the peak I_{Ca} associated with each pulse is plotted as a function of pulse number. In this cell 15 consecutive voltage-clamp pulses of 150 ms in duration, from a holding potential of -40 mV to a test potential of $+10$ mV were applied at 0.5 and 1 Hz (5 min between trains) in the absence and presence of 5×10^{-6} M propafenone. Frequency-dependent blockade was measured as the difference between peak I_{Ca} for the first and 15th pulses in control and after drug exposure. The results plotted in Figure 4 show that under control conditions the magnitude of the peak I_{Ca} declined after 15 pulses by 7% and 20% for 0.5 and 1 Hz, respectively, probably because of an incomplete recovery of I_{Ca} from inactivation. However, in the presence of 5×10^{-6} M propafenone there was a dramatic decline in the peak I_{Ca} during the train of 15 depolarizing pulses (57.1% and 82.8% for 0.5 and 1 Hz respectively). In 3 cells driven at 0.5 and 1 Hz propafenone decreased the I_{Ca} by 57.2 ± 5.5 $(P < 0.01)$ and 82.4 ± 0.2 $(P < 0.01)$, respectively.

Effect of 5×10^{-6} M propafenone on the voltage-dependence of I_{Ca} inactivation

Because the I_{Ca} blockade by Ca channel blocking drugs can be modulated by membrane potential (McDonald et al., 1980; Hondeghem & Katzung, 1984; Sanguinetti & Kass, 1984), the interaction between propafenone block and calcium channel availability over a range of potentials was analyzed. For kinetic analysis of I_{Ca}, all experiments were performed in zero K^+ external solution and in the presence of 15 μM TTX and patch pipette contained CsCl. The voltage-dependence of I_{Ca} availability was examined by use of a double-pulse protocol: 2 s conditioning pulses of variable amplitude $(-55$ to $+10$ mV) which produced steady state

Figure 2   Dose-response plot of propafenone in single ventricular cells. I_{Ca} was elicited by depolarization from -40 to $+10$ mV at 0.03 Hz. The peak I_{Ca} in control was taken as 1.0 and compared with the peak I_{Ca} measured 7 min after beginning the drug superfusion. The symbols and vertical bars indicate the mean and s.e.mean, respectively. The number of observations for each concentration is indicated in parentheses. The approximate IC_{50} was 5×10^{-6} M.
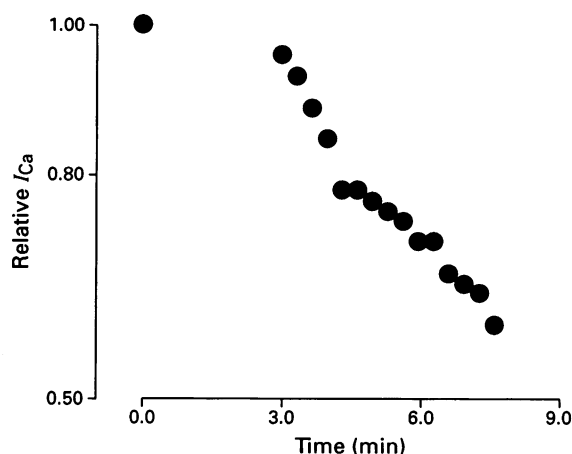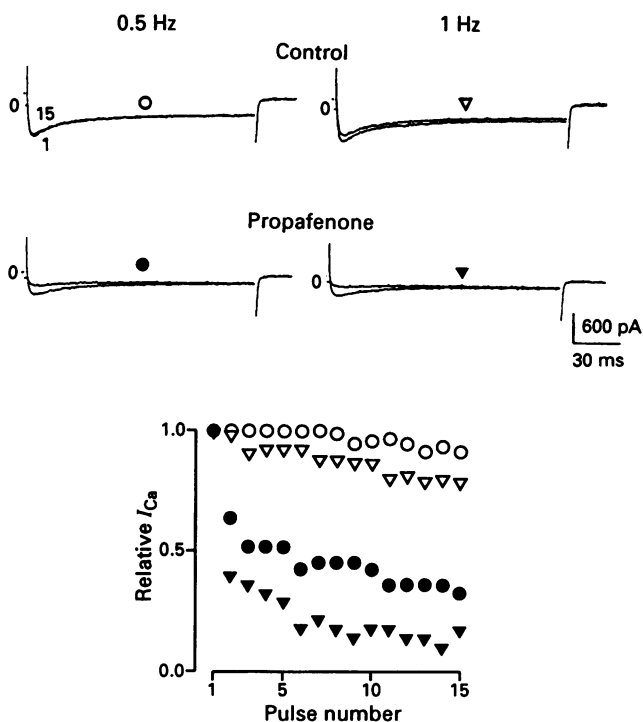
Figure 4 Effects of repetitive pulses at 0.5 and 1 Hz on the modulation of I_{Ca} by propafenone (5×10^{-6} M). Fifteen depolarizing pulses of 150 ms duration were applied to $+10$ mV from a holding potential of -40 mV at the frequency of 0.5 (circles) and 1 Hz (triangles) during control (open symbols) and after drug superfusion (closed symbols). Upper panel shows current records for pulse 1 and 15 with and without the drug. Bottom panel summarizes the results of plotting normalized I_{Ca} values for individual pulse numbers. The external solution was normal Tyrode.

Figure 5 Effects of propafenone on the steady-state inactivation curve for I_{Ca}. The membrane potential was held at -40 mV. After the application of 2 s conditioning pulses to various levels ranging from -55 to $+10$ mV in 5 mV steps, the membrane was depolarized to $+10$ mV for 150 ms. (a) Shows the I_{Ca} values at individual conditioning potentials in control (O) and after applying propafenone (5×10^{-6} M) (●). In (b), the steady-state inactivation curve of I_{Ca} was constructed by normalizing the I_{Ca} values. The line drawn through the data points represents the equation:

$$I = I_{max}/(1 + \exp[(V_m - V_h)/K]$$

Where V_h is the potential of half inactivation, V_m is the conditioning potential and K is the slope factor of the curve. Under control conditions, $V_h = -26.0$ mV and $K = 4.5$. After exposure to propafenone (5×10^{-6} M), $V_h = -29.7$ mV and $K = 4.0$.

inactivation of I_{Ca} (Yatani *et al.*, 1986) under control conditions were followed by a 10 ms return to the holding potential and a 150 ms test pulse to $+10$ mV. Figure 5a shows a representative steady-state inactivation curve for I_{Ca}. The data were fitted with a nonlinear least-squares regression analysis to a Boltzmann distribution (Hodgkin & Huxley, 1952):

$$I = I_{max}/(1 + \exp[(V_m - V_h)/K])$$

where V_h is the potential of half inactivation, V_m is the prepulse potential and K is the slope factor of the curve. The curve was S-shaped, with conditioning pulses to more negative potentials being associated with greater I_{Ca} values. Propafenone (5×10^{-6} M) decreased I_{Ca} at -55 mV from 697 to 368 pA as expected from the concentration-response curve. Figure 5b shows the resultant steady-state inactivation curves obtained after individual tail currents (I_{test}) were normalized to the tail currents elicited by conditioning pulse to -55 mV (I_{max}) and plotted against the conditioning potential. In this individual cell, propafenone shifted the V_h value from -26.0 mV to -29.7 mV, while the slope factor was almost similar to the control value (4.5 vs 4.0, respectively). The mean control value for V_h in 5 cells was -25.3 ± 0.8 mV and in the presence of 5×10^{-6} M propafenone the curve shifted significantly in the hyperpolarizing direction with a mean value for V_h of -32.7 ± 1.7 mV ($P < 0.01$). It is worth noting that no change in the slope factor of the curve under the two conditions was observed (3.7 ± 0.3 during control and 3.9 ± 0.6, in the presence of propafenone, $n = 5$).

The results suggest that propafenone possesses a high affinity for the inactivated state of the Ca channel. However, it is of interest to analyze the possibility as to whether propafenone can block calcium channels in the open state. In
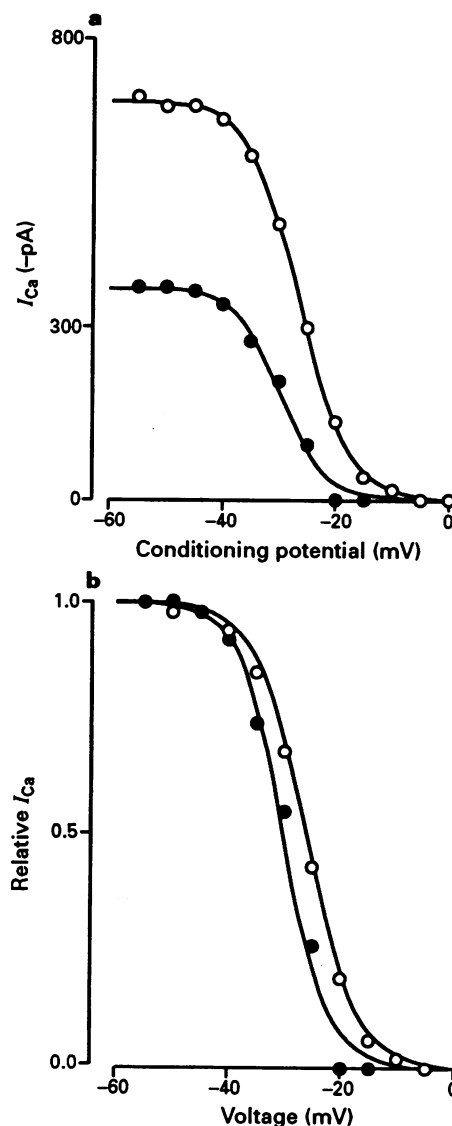
the simplest case, in which the inactivated state is assumed to be absorbing, the open channel block can be measured by comparing the waveform of the currents during depolarizing voltage pulses before and after exposure to propafenone (Yantani *et al.*, 1986). Drugs that bind to the open state of the channel have been shown to increase the inactivation rate of current through the channels during depolarizing voltage pulses after applying the drug (Lee & Tsien, 1983; Colquhoun & Hawkes, 1983). Figure 6a shows the time course of I_{Ca} before and after the application of propafenone (5×10^{-6} M). The Ca currents recorded with a faster time scale (Figure 6b) revealed that propafenone not only reduced the

peak magnitude of the I_{Ca} but also hastens its decay. The relaxation process was well described by a double exponential fit. The fast time constant was similar in the presence and absence of propafenone (5.1 ± 0.3 and 5.5 ± 0.1 ms, respec-
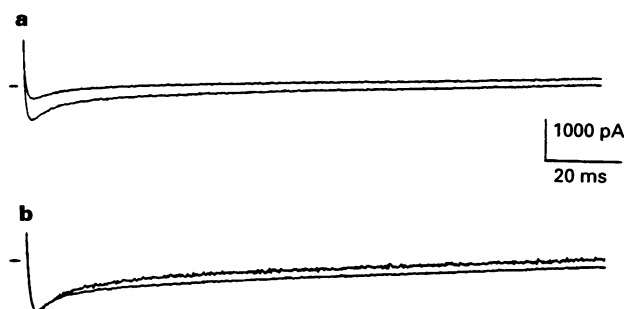

Figure 6 Effects of propafenone (5 × 10⁻⁶ M) on the rate of inactivation of I_{Ca} during depolarizing voltage pulse. (a) Current records in response to 100 ms clamp step to +10 mV from a holding of −40 mV at a rate of 0.03 Hz, control (O) and after applying propafenone (●) are superimposed. (b) The current traces before (O) and after applying propafenone (●) were scaled and were superimposed to compare the inactivation time course of I_{Ca}. The relaxation process was well described by a double exponential fit. The fast time constant was similar in the presence and absence of propafenone (5.1 vs 5.0 ms) but the slow time constant was shortened by propafenone (42.8 vs 25.6 ms).
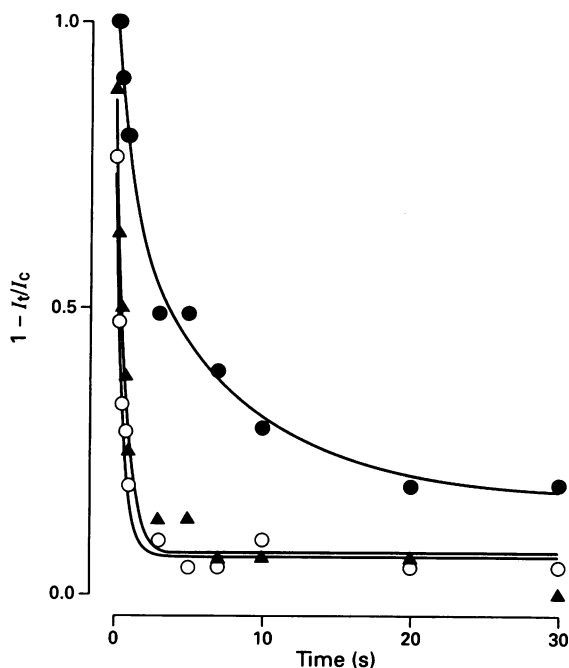

Figure 7 Effects of propafenone (5 × 10⁻⁶ M) on the recovery of I_{Ca}. A 2 s conditioning pulse which produced apparent inactivation at +10 mV was followed by a test pulse of 150 ms duration from a holding potential of −40 mV to +10 mV at various intervals (from 100 ms to 30 s). The peak current for each test pulse (I_t) was normalized to that for the control pulse applied 30 s before the conditioning pulse (I_c). The data were fitted by a least squares method of a sum of two exponentials: $y = A_1e^{-1/\tau 1} + A_2e^{-1/\tau 2}$, where y is the ratio of unrecovered current ($1-I_t/I_c$). In the absence of propafenone (O) the time course was best fitted by one exponential with a recovery time constant of 503 ms. With propafenone (●), the recovery of I_{Ca} was best fitted by two exponentials, 1100 ms and 8 s for the fast and slow components, respectively. After washout (▲), the time course of recovery was again fitted by a single exponential, with a recovery time constant similar to the control (658 ms).

tively, n = 6) but the slow time constant was shortened by the drug (41.3 ± 1.6 ms in control and 26.9 ± 1.9 ms in the presence of propafenone, n = 6, P<0.001).

Effects of propafenone on I_{Ca} reactivation

The frequency-dependent blocking action of I_{Ca} observed in the presence of the drug suggested that the recovery from inactivation after depolarizing test pulse may also be slowed by propafenone. In order to examine this possibility the time course of recovery from inactivation of I_{Ca} was investigated by use of a double-pulse protocol. A 2 s conditioning pre-pulse which produced apparent inactivation at +10 mV was followed by a test pulse of 150 ms duration from a holding potential of −40 mV to +10 mV at various intervals (from 100 ms to 30 s). The peak current for each test pulse was normalized to that for the control pulse applied 30 s before the conditioning pulse and is plotted as a function of the recovery period in Figure 7. In the absence of propafenone, the time course of recovery was best fitted by a single exponential function with a time constant of recovery (τ) of 503 ms while in the presence of 5 × 10⁻⁶ M propafenone the recovery process was markedly slowed being better described by two exponential functions (time constant of 1100 ms and 8 s for the fast and slow component respectively). The figure also shows that when the same experimental protocol was repeated after perfusing the cell with drug-free solution, the time course of recovery was again fitted by a single exponential which reached values similar to the control (τ = 658 ms). Similar findings were observed in another two cells. Then, it may be suggested that the slow component of the Ca current recovery with propafenone reflects the time course of drug dissociation from the inactivated Ca channel, whereas the fast component seems to reflect, at least in part, the recovery of drug-unbound channels from inactivation. The slow recovery of propafenone-bound channels from inactivation is consistent with the observation that propafenone produced a profound frequency-dependent I_{Ca} block even at 0.5 Hz.

Discussion

The results of the present investigation provide evidence that L-type Ca channels of guinea-pig isolated ventricular myocytes are blocked reversibly by propafenone. This conclusion is based on the finding that this drug blocks in a concentration-dependent manner the calcium inward current activated from potentials positive to −40 mV, the half-maximum inhibition (IC₅₀) occurring at a concentration of about 5 × 10⁻⁶ M. At the holding potential used in this study most T-type Ca channels are inactivated (Bean, 1985; Mitra & Morad, 1986), and thus the contribution of I_{Ca} through these channels can be ruled out. Propafenone reduced the peak amplitude of I_{Ca} without affecting the voltage-dependence of Ca channel activation or the slope factor of the inactivation curve. These results suggested that propafenone reduced the maximum conductance of I_{Ca}. The finding that propafenone blocks I_{Ca} agrees well with previous studies on cardiac tissues in which at the same range of concentrations, propafenone produced concentration-dependent decrease of the V_{max} of the slow action potentials, a valid index of the I_{Ca} (Malecot & Trautwein, 1987), both in guinea-pig ventricular muscle fibres (Delgado et al., 1985) and in dog Purkinje fibres (Malfatto et al., 1988). Moreover, voltage-clamp experiments on rabbit sinus node cells (Satoh & Hashimoto, 1984) have demonstrated that propafenone inhibited the I_{Ca}, but only at concentrations higher than 10⁻⁵ M. The discrepancy in the concentrations of propafenone required to inhibit the I_{Ca} can probably be attributed to the different methodology used (voltage-clamp vs patch-clamp and experimental protocols), even though species and/or tissue differences can also account for this discrepancy.

Furthermore, propafenone exerted voltage- and frequency-

dependent blockade of I_{Ca}. The inhibitory effects of a variety of Ca channel antagonists on myocardial Ca channels may be explained according to the modulated receptor hypothesis (Hondeghem & Katzung, 1977; Hille, 1977a,b; Uehara & Hume, 1985) which postulated that drug affinity for the Ca channel is modulated by channel state (i.e. rested, activated, inactivated). In the present experiments, propafenone produced little or no tonic I_{Ca} block, i.e. block of the Ca channels in the absence of stimulation, which indicated that it exhibits a low affinity for the resting state of the channels. In addition, the block of I_{Ca} by propafenone was markedly dependent on the frequency of stimulation. Use or frequency-dependence block of I_{Ca} has been hypothesized to occur because depolarizing pulses cause channel to cycle between resting, open, and inactivated states. If the affinity of a drug is much greater for open and/or inactivated channels than resting channels, then significant drug binding may occur during depolarizing steps even though little drug was bound under resting conditions. If during a train of depolarizing pulses the time available between pulses is not enough to allow drug unbinding from the inactivated channels, then block will accumulate until a steady-state level is reached. In the present experiments propafenone shifted the steady-state Ca inactivation curve toward more negative potentials, which indicated that the block of I_{Ca} by propafenone is also modulated by membrane potential. This shift suggested that propafenone-induced blockade of I_{Ca} is more pronounced when the channels are in the inactivated state and thus, that it exhibits a higher affinity for the inactivated state than for the resting state of the Ca-channel. According to Bean et al. (1983), the shift in the middle point of the steady state availability curve (V_h) is related to the drug concentration and dissociation constants for binding to inactivated (K_I) and rested state channels (K_R) as follows:

$$V_h = K \ln [(1 + [B]/K_R/(1 + [B]/K_I)]$$

where K is the slope factor of the inactivation curve ($K = 4.0$ in Figure 5). Plugging in the shift (ΔV_h) of -7 mV produced by 5×10^{-6} M propafenone in Figure 5, along with the value for K_R from Figure 2, gives a value of $K_I = 5 \times 10^{-7}$ M for 'binding' to the inactivated Ca channels.

The frequency-dependent block of I_{Ca} by propafenone can also be explained by a decrease in the recovery of inactivation. Several organic Ca antagonists have been shown to prolong the recovery from inactivation (Sanguinetti & Kass, 1984; Uehara & Hume, 1985). In the present study, the recovery process was slowed by propafenone being better described by a double exponential, the slower phase possibly indicating a slow dissociation of drug molecules from the inactivated Ca channels (Hondeghem & Katzung, 1984; Uehara & Hume, 1985). This finding can explain the marked use-dependent block of I_{Ca} induced by propafenone at slow frequencies of stimulation (0.5 and 1 Hz). However, it cannot explain by itself the shift of the inactivation curve to more negative potentials as the slope factor remains unchanged. On the other hand, within the context of a modulated-receptor hypothesis, the kinetics of I_{Ca} recovery for drugs that are predominantly charged at physiological pH are slower than for drugs that are predominantly neutral (Hille, 1977b; Uehara & Hume, 1985). The apparent pK_a of propafenone has been calculated as 9.0 (Courtney et al., 1987) and is similar to that of D-600 ($pK_a = 8.6$) (Uehara & Hume, 1985) indicating that both drugs are predominantly charged at physiological pH. In addition, this property suggests that propafenone must cross the cell membrane to gain access to its site of action (Hescheler et al., 1982). Further experiments will be necessary to prove this possibility.

Finally, the concentration of propafenone used in this study (10^{-6} M–5×10^{-5} M) included not only the therapeutic plasma levels (482 μg l^{-1}–1812 μg l^{-1}: 1.3×10^{-6} M–5.3×10^{-6} M; Keller et al., 1978; Karaguezian et al., 1983; Connolly et al., 1983) but also the toxic plasma level of drug. Therefore, the observed I_{Ca} block produced by propafenone may be responsible for some of its cardiodepressant effects as well as for its antiarrhythmic effectiveness on supraventricular arrthythmias. Propafenone can depress cardiac automaticity (Tamargo & Delgado, 1985) and marked bradycardia, sino-atrial block and sinus arrest have been reported after the administration of propafenone (Connolly et al., 1983; Schlepper, 1987). On the other hand, Honjo et al. (1989) found that propafenone at concentrations $>10^{-6}$ M shortened action potential duration in single ventricular myocytes from guinea-pig and this shortening was accompanied by an attenuation of sarcomere shortening during twitch contraction suggesting that the Ca-antagonist effect of propafenone could be involved. Furthermore at high concentrations in vitro, propafenone exerted a negative inotropic effect in isolated atrium or ventricle compatible with calcium-channel blockade (Dukes & Vaughan Williams, 1984; von Philipsborn et al., 1984). Moreover, since the I_{Ca} plays a major role in both conduction and automaticity of the A-V node, the marked frequency-dependent I_{Ca} block appears also to be important to explain the clinical effectiveness of propafenone against paroxysmal and non-paroxysmal A-V nodal reentrant tachycardia (Funk-Brentano et al., 1990).

In conclusion, the present data demonstrate that in guinea-pig ventricular myocytes propafenone produces a concentration-dependent inhibition of I_{Ca} which can be involved in its antiarrhythmic mechanism.

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