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# Inhibition of L-type calcium current by propafenone in single myocytes isolated from the rabbit atrioventricular node

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**1** The atrioventricular node (AVN) is an important part of the conduction system in the heart and is a significant site of antiarrhythmic drug action. The class 1 antiarrhythmic propafenone is effective in treating a variety of arrhythmias, including those involving the AVN. In this study, we have investigated the effects of propafenone on ionic currents in single rabbit AVN cells, focusing in particular on those on L-type calcium current ($I_{Ca,L}$).

**2** With a standard K-based internal dialysis solution, exposure to 5 $\mu$M propafenone reduced significantly the amplitude of $I_{Ca,L}$. In spontaneously active AVN myocytes, action potential upstroke velocity was decreased by propafenone exposure, consistent with the observed change in $I_{Ca,L}$.

**3** By use of a Cs-based internal dialysis solution to record $I_{Ca,L}$ selectively, voltage clamp test pulses were applied from a holding potential of $-40$ mV to $+10$ mV (stimulation frequency 0.33 Hz). Propafenone 5 $\mu$M reduced mean $I_{Ca,L}$ density at $+10$ mV from $-9.58 \pm 1.05$ pA/pF to $-4.19 \pm 0.60$ pA/pF ($P < 0.002$). A range of propafenone concentrations were applied which reduced $I_{Ca,L}$ in a dose-dependent manner ($IC_{50}$ 1.7 $\mu$M). When test pulses were applied to a range of potentials, propafenone reduced $I_{Ca,L}$ at each potential without significantly affecting the activation curve for this current. Thus, propafenone reduced $I_{Ca,L}$ conductance, without affecting the voltage-dependent activation properties of the current.

**4** $I_{Ca,L}$ block by propafenone exhibited tonic-, use- and frequency-dependent characteristics.

**5** In the presence of propafenone, the voltage-dependence of inactivation of $I_{Ca,L}$ was shifted 8 mV in the hyperpolarizing direction. Also, the recovery of $I_{Ca,L}$ from inactivation was slowed by propafenone.

**6** The $I_{Ca,L}$ blocking properties of propafenone may mediate some of the antiarrhythmic properties of this agent, particularly in regions of the heart such as the AVN in which $I_{Ca,L}$ contributes significantly to the action potential upstroke.

**Keywords:** Atrioventricular node; single cell; L-type calcium current; propafenone; antiarrhythmic

## Introduction

Propafenone is an antiarrhythmic agent used clinically for treatment of ventricular and supraventricular arrhythmias (Hammill *et al*., 1987; Funck-Brentano *et al*., 1990; Kishore & Camm, 1995). As a class 1c antiarrhythmic agent, propafenone exhibits potent 'fast sodium channel' ($I_{Na}$) blocking effects (e.g. Kohlhardt *et al*., 1983; Delgado *et al*., 1985) which are important for mediating its action on atrial and ventricular tissues, in which the action potential upstroke is $I_{Na}$-dependent. Propafenone also exerts other actions: it shows $\beta$-blocking actively *in vitro* and *in vivo* (e.g. Muller-Pelzer *et al*., 1983; Delgado *et al*., 1985), potassium channel blocking activity (e.g. Duan *et al*., 1993; Delpon *et al*., 1995) and two studies have suggested that propafenone can also inhibit L-type calcium current ($I_{Ca,L}$) in guinea-pig ventricular myocytes (Delgado *et al*., 1993; Fei *et al*., 1993). These additional effects might be expected to play a role, particularly in areas of the heart which are not critically dependent on $I_{Na}$, to generate the action potential upstroke.

Slow conduction through the atrioventricular node (AVN) plays an important role in generating the correct timing between atrial and ventricular contraction (De Carvalho & De Almeida, 1960). The AVN can provide a protective function during atrial fibrillation and flutter, since the slow conduction through this region allows only a fraction of impulses to be transmitted to the ventricles thereby protecting, to some extent, cardiac function. Moreover, abnormalities in AVN conduction can give rise to arrhythmias (Goy & Fromer, 1991). The AVN, therefore, is an important site of antiarrhythmic drug action. Propafenone is effective in chronic treatment of some atrioventricular tachycardias (Goy & Fromer, 1991) and, administered intravenously, this drug has been shown to terminate atrioventricular nodal (AVNRT) and atrioventricular re-entrant (AVRT) tachycardias (Santinelli *et al*., 1989; Mannino *et al*., 1994). However, little if anything is known at present about the cellular mechanisms underlying the actions of propafenone in the AVN. The availability of Ca tolerant single AVN cells (e.g. Hancox *et al*., 1993; Martynyuk *et al*., 1995; 1996; Habuchi *et al*., 1995; Hancox & Levi, 1996; Munk *et al*., 1996) now makes it possible to investigate cellular mechanisms of drug action on the AVN. There is some heterogeneity of the action potential throughout the AVN (Akiyama & Fozzard, 1979; Kokubun *et al*., 1980). However, evidence from single AVN cell experiments, as well as from multicellular preparations and the intact perfused heart, suggests that in a significant proportion of cells the action potential upstroke is carried by L-type calcium current (Zipes & Fischer, 1974; Kokubun *et al*., 1982; Hancox & Levi, 1994). In this study we have investigated the actions of propafenone at the single cell level, focusing in particular on its actions on $I_{Ca,L}$.

## Methods

### *Preparation of cells for recording*

Rod and spindle shaped AVN myocytes were isolated from the hearts of male New Zealand White rabbits (1.5–2.5 kg) by the method described by Hancox *et al*. (1993). (Note that the EGTA concentration added to the perfusate during the

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isolation was 100 $\mu$M and not 100 mM as published). AVN cells were placed in a chamber mounted on an inverted microscope (Nikon Diaphot) and superfused with Tyrode solution containing (in mmol $l^{-1}$): NaCl 130, KCl 2.7, $CaCl_2$ 2, $MgCl_2$ 1, glucose 10, HEPES 5 and titrated to a pH of 7.4 by addition of 4 mmol of NaOH (calculated from the volume of 1 M NaOH needed to bring the solution to the desired pH, monitored with a Corning 140 pH meter). For protocols investigating the steady state inactivation of $I_{Ca,L}$, N-methyl-D-glucamine (NMDG) was substituted for Na to block $I_{Na}$ and 40 $\mu$M Ni was added to block T-type $I_{Ca}$ (Hagiwara *et al*., 1988). The experimental chamber had a volume of 75 $\mu$l and a flow rate of 2–4 ml $min^{-1}$ (Levi, 1991). AVN cells were placed in the chamber as a cell suspension in 'KB' medium (Isenberg & Klockner, 1982a), and allowed to settle on the glass bottom for 20 s. Perfusion was then started at a slow rate and gradually increased. In this way, flow rate could be increased without cells becoming dislodged and as the Ca-containing bath solution replaced KB, the cells were slowly re-exposed to Ca.

### *Electrophysiological recording*

For electrophysiological recording, the whole cell patch clamp technique was used. Patch-pipettes (Corning 7052 glass, AM Systems Inc.) were pulled to resistances of 1–3 MΩ (Narashige PP83 puller) and fire-polished to 4–6 MΩ (Narishige MF83 microforge). The K-based internal dialysis solution contained (in mmol $l^{-1}$): KCl 110, NaCl 10, $K_2ATP$ 5, Tris-GTP 0.5, $MgCl_2$ 0.4, HEPES 10 and glucose 5, titrated to pH of 7.1 by adding 10 mmol KOH (added from 1 M KOH stock solution). The 'pipette-to-bath' liquid junction potential was measured for this filling solution and was found to be −2.7 mV. Since this value was small, no corrections of membrane potential were made. It has been shown previously that a caesium ($Cs^+$)-based internal dialysis solution blocks effectively outward potassium currents in single AVN myocytes, and this was used for selective recording of L-type calcium current (Hancox & Levi, 1994; 1996). In the Cs-based solution KCl was replaced by CsCl in an equimolar fashion, and 5 mM BAPTA (1,2,*bis* (2 aminophenoxy)ethane N,N,N′,N′-tetraacetic acid) was used to eliminate the intracellular $Ca_i$ transient and resulting Ca-activated currents (e.g. Hancox & Levi, 1996). For all experiments a holding potential of −40 mV was used, since this lies near the 'zero-current' potential for AVN cells (Hancox *et al*., 1993) and inactivates fast sodium current. All recordings of membrane currents and action potentials were made with an Axopatch 1D amplifier (Axon Instruments) and a CV-4 headstage. Normally 80 to 90% of the electrode series resistance could be compensated.

### *Experimental solutions*

Propafenone (Sigma) was dissolved in ethanol to give a 10 mM stock solution, which was kept at 4°C. Aliquots of this were added to normal Tyrode solution to produce a solution with the final concentrations described in the text. Nickel chloride was obtained from Sigma, dissolved in de-ionised water to give a 0.5 M stock solution and serial dilutions were made in Na-free Tyrode solution to give the final concentration of 40 $\mu$M. Test solutions were applied to cells by a multi-barralled 'in-house' built solution application device which could change bulk solution around a single myocyte within 1 s (Levi *et al*., 1996). All experiments were performed at temperatures between 35–37°C.

### *Generation of protocols, data acquisition and analysis*

Specific voltage clamp protocols are detailed in the relevant Results sections. Voltage clamp command signals were generated by a Cambridge Electronic Design (CED) 1401 interface and 'WCP', a programme written and supplied free of charge by John Dempster of Strathclyde University. Data were recorded directly onto the 'hard-disk' of a Viglen EX personal computer for off-line analysis by 'WCP'. Membrane currents were sampled at 2 kHz. Calcium current amplitudes were measured as the difference between the peak inward current at the start of the test pulse and the steady-state value at the end of the pulse. Graphs were drawn by use of 'FigP for Windows' software (Biosoft) and statistical analysis was performed with 'Systat for Windows' (Systat, Inc.). Data are presented as means ± s.e.mean and statistical comparisons were made by Student's *t* test.

## Results

### *Effects of propafenone on $I_{Ca,L}$ and action potentials recorded with a K-based internal dialysis solution*

In initial experiments, recordings were made with K-based internal dialysis solution. We applied a series of 500 ms duration voltage clamp pulses from −40 mV to +10 mV at a stimulation frequency of 0.33 Hz, in normal Tyrode solution and in the presence of 5 $\mu$M propafenone. Figure 1a shows representative records from a single AVN myocyte. In normal Tyrode solution, a pulse to +10 mV elicited a rapidly activating inward current, which attained a peak amplitude near −430 pA and then inactivated during the pulse (left panel). This current is known to be largely comprised of

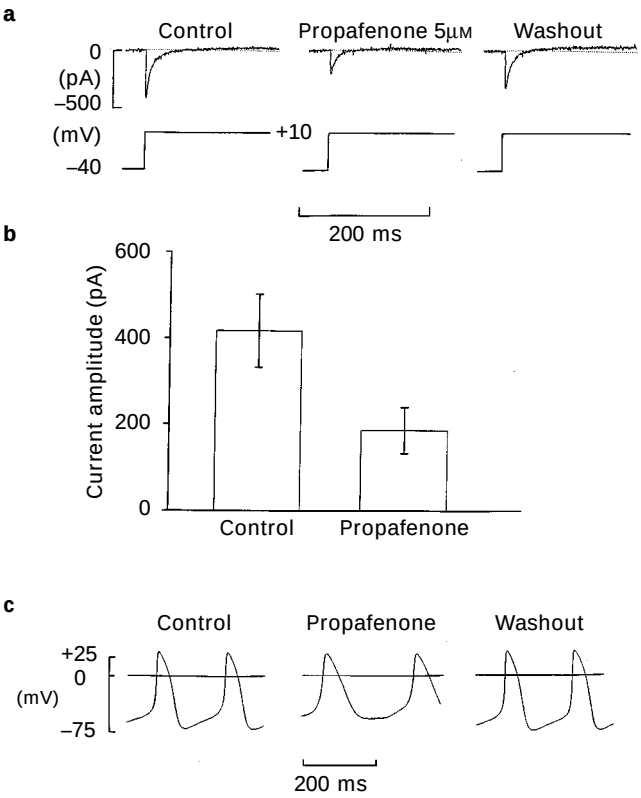



**Figure 1** Effects of propafenone observed with a K-based internal dialysis solution. In (a) and (b) 500 ms duration voltage clamp test pulses were applied from −40 to +10 mV. Pulse frequency 0.33 Hz. (a) Inward current ($I_{Ca,L}$: upper trace) in normal Tyrode solution (control), two minutes after exposure to 5 $\mu$M propafenone and 5 min after washout (lower panel shows test pulse voltage). (b) Histogram shows mean amplitude of $I_{Ca,L}$ in Tyrode solution and in the presence of propafenone ($n=7$). (c) Records of spontaneous action potentials recorded in 'current clamp' mode in normal Tyrode solution (Control), 30 s after a rapid switch to 5 $\mu$M propafenone (Propafenone) and one minute after 'Washout'.

$I_{Ca,L}$ (Hancox *et al*., 1993). When the perfusate was switched to one containing 5 $\mu$M propafenone the amplitude of this current declined rapidly, reaching an amplitude of 205 pA within 2 min (middle panel), after which no further reduction in current amplitude was observed. Similar results were seen in each of seven cells. This effect was partially reversible on changing the superfusate back to normal Tyrode solution (right panel; 5 min after return to normal Tyrode solution). The amplitude of the inward current after 5 min washout with normal Tyrode solution was 75% of the control value, which is similar to the 80% value obtained by Delgado *et al.* (1993) after applying 5 $\mu$M propafenone to guinea-pig ventricular cells. Mean data for seven cells are shown in Figure 1b, a significant decline of current amplitude ($P<0.002$) was seen after 2 min exposure to propafenone. We also tested the effects of propafenone on spontaneous action potentials, recorded in 'current-clamp' mode, from cells dialysed with the K-based internal dialysis solution. Figure 1c shows recordings from a representative cell. Under control conditions (left panel), this cell exhibited spontaneous action potentials with a maximal upstroke velocity ($V_{max}$) of 11.7 V $s^{-1}$, a duration (measured at 50% repolarization: $APD_{50}$) of 49 ms, and a maximal diastolic potential (MDP) near $-69$ mV. Thirty seconds after a rapid switch to 5 $\mu$M propafenone (middle panel), $V_{max}$ had decreased to 6.4 V $s^{-1}$, the $APD_{50}$ had increased to 60 ms, and the MDP had depolarized to $-57$ mV. There was also a slight lengthening (27%) of the spontaneous action potential cycle length. After 45 s of exposure the solution was rapidly switched back to normal Tyrode solution, and within 1 min spontaneous activity was similar to that observed under control conditions. Qualitatively similar effects of propafenone were observed in each of three cells. The decreased $V_{max}$ in the presence of propafenone was consistent with the changes to $I_{Ca,L}$ observed in voltage clamped cells.

### *Inhibition of* $I_{Ca,L}$ *in cells dialysed with a Cs-based internal dialysis solution*

With a K-based internal dialysis solution, propafenone inhibited the early inward current (largely $I_{Ca,L}$) and exerted a number of effects on spontaneous action potentials. Some of the effects (eg depolarization of the MDP and increase in $APD_{50}$) might have reflected actions of propafenone on current systems other than $I_{Ca,L}$. Therefore, to investigate the effect of propafenone on $I_{Ca,L}$ more selectively, a Cs-based internal dialysis solution was used in subsequent experiments (Hancox & Levi, 1994; 1996). Figure 2a shows the effects of 5 $\mu$M propafenone on $I_{Ca,L}$. In normal Tyrode solution a pulse from $-40$ to $+10$ mV elicited an $I_{Ca,L}$ with an amplitude near 400 pA. After 2 min exposure to propafenone, the current amplitude declined to 45% of the control value. Figure 2b shows mean data for 10 cells. For each cell, $I_{Ca,L}$ amplitude at $+10$ mV was normalized to cell capacitance. The degree of $I_{Ca,L}$ block by 5 $\mu$M propafenone under selective recording conditions was significant ($P<0.001$), and comparison of the level of current block between K- and Cs-based internal dialysis solutions showed that it was similar for the two internal dialysis solutions ($P>0.1$).

It was important to test whether propafenone actually reduced peak $I_{Ca,L}$ or whether it merely shifted the voltage-dependence of $I_{Ca,L}$. Current-voltage (*I*-V) relations for $I_{Ca,L}$ were obtained in normal Tyrode solution and in the presence of propafenone, by applying 500 ms duration voltage clamp pulses from $-40$ mV to a variety of test potentials (pulse frequency: 0.33 Hz). Figure 2c shows mean data (normalized to cell capacitance) for four cells. Propafenone 5 $\mu$M reduced $I_{Ca,L}$ at all test potentials between $-30$ and $+50$ mV. In both normal Tyrode solution and propafenone-containing solution the *I*-V relationship peaked at $+10$ mV. The effect of propafenone on $I_{Ca,L}$ activation was also determined by using *I*-V data from each cell to construct activation curves for this current. Activation variables at each potential for each of four cells were determined by Tyrode solution and after propafenone exposure by use of methods described previously (Isen-

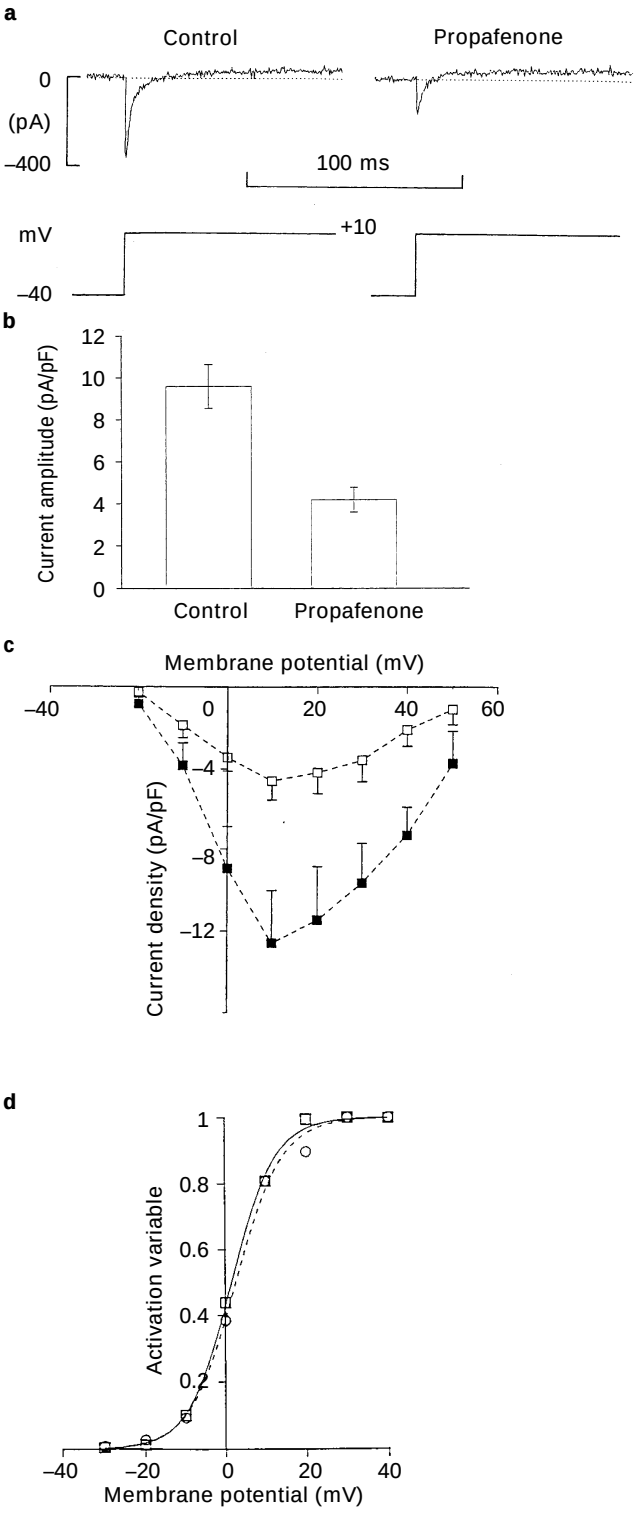



**Figure 2** Effects of 5 $\mu$M propafenone on $I_{Ca,L}$ recorded selectively with a Cs-based internal dialysis solution (a) $I_{Ca,L}$ in control conditions (left panel) and after 2 min exposure to propafenone (right panel; same pulse protocol as described in Figure 1 legend). (b) Histogram shows mean $I_{Ca,L}$ density for 10 cells in normal Tyrode solution and in propafenone-containing solution. (c) Current-Voltage (*I*-V) relation for $I_{Ca,L}$ in normal Tyrode solution (■) and in the presence of propafenone (□), obtained by applying test pulses to potentials between $-30$ and $+50$ mV ($n=4$). (d) Steady state activation data for $I_{Ca,L}$ in an AVN cell in normal Tyrode solution (□) and after 5 $\mu$M propafenone (○) (curves fitted by use of equation described in Results section). Propafenone did not significantly affect $I_{Ca,L}$ activation in each of 4 cells.

berg & Klockner, 1982b; Hancox & Levi, 1994). The data were fitted by a Boltzmann charge distribution.

$$\text{Activation variable} = 1/\{1+\exp[(V_{0.5}-V_m)/k]\} \quad (1)$$

where $V_{0.5}$ is the membrane potential at which the $I_{Ca,L}$ conductance is half-maximal, $V_m$ is the potential at which $I_{Ca,L}$ was measured and $k$ is the slope factor describing the steepness of the activation curve. Figure 2d shows $I_{Ca,L}$ activation data for an AVN myocyte in Tyrode solution and after 5 min exposure to propafenone. At most membrane potentials the activation variables obtained in control and drug-containing solutions are superimposed, and the activation curves differ very little between the two experimental conditions. When data from four cells were combined the mean value of $V_{0.5}$ was $0.61 \pm 2.4$ mV in control solution and $-0.52 \pm 3.8$ mV in the presence of propafenone ($P > 0.8$), whereas $k$ was $6.27 \pm 0.4$ mV in control solution and $5.8 \pm 0.9$ mV in the presence of propafenone ($P > 0.64$). Thus, propafenone reduced the magnitude of $I_{Ca,L}$ at a given membrane potential without affecting significantly the voltage-dependence of $I_{Ca,L}$ activation.

Figure 3 shows the effects of a range of concentrations of propafenone on peak $I_{Ca,L}$. We applied four propafenone concentrations between 50 nM and 0.5 mM, with command pulses from $-40$ mV to $+10$ mV used to elicit $I_{Ca,L}$. The magnitude of $I_{Ca,L}$ between two and three minutes after switching to propafenone (at which time $I_{Ca,L}$ had declined to a steady level in the presence of drug) was normalized to the amplitude in normal Tyrode solution for each cell, and the mean relative current plotted against the log of each concentration. Over this range of drug concentrations, the log-dose response relation was approximately linear and therefore difficult to fit reliably to a Hill plot (Hill plot analysis gave a $K_d$ of 1.5 $\mu$M with a curve of shallow slope and a Hill coefficient of 0.3). Delgado *et al.* (1993) similarly observed a linear dose-response relationship and performed least square analysis of the experimental data, with the regression function obtained to estimate the drug concentration correlating to a relative $I_{Ca,L}$ value of 0.5. We adopted this method to analyse our dose-response data and obtained an approximate $IC_{50}$ value of 1.7 $\mu$M.

### *Tonic- and use-dependent block of* $I_{Ca,L}$ *by propafenone*

We used a standardized protocol similar to that used in a previous study (Delgado *et al.*, 1993) to investigate whether propafenone showed tonic- and/or use-dependent properties.

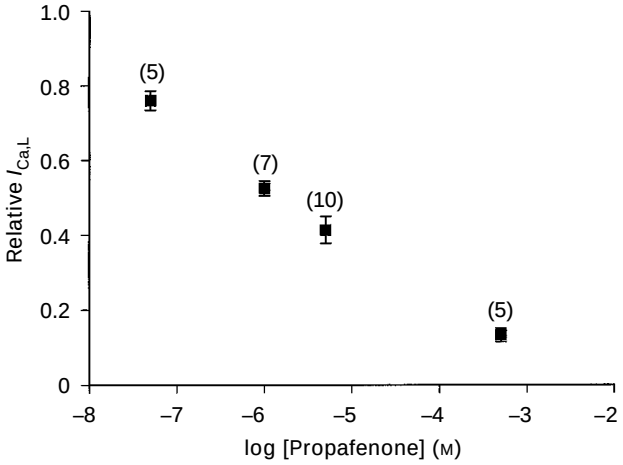



**Figure 3** Log dose-response relation for inhibition of $I_{Ca,L}$ by propafenone. For each concentration of propafenone (50 nM, 1 $\mu$M, 5 $\mu$M and 0.5 mM) $I_{Ca,L}$ amplitude in the presence of the drug was expressed relative to the current amplitude in normal Tyrode solution. The numbers in parentheses above each point indicate the number of cells from which the data-point was derived. Least square analysis of the relation gave an approximate $IC_{50}$ of 1.7 $\mu$M.

Ten 200 ms voltage clamp pulses from $-40$ to $+10$ mV were applied at a frequency of 0.33 Hz in normal Tyrode solution, and the mean $I_{Ca,L}$ amplitude for the last three pulses was taken as the control value. Solution was then switched to one containing 1 $\mu$M propafenone and pulsing was discontinued for a period of three minutes. After three minutes had elapsed (during which time no pulses were applied) pulsing was resumed in the presence of propafenone. Tonic blockade was measured as the difference between the control $I_{Ca,L}$ amplitude and that exhibited by the first pulse on re-starting stimulation. The amplitude of $I_{Ca,L}$ was then monitored until the current had declined to a steady level (between twelve and sixteen pulses). For each of seven cells, the amplitude of $I_{Ca,L}$ elicited by each pulse in the presence of propafenone was expressed as a proportion of control $I_{Ca,L}$ for each pulse in the series (Figure 4a). The mean current with the first pulse after propafenone exposure was $0.72 \pm 0.04$ of the control amplitude, and the relative amplitude declined progressively with further stimulation until $I_{Ca,L}$ was near $0.52 \pm 0.02$ of control after 13 pulses (it did not decline significantly after this: $P > 0.8$ between pulse 13 and 16). The histogram in Figure 4b shows mean relative $I_{Ca,L}$ for the first and sixteenth pulse in this protocol. The re-

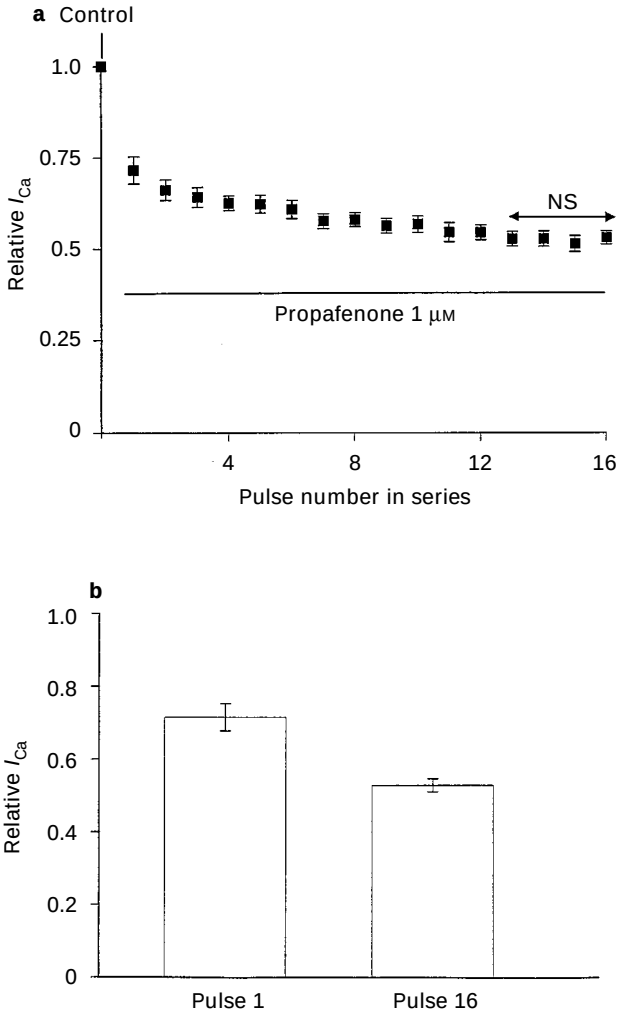



**Figure 4** Tonic- and use-dependent block of $I_{Ca,L}$ by propafenone. Protocol described in Results section. (a) Plot of mean $I_{Ca,L}$ amplitude (normalized to control amplitude in normal Tyrode solution) during a train of pulses applied after 3 min exposure to 1 $\mu$M propafenone (during which no pulses were applied). Each point shows mean data from 7 cells. $I_{Ca,L}$ amplitude was significantly reduced with the first pulse on resumption of stimulation, and declined further over 13 pulses, after which there was no significant further reduction. (b) Histogram shows relative current activated by pulse 1 and pulse 16 on resumption of stimulation (significance levels given in text).

lative $I_{Ca,L}$ on the first pulse in propafenone was significantly reduced compared to control ($P<0.002$) and there was a significant difference between the relative $I_{Ca,L}$ on the first and sixteenth pulses ($P<0.01$). In order to determine whether some of the changes in $I_{Ca,L}$ could be due to current 'run-down', we applied the same test pulse protocol to three cells which were not exposed to propafenone. The relative current after 3 min was $0.89\pm0.05$ of control ($P<0.05$ compared to propafenone-treated cells). There was no significant further decline over a further 20 pulses ($P>0.9$), and these results indicate that the changes to $I_{Ca,L}$ with propafenone exposure cannot simply be explained on the basis of current run-down, alone. The data suggest (a) that propafenone showed some affinity for binding to the resting state of the channel for $I_{Ca,L}$ and, therefore, showed tonic blocking properties and (b) that inhibition of $I_{Ca,L}$ by propafenone also exhibited use-dependence. In 5 propafenone-treated cells we increased the stimulation rate from 0.33 Hz to 1 Hz, once $I_{Ca,L}$ had declined to a steady level in the presence of propafenone at the lower stimulation rate. We observed a further decline in relative $I_{Ca,L}$ amplitude to 0.37 of control amplitude ($\pm0.04$), which was significantly greater than the level of inhibition at 0.33 Hz ($P<0.002$). This observation suggests that propafenone showed frequency as well as use-dependent blocking properties under these experimental conditions.

### *Effects of propafenone on inactivation properties of* $I_{Ca,L}$

To determine whether propafenone affected steady state inactivation of $I_{Ca,L}$, inactivation data were obtained in normal Tyrode solution and in the presence of propafenone by use of a protocol similar to that used by Hancox and Levi (1994). The voltage protocol is depicted in a schematic form in Figure 5a (inset). A 1 second pulse was applied from $-40$ mV to various conditioning potentials between $-80$ and $+30$ mV and this conditioning pulse was followed by a 250 ms test pulse to $+10$ mV. Consecutive pairs of pulses were separated by an interval of 20 s. For each cell, $I_{Ca,L}$ amplitude activated by the test pulse to $+10$ mV following each conditioning pulse was measured, and expressed for each cell as a proportion of the maximal $I_{Ca,L}$ recorded from the cell with this protocol. To remove any contamination by $I_{Na}$ or T-type $I_{Ca}$ which might be activated following a pre-pulse to a negative membrane potential, Na-free, nickel-containing Tyrode solution was used for these experiments (see Methods). Inactivation variables for $I_{Ca,L}$ in this Tyrode solution and after addition of 5 $\mu$M propafenone were obtained and fitted by a Boltzmann charge distribution:

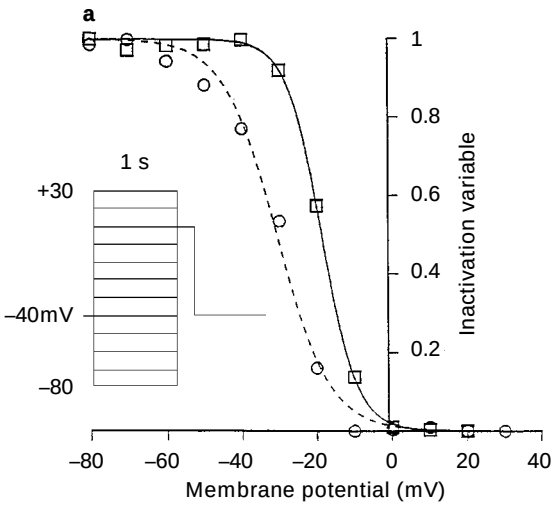
$$\text{Inactivation variable} = 1-(1/\{1+\exp[(V_{0.5}-V_m)/k]\}) \quad (2)$$

where $V_m$ has the meaning in equation (1), $V_{0.5}$ is the potential at which $I_{Ca,L}$ was half maximally inactivated and $k$ was the slope factor of the inactivation curve. Figure 5a shows data for a single AVN myocyte. The inactivation relation was shifted to the left along the voltage axis following exposure to propafenone, indicating that for a given value of $V_m$ the degree of current inactivation was larger in the presence of propafenone. Similar results were observed in each of five cells. The $V_{0.5}$ in control solution was $-18.76\pm1.79$ mV and that in propafenone-containing solution was $-26.82\pm1.97$ mV ($P<0.02$). There were individual variations in $k$ from cell-to-cell, but the difference in this parameter between control ($4.76\pm0.52$ mV) and propafenone-containing ($6.5\pm0.58$ mV) solution ($P>0.05$) was not significant.

We also investigated the effect of propafenone on the recovery of $I_{Ca,L}$ from inactivation (also known as restitution of current). The protocol for this experiment is shown schematically in the inset of Figure 5b. A 1 s pre-pulse was applied from $-40$ mV to $+10$ mV in order to inactivate $I_{Ca,L}$, followed by a second 300 ms test-pulse applied at various intervals ($\Delta t$) between 100 ms and 6 s after the first pulse. Consecutive pairs of pulses were separated by an interval of 20 s. The effect of propafenone on the recovery of $I_{Ca,L}$ from inactivation is shown in Figure 5b (main figure, each point showing mean data from 7 cells). $I_{Ca,L}$ restitution was fitted by a double-exponential function in both experimental conditions, with fast ($\tau_f$) and slow ($\tau_s$) time-constants as follows: control; $\tau_f=183.3\pm7.4$ ms and $\tau_s=1324\pm51$ ms; propafenone $\tau_f=304\pm30.1$ ms and $\tau_s=2911\pm263$ ms. The changes to both fast and slow time constants following propafenone exposure were significant ($P<0.005$ in each case). Thus, in addition to changing the voltage-dependence of inactivation of $I_{Ca,L}$, propafenone also slowed down the recovery of this current from inactivation.

## Discussion

In this study, we have shown that propafenone inhibits $I_{Ca,L}$ in single rabbit AVN cells. Despite the clinical importance of this drug, there appear to be few studies which have examined the



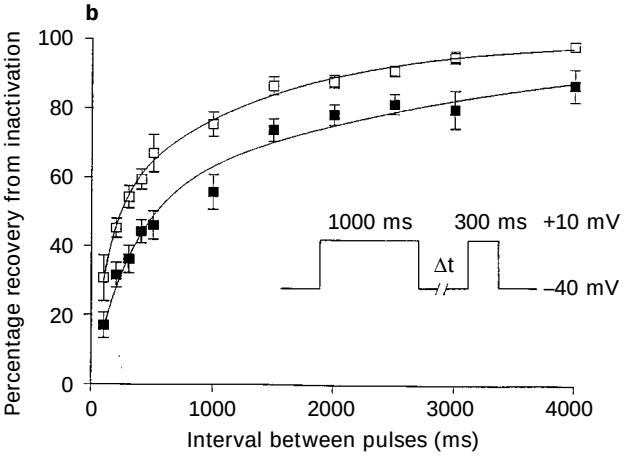




**Figure 5** (a) Steady state $I_{Ca,L}$ inactivation relation for an AVN cell in the two experimental conditions, (□) control, (○) propafenone. In each of five such cells the inactivation curve was shifted along the voltage axis in the negative direction. The inset shows the voltage protocol used: 1 s pre-pulses to different potentials were followed by a 250 ms test pulse to $+10$ mV. (b) Recovery of $I_{Ca,L}$ from inactivation in normal Tyrode solution (□) and in propafenone containing solution (■). Inset shows in a schematic form the protocol used. For each $\Delta t$ the amplitude of $I_{Ca,L}$ activated by the second pulse was expressed as a percentage of that activated by the first. Fast and slow time constants of recovery from inactivation under both conditions are given in Results section. Data-points represent mean data from 7 cells.

effect of propafenone on $I_{Ca,L}$ (Satoh & Hashimoto, 1984; Fei *et al*., 1993; Delgado *et al*., 1993) and the present study is the first examining directly the effects of this agent on ionic currents in the AVN. Satoh and Hashimoto (1984) showed effects of propafenone on $I_{Ca}$ in rabbit sinoatrial nodal (SAN) tissue and our results obtained with a K-based internal dialysis solution are in broad agreement with this. However, Satoh and Hashimoto (1984) obtained significant inhibition of $I_{Ca,L}$ only at concentrations of propafenone greater than $10^{-5}$ M, which differs from the present study, in which we observed $I_{Ca,L}$ inhibition in the nM range, with an $IC_{50}$ of 1.7 $\mu$M. One possible explanation for the apparent difference between the two studies is that Satoh and Hashimoto used a small multi-cellular preparation from the sinoatrial node (SAN), whilst we used a single AVN cell preparation. Multicellular cardiac muscle preparations confer advantages for certain kinds of investigation, but are less ideal for precise pharmacological measurements because it can be difficult to ensure equal access of drugs to all cells within the preparation (Noble, 1984). This might account for the apparently lower sensitivity of $I_{Ca,L}$ to propafenone in the study of Satoh and Hashimoto (1984), although genuine differences in propafenone sensitivity between the SAN and AVN cannot be ruled out.

### *Comparison with previous single cell studies*

Useful comparisons with the present study come from the two previous investigations of propafenone on $I_{Ca,L}$ with single cells (Fei *et al*., 1993; Delgado *et al*., 1993). Both these studies examined the action of propafenone on single guinea-pig ventricular cells and showed half-maximal block of $I_{Ca,L}$ in the $\mu$M range (1.5 $\mu$M: Fei *et al*., 1993; 5 $\mu$M: Delgado *et al*., 1993), which agrees well with our results on rabbit AVN cells. Another area of close agreement between these findings and our own is that all three investigations obtained $I_{Ca,L}$ inhibition without a shift in the *I*-V relation or activation curve for this current. Thus, it seems that propafenone may decrease $I_{Ca,L}$ conductance without significantly affecting voltage-dependent activation. The $I_{Ca,L}$ inhibition described in our study occurred more rapidly than that obtained in the earlier two studies. However, with our method of solution-change we were able to apply solutions to a myocyte very rapidly (Levi *et al*., 1996) and our experiments were performed at 35–37°C rather than at room temperature. These factors might be significant in determining the rapidity of observed effects. There is less agreement between the data of the two previous studies (despite being performed on the same cell type from the same species) in relation to effects of propafenone on the voltage-dependence of $I_{Ca,L}$ inactivation or with respect to tonic-, use- and frequency-dependent block. Fei *et al*. (1993) showed that $I_{Ca,L}$ block by propafenone was 'channel state-, use- or frequency-independent', whilst Delgado *et al*. (1993) found evidence for use- and frequency-dependent block, but observed relatively little tonic blockade. We cannot explain these fundamental differences between two studies on ventricular myocytes from the same species, but our own results on rabbit AVN cells are consistent with the findings of Delgado *et al*. (1993) in terms of observing use- and frequency-dependent blockade. In addition, we also saw a significant tonic blockade. Together, these results suggest affinity of propafenone for both the activated and inactivated states of the Ca channel in the AVN. Propafenone and other class I antiarrhythmic agents have been shown to exert effects on the inactivated and activated states of the Na-channel, showing both tonic- and use-dependent blocking actions (e.g. Campbell, 1983; Clarkson & Hondeghem, 1985). Whilst it is possible that there are cell type-specific, or species-specific effects of propafenone on $I_{Ca,L}$, in rabbit AVN cells at least the mechanism of propafenone blockade of $I_{Ca,L}$ seems to share similarities with its actions on $I_{Na}$.

Whilst we saw little evidence for a shift in the voltage-dependence of $I_{Ca,L}$ activation with propafenone, we did observe a $-8$ mV shift in the $V_{0.5}$ of the steady state inactivation relation. Fei *et al*. (1993) saw little shift in the steady-state inactivation curve for $I_{Ca,L}$ but Delgado *et al*. (1993) obtained a $-7$ mV shift in the $V_{0.5}$ value, which is close to that observed for AVN cells. Such a displacement of the inactivation curve indicates an affinity of propafenone for the inactivated state of the Ca channel and on the basis of their data Delgado *et al*. (1993) calculated a dissociation constant ($K_i$) for binding to the inactivated Ca channel of $5 \times 10^{-7}$ M. By use of the method described by Delgado *et al*. (1993), our data suggest a $K_i$ of between 3 and $5 \times 10^{-7}$ M in the AVN.

### *Implications of this study*

It is of interest to relate the effects of propafenone on $I_{Ca,L}$ to those on spontaneous action potentials recorded under current clamp. Previous data from this laboratory suggest that $I_{Ca,L}$ is very important in generating the action potential upstroke in AVN cells (Hancox & Levi, 1994). Action potential upstroke velocity is contingent upon inward current flux and decreased $I_{Ca,L}$ following exposure to propafenone might be predicted to slow action potential upstroke rate. Our action potential data are consistent with this prediction. Slowed recovery of $I_{Ca,L}$ from inactivation might also have contributed to an increased cycle length between consecutive action potentials. Other effects, such as increased action potential duration and a depolarization of the MDP probably involve additional effects of propafenone on other membrane currents. Delayed rectifier K current ($I_K$) in both atrial and ventricular myocytes has been shown to be inhibited by propafenone (Duan *et al*., 1993; Delpon *et al*., 1995), and an action on this current would be consistent with both increased APD and a more depolarized MDP (although $I_K$ block in AVN cells remains to be determined experimentally). In the *in vivo* situation, pacemaking properties of the AVN are normally suppressed (since the dominant pacemaker is normally the SAN) and the conduction of activity through the AVN is of particular importance. Slowed action potential upstrokes and slowed action potential repolarization following propafenone exposure might be expected to slow AVN conduction and prolong refractoriness.

The increase in $I_{Ca,L}$ block we observed by raising the pulse frequency from 0.33 Hz to 1 Hz can be explained by the slower recovery of $I_{Ca,L}$ from inactivation in the presence of propafenone. Although the precise degree to which $I_{Ca,L}$ restitution was slowed by propafenone differs between studies, our results and those obtained for guinea-pig ventricular cells (Fei *et al*., 1993; Delgado *et al*., 1993) are in qualitative agreement regarding this effect. At higher stimulation frequencies, less time would be available for the recovery of $I_{Ca,L}$ between pulses, whilst greater time would be required for a given level of restitution in the presence of propafenone. Such an effect might be of relevance to the clinical situation during supraventricular or AVN tachycardias: at faster stimulation rates of AVN cells, the $I_{Ca,L}$ blocking effects of propafenone might be expected to increase, and this would be anticipated to decrease impulse transmission to the ventricles.

It is important to note that the $IC_{50}$ value for $I_{Ca,L}$ block by propafenone in the present study lies within the therapeutic concentration range (1–5 $\mu$M; Keller *et al*., 1978; Karaguezian *et al*., 1983) and, therefore, it is likely that $I_{Ca,L}$ block does mediate some of the clinical actions of this drug, perhaps especially so in the AVN. The action potential upstroke differs in its velocity in different regions of the AVN (e.g. Akiyama & Fozzard, 1979; Kokubun *et al*., 1980), but evidence from single cell, multicellular and whole heart studies suggests that in a significant proportion of AVN cells it is primarily dependent on $I_{Ca,L}$ (Zipes & Fischer, 1974; Kokubun *et al*., 1982; Hancox & Levi, 1994). Indeed, a recent study on single AVN cells (Munk *et al*., 1996) indicates that cells isolated from the mid-nodal region exhibit very slow action potential upstroke rates, and in this region $I_{Ca,L}$ may be almost exclusively responsible

for carrying the action potential upstroke. Therefore, actions of propafenone on $I_{Ca,L}$ are likely to be more important than actions on $I_{Na}$ when considering anti-arrhythmic effects of this drug on the AVN. In discussing the results of their study, Delgado *et al*. (1993) conjectured 'since $I_{Ca}$ plays a major role in both conduction and automaticity of the AV 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 AV nodal re-entrant tachycardia (Funck-Brentano *et al*., 1990)'. The findings of the present study support this speculation with the direct evidence that therapeutic concentrations of propafenone do block $I_{Ca,L}$ in the AVN in a fashion consistent with the known clinical effects of this agent. Indeed results from this, and other recent studies on the AVN (Hancox & Levi, 1996; Hancox, 1997) suggest that $I_{Ca,L}$ blockade in this region may be an important mechanism of anti-arrhythmic action of classes of drug which are not primarily considered Ca-channel antagonists.

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