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Effects of propafenone on calcium currents in single ventricular myocytes of guinea-pig

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1 The effects of propafenone on the inward calcium current (I_{Ca}) were investigated in isolated single ventricular myocytes of the guinea-pig by the whole-cell clamp method. Propafenone inhibited I_{Ca} in a dose-dependent manner at concentrations of propafenone ranging from 1×10^{-8} to 1×10^{-3} M and half maximal block of I_{Ca} occurred at a propafenone concentration of 1.5×10^{-6} M. Propafenone did not change the current-voltage relation of I_{Ca} other than a reduction in amplitude and showed no clear use- or frequency-dependent effects upon I_{Ca} (stimulation frequencies from 0.03 to 2 Hz). Propafenone did not alter the steady-state inactivation process: the half maximal activation potentials were 18.5 ± 2.2 mV in the control state and 20.9 ± 5.0 mV in the presence of 1×10^{-6} M propafenone ($n = 12$, NS). Propafenone (1×10^{-6} M) increased the half-time of reactivation by 73.9% ($n = 6$, 212.3 ± 1.2 ms vs 369.2 ± 1.5 ms, $P < 0.05$).

2 We conclude that propafenone blocks I_{Ca} in a concentration-dependent and a channel state-, use- or frequency-independent manner. The I_{Ca} blockade elicited by propafenone at clinically therapeutic plasma concentration is significant and may be involved in its anti-arrhythmic effects.

Keywords: Propafenone; whole-cell clamp technique; calcium currents; cardiac myocytes; drug actions

Introduction

Calcium influx into the cardiac cells through voltage-dependent calcium channels is important in the initiation of both mechanical and electrical events (Hess, 1990). In well polarized tissues, the principal action of Class Ic anti-arrhythmic agents is the reduction of impulse propagation by inhibition of the fast inward sodium current (I_{Na}). However, in depolarized tissues, the availability of the fast sodium channel is reduced, and at levels more positive than − 65 mV during ischaemia, premature beats and membrane potential oscillations relate to the inward calcium current (I_{Ca}) and the ascending phase of the action potential is markedly dependent on I_{Ca} (Sperelakis, 1984). It has been demonstrated that frog atrial and mammalian ventricular muscles develop slow oscillatory potentials in response to a depolarizing current which are strongly dependent on the external Ca^{2+} concentration and are antagonized by verapamil and quinidine (Ducouret, 1976; Grant & Katzung, 1976). Since the intracellular Ca^{2+} fluctuations appear to be responsible for the generation of arrhythmias directly and/or indirectly, the modulation of I_{Ca} by anti-arrhythmic drugs has become a matter of increasing interest (Sperelakis, 1984).

Propafenone is an effective anti-arrhythmic agent for the treatment of supraventricular and ventricular arrhythmias (Hammill *et al.*, 1987; Jonason *et al.*, 1988). It is a class I antiarrhythmic drug because of its potent effects upon the fast inward sodium currents (I_{Na}) with reductions of the maximal depolarization of the action potential (V_{max}) (Delgado *et al.*, 1985; Malfatto *et al.*, 1988). This block of I_{Na} demonstrates strong rate-dependent and voltage-dependent properties (Honjo *et al.*, 1989). There is indirect evidence (Ledda *et al.*, 1981; Dukes & Vaughn-Williams, 1984; Delgado *et al.*, 1985) that propafenone has an inhibitory effect on I_{Ca} and this may have an important role in its electrophysiological actions. However, the effects of propafenone on I_{Ca} in single cardiac myocytes remain to be adequately defined. We have therefore examined the effects of propafenone on I_{Ca} and the mechanisms of its calcium channel blockade in single ventricular myocytes of the guinea-pig using the whole-cell clamp technique.

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Methods

Cell isolation

Isolated ventricular myocytes of the guinea-pig were prepared by a modification of the method of Trautwein & Kameyama (1987). Female guinea-pigs (weight 300–400 g) were killed by cervical dislocation (Schedule 1, Home Office Regulations). The heart was removed rapidly and transferred into a cold 'low calcium solution' of the following composition (mM): NaCl 120, KCl 5.4, $MgSO_4$ 5, pyruvate 5, glucose 20, taurine 20, HEPES 10; pH 6.96 with NaOH. The aorta was cannulated and the heart was retrogradely perfused on a Langendorff apparatus with the 'low calcium solution' at 37°C for approximately 4 min, then with protease solution (1.6 units ml^{-1}, Sigman type XXIV) for approximately 2 min. Collagenase (42.75 units ml^{-1}, Worthington class II) and hyaluronidase solutions (136 units ml^{-1}, Sigma) were then perfused for 2.5 min. The enzymatic solutions had the same composition as the low calcium solution but were adjusted with 100 mM $CaCl_2$ to give a free Ca^{2+} level of 200 μM. Finally, the heart was washed with a 'KB solution' (mM: glutamic acid 50, KOH 85, KCl 30, KH_2PO_4 30, $MgSO_4$ 3, taurine 20, EGTA 0.5, HEPES 10, glucose 10; pH 7.4 with KOH) for 1.5 min. The ventricles were cut into fresh KB solution, chopped and triturated briefly to release single myocytes. The KB solution containing single cells was filtered through a coarse mesh, centrifuged at 1000 r.p.m. for 1 min, and the pellet of cells resuspended in fresh KB solution and stored at room temperature for 30 min. The cells were then washed with 'recording solution' (mM: NaCl 112, $NaHCO_3$ 24, NaH_2PO_4 1, KCL 5.4, $CaCl_2$ 1, $MgCl_2$ 1, HEPES 5, glucose 10; pH 7.4 with NaOH) prior to transfer to the bath for studies.

Propafenone hydrochloride (Sigma Chemicals, Dorset) was dissolved in distilled water as 10^{-3} or 10^{-4} M solutions and then diluted to the different concentrations required in the recording solution immediately prior to use.

Electrophysiological experiments

Calcium currents were studied in these isolated myocytes by the whole-cell voltage clamp technique (Hamill *et al.*, 1981). The cell suspension was placed in a bath (10 × 25 × 4 mm)

which was constantly perfused with oxygenated recording solution with 95% O_2 and 5% CO_2 on the stage of an inverted microscope (STC-B1, Correct, Tokyo, Japan) at room temperature (19–25°C). Patch electrodes (1–4 MΩ) were pulled from glass micropipettes (GC150TF-10, 1.5 mm o.d.; 1.17 mm i.d., Clark Electromedical Instruments, England) on a micropipette puller (PB-7, Narishige Co, Ltd, Japan) and fire-polished. The electrodes were filled with the following internal solution (mM): CsCl 130, HEPES 15, $MgCl_2$ 1, Na_2ATP 2, EGTA 1; pH 7.2 with CsOH. After junction potential nulling, the electrodes were positioned onto the cells using a hydraulic micromanipulator (MO-203, Narishige Co, Ltd, Japan). Following the formation of a giga-ohm seal, the patch was disrupted by a suction to get the whole-cell clamp configuration, and the capacitance and series resistances were compensated.

Voltage-clamp protocols were applied to the myocytes through a patch clamp amplifier (RK 300, Biologic, France) from a programmable digital stimulus generator (Active 2, Intracel, England). The myocytes were clamped at a holding potential of − 80 mV and I_{Ca} was elicited by a 300 ms depolarization from − 40 mV to 0 mV at 0.18 Hz. Potassium and sodium currents were suppressed by intracellular caesium and a 100 ms pre-pulse from a holding potential of − 80 mV to − 40 mV, respectively.

Data were sampled at 5000 Hz by an analog-to-digital converter and saved on the hard disk of a microcomputer (Tandon PCA-sl) for off line analysis (Active 2, Intracel, England). Peak I_{Ca} was calculated as the difference between peak inward current and late current at the end of a 300 ms depolarization. All data were expressed as mean ± s.d. Paired or unpaired t test and linear regression analysis were used when appropriate. A P value less than 0.05 was considered to be statistically significant.

Results

The effects of propafenone on I_{Ca}

In the whole-cell configuration, 1 to 2 min was allowed to complete the exchange between intrapipette solution and intracellular liquid of the myocytes. The I_{Ca} which was stable at a stimualtion frequency of 0.18 Hz while the cell was being perfused with oxygenated recording solution was taken as the control value. I_{Ca} did not run-down for at least 30 min in these myocytes. After the measurements of I_{Ca} in the control condition, the propafenone solutions of concentrations varying from 10^{-8} M to 10^{-3} M were perfused at a speed of 1.5 ml min^{-1} while I_{Ca} was monitoring by use of the same depolarization protocol as above at a frequency of 0.03 Hz. The magnitude of the drug effect was defined as the differences between the amplitudes of I_{Ca} in the control state and in the presence of drug once I_{Ca} has reduced to a stable level and this was expressed as the percentage of its control value. About 15 (range 10 to 20) min were required to obtain a steady state of the inhibitory effect of propafenone on I_{Ca}, which could be reversed by washing with the recording solution (Figure 1a). In order to examine the frequency-dependency of the effect of propafenone on the I_{Ca}, cells were subject to depolarization frequencies varying from 0.03 to 2 Hz in the presence of the different concentrations of propafenone. Propafenone at the concentration from 1×10^{-7} to 10^{-3} M had no clear use- or frequency-dependent effects upon I_{Ca} within the depolarization frequency range of 0.3 to 2 Hz (Figure 1b).

Dose-response curve of propafenone on I_{Ca}

The dose-response curve of the effect of propafenone on I_{Ca} was measured at 10 different concentrations from 1×10^{-8} to 1×10^{-3} M, each of which was repeated in 3 to 11 (mean 5) cells from 2 to 4 hearts (Figure 1c). I_{Ca} was inhibited in a

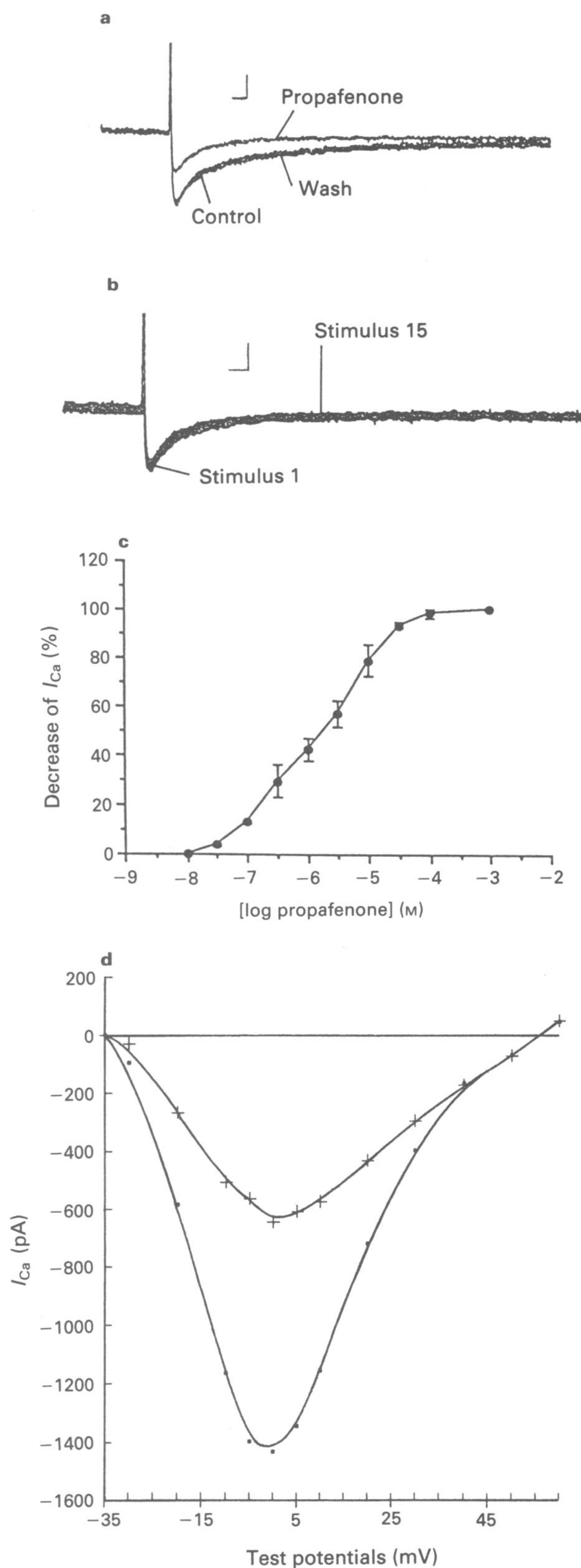

Figure 1 (a) The effect of propafenone (1×10^{-6} M) on I_{Ca}. This was reversed by washing with the recording solution. Scale bar: 200 pA (vertically) and 10 ms (horizontally). (b) Propafenone showed no distinct use-dependent blockade of I_{Ca}. The trace represents a stack of I_{Ca} elicited by 15 stimulations at 2 Hz in the presence of 1×10^{-6} M propafenone. Scale bar: 100 pA (vertically) and 10 ms (horizontally). (c) The dose-response curve of propafenone on I_{Ca}. Each dose level represents experiments of 3 to 5 cells (mean = 5) from 2 to 4 hearts. The half-maximal blockade of I_{Ca} occured at 1.5×10^{-6} M. (d) The current-voltage relationship of propafenone on I_{Ca} in the control state (●) and in the presence of 1×10^{-5} M propafenone (+).

dose-dependent manner at propafenone concentrations above 1×10^{-7} M with a near maximal blockade at the concentration of 1×10^{-4} M. Half maximal block of I_{Ca} occured at 1.5×10^{-6} M. In these experiments, one cell was used once for only one concentration of propafenone because of the duration of the experiments.

The effect of verapamil on I_{Ca}

Verapamil (1×10^{-6} M) showed use-dependent as well as tonic or rest inhibition of I_{Ca} as previously reported (Lee & Tsien, 1983). Tonic or rest block was defined by the first depolarization of a train of 15 stimulations. The further decrease in I_{Ca} on the last of the depolarizations gave the contribution of use-dependent block. Fifteen pulses were enough to produce steady state blockade of I_{Ca} (Uehara & Hume, 1985). Propafenone and verapamil at concentration of 1×10^{-6} M blocked I_{Ca} in the same experimental conditions by $42.1 \pm 4.7\%$ ($n = 11$) and $48.6 \pm 7.7\%$ ($n = 8$ including tonic and use-dependent block at 0.18 Hz), respectively ($P < 0.05$). In 5 cells propafenone (1×10^{-6} M) was applied after the application of verapamil (1×10^{-6} M). There was no increase in the degree of block of I_{Ca} in this situation ($46.2 \pm 8.2\%$ with verapamil; 48.8 ± 8.9 after further addition of propafenone).

The effect of propafenone on the current-voltage relationship and inactivation of I_{Ca}

Propafenone reduced the amplitude of I_{Ca}, but did not change the shape of the current-voltage relation and did not shift the position of its peak, which was 0 mV (Figure 1d). Steady-state inactivation of I_{Ca} was measured by a double-pulse protocol shown in Figure 2. A 300 ms pre-pulse from − 40 mV to various potentials was followed by a 300 ms test-pulse to 0 mV separated by a 3 ms return to − 40 mV. The amplitude of I_{Ca} evoked by the test pulse was expressed as the percentage of the I_{Ca} evoked by a step from − 40 mV to 0 mV in the absence of a pre-pulse. Propafenone did not significantly alter the steady-state inactivation process (Figure 2a). The half maximal inactivation potentials were 18.5 ± 2.2 mV in the control and 20.9 ± 5.0 mV in the presence of 1 ± 10^{-6} M propafenone ($n = 12$, NS).

The effect of propafenone on the reactivation of I_{Ca}

We examined the effect of propafenone on the reactivation process of I_{Ca} in order to obtain more information about its use-dependent effect on I_{Ca}. Reactivation of I_{Ca} was measured by a double-pulse protocol with intervals between the pre-pulse and the test pulse varying from 32 to 4096 ms and expressed as the percentage of I_{Ca} elicited by the pre-pulse (Figure 2b). The half-times of reactivation ($t_{1/2}$, the time at which 50% of I_{Ca} had recovered) were 212.3 ± 1.2 ms in the control and 369.2 ± 1.5 ms in the presence of 1×10^{-6} M propafenone ($n = 6$, $P < 0.05$), an increase in the $t_{1/2}$ of 73.9%.

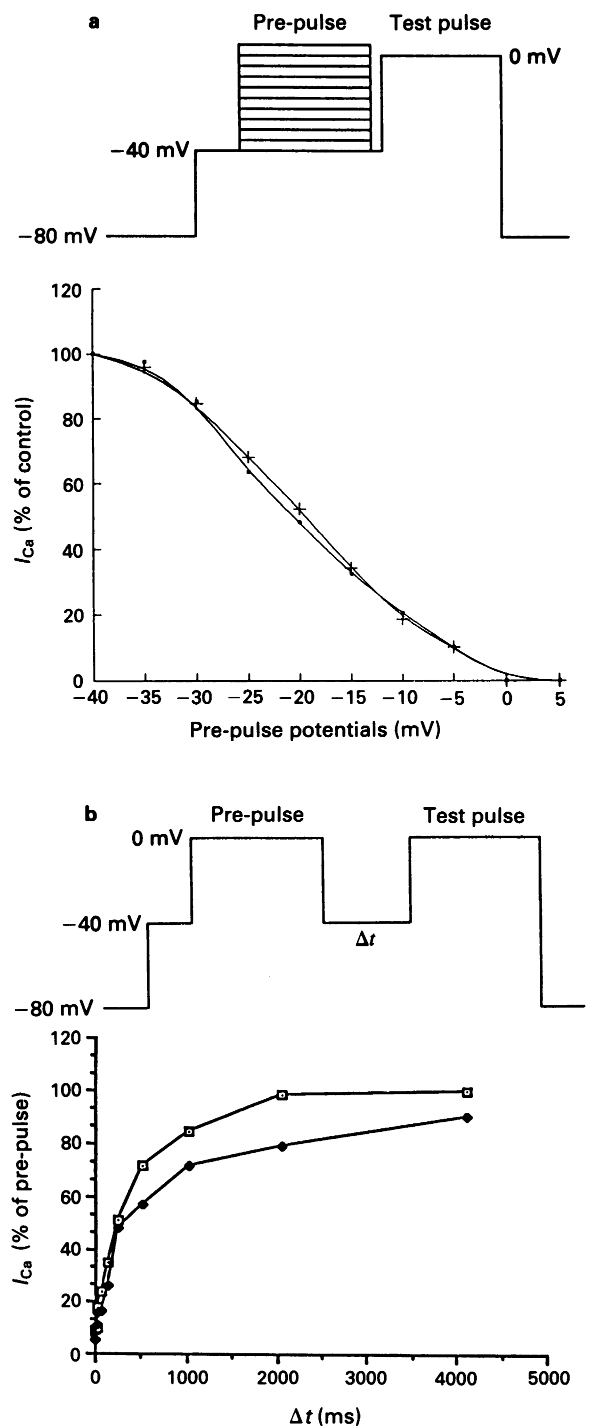

Figure 2 (a) The steady-state inactivation curve of I_{Ca}. Inactivation was measured by a double-pulse protocol. I_{Ca} was plotted as a percentage of the I_{Ca} elicited during a pulse from − 40 mV to 0 mV in the absence of a pre-pulse. Propafenone did not alter the steady-state inactivation curve of I_{Ca}. (●) Control; (+) propafenone. (b) The effect of propafenone on reactivation of I_{Ca}. Reactivation was measured by a double-pulse protocol. Both pre- and test-pulse were 300 ms. I_{Ca} was plotted as the percentage of I_{Ca} evoked by the pre-pulse. (□) Control; (◆) propafenone.

Discussion

We have demonstrated in the present study that propafenone is a potent blocker of I_{Ca}. The I_{Ca} in this study can be assumed to be entirely the L-type current because T-type current was inactivated by the pre-pulse from − 80 mV to − 40 mV (Nilius *et al.*, 1985). The L-type I_{Ca} is the main inward current contributing to the maintenance of the action potential plateau, determining the rise rate of the slow action potentials and supplying the Ca^{2+} required for normal excitation-contraction coupling (Noble, 1984). Propafenone inhibits the I_{Ca} with a relatively slow kinetics. Its inhibitory effects on I_{Ca} reached the maximal level after being perfused for a relatively long time (approximately 15 min). This was consistent with the results reported by Campbell (1983) demonstrating that class Ic anti-arrhythmic agents had a 'slow' kinetics of rate-dependent effects on V_{max} of guinea-pig ventricular action potentials. There is also evidence that propafenone needs a long wash-out period for reversal of its electrophysiological effects (Schlepper, 1987), consistent with

our results and its slow kinetics of action (Rhodes *et al.*, 1985). Two distinct models have been postulated for the possible mechanisms for drugs to reach their acting sites (Rhodes *et al.*, 1985): the 'aqueous approach', the drug reaches the receptor by diffusion through the bulk solvent, and the 'membrane approach', the drug partitions into the membrane bilayer and then diffuses laterally to a specific receptor site. Whether the slow kinetics of its action and wash-out is attributed to its slow receptor kinetics or to another mechanism remains to be investigated. It is also not clear whether the binding site of the drugs is the calcium channel itself or an associated subunit for an adjacent receptor complex but our data suggest that propafenone may act at a site similar to verapamil.

Scamps *et al.* (1989) reported that the positive staircase effects of stimulation frequency on I_{Ca} were suppressed by quinidine and flecainide in frog cardiac cells. We confirmed that propafenone shared the same effect as quinidine and flecaininde in guinea-pig ventricular myocytes. We noted that the effect of propafenone on I_{Ca} was roughly parallel to its effect on the I_{Na} evoked by the pre-pulse. This suggests that both the effects of propafenone upon I_{Na} and I_{Ca} occur at concentrations within the clinically therapeutic concentration-range of propafenone.

Half maximal block of I_{Ca} occurred at 1.5×10^{-6} M (566.9 ng ml^{-1}), which is within the clinically therapeutic plasma concentration range of propafenone (300–1000 ng ml^{-1}) (Schlepper, 1987). We were surprised to find that 1×10^{-6} M propafenone achieved 87% of the blockage of I_{Ca} elicited by 1×10^{-6} M verapamil, suggesting the potent inhibitory effects of propafenone upon the calcium channel. Scamps *et al.* (1989) studied the dose-response curves of flecainide and quinidine on I_{Ca} in single frog myocytes. Comparing our results with the concentration of drugs required for half maximal blockade of I_{Ca} in their studies, propafenone was over 13 times and 7 times more potent in blockade of I_{Ca} than flecainide and quinidine, respectively. Although the differences in the results may be due to differences in the species of animal and the experimental conditions, these results suggest that the effects of propafenone on I_{Ca} at clinically therapeutic plasma levels are significant and may be involved in its antiarrhythmic effects directly or indirectly by changing the membrane calcium influx.

Propafenone did not show clear use-dependent inhibition of I_{Ca} at the concentrations used in this study. Propafenone did not change the shape of the current-voltage relationship or shift its peak. This suggests that the effects of propafenone on I_{Ca} are independent of the channel-state. Two models have been proposed to explain the effects of anti-arrhythmic agents on I_{Ca} the modulated receptor hypothesis, which was originally developed for local anaesthetic agents interaction with the sodium channel (Hondeghem & Katzung, 1984) and the guarded receptor hypothesis (Starmer & Grant, 1985). Propafenone and other class I drugs are known to block sodium current both in tonic and use-dependent ways, binding to the sodium channel during activated and inactivated states (Campbell, 1983; McLeod *et al.*, 1984; Clarkson & Hondeghem, 1985). In the present study, propafenone reduced I_{Ca} without changing the shape of the current-voltage relationship. This is similar to the block of I_{Ca} by flecainide, another class I anti-arrhythmic agent (Scamps *et al.*, 1989). The concentration of flecainide for the half maximal block of I_{Ca} was 20 μM and the $t_{1/2}$ increased by $71.3 \pm 12.8\%$ in the presence of 30 μM flecainide in their experiments. Our results are comparable: $t_{1/2}$ was increased by 73.9% in the presence of 1 μM propafenone. The mechanism of the blockade by propafenone upon I_{Ca} (channel state-independent) appears to differ from the inhibition of I_{Na} (channel state-dependent).

We conclude that propafenone blocks I_{Ca} in a concentration-dependent and in a channel state-, and use- or frequency-independent manner. The I_{Ca} blockade by propafenone at clinically therapeutic plasma concentrations is significant and may be involved in its anti-arrhythmic effects and its negative inotropic actions on the myocardium.

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References

CAMPBELL, T.J. (1983). Kinetics of onset of rate-dependent effects of class I antiarrhythmic drugs are important in determining their effects on refractoriness in guinea-pig ventricle, and provide a theoretical basis for their subclassification. *Cardiovasc. Res.*, **17**, 344–352.

CLARKSON, C.W. & HONDEGHEM, L.M. (1985). Evidence for a specific receptor site for lidocaine, quinidine, and bupivacaine associated with cardiac sodium channels in guinea pig ventricular myocardium. *Circ. Res.*, **56**, 496–506.

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.

DUCOURET, P. (1976). The effect of quinidine on membrane electrical activity in frog auricular fibres studied by current and voltage activity clamp. *Br. J. Pharmacol.*, **57**, 163–184.

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

GRANT, A.O. & KATZUNG, B.G. (1976). The effects of quinidine and verapamil on electrically induced automaticity in the ventricular myocardium of guinea pig. *J. Pharmacol. Exp. Ther.*, **196**, 407–419.

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.

HAMMILL, S.C., MCLARAN, C.J., WOOD, D.L., OSBORN, M.J., GERSH, B.J. & HOLMES, D.R. Jr. (1987). Double-bind study of intravenous propafenone for paroxysmal supraventricular reentrant tachycardia. *J.A.C.C.*, **9**, 364–368.

HESS, P. (1990). Cardiac calcium channels, In *Cardiac Electrophysiology, from Cell to Bedside*. ed. Zipes D.P. & Jalife J. pp. 10–7. Philadelphia: W B Saunders Company.

HONDEGHEM, L.M. & KATZUNG, B.G. (1984). Antiarrhythmic agents: the modulated receptor mechanism of action of sodium and calcium channel-blocking drugs. *Annu. Rev. Pharmacol. Toxicol.*, **24**, 387–423.

HONJO, H., WATANABE, T., KAMIYA, K., KODAMA, I. & TOYAMA, J. (1989). Effects of propafenone on electrical and mechanical activities of single ventricular myocytes isolated from guinea-pig hearts. *Br. J. Pharmacol.*, **97**, 731–738.

JONASON, T., RINGQVIST, I., BANDH, S., NILSSON, G., NILSSON, H., LIDELL, C., BJERLE, P. & OLOFSSON, B.-O. (1988). Propafenone vs disopyramide for treatment of chronic symptomatic ventricular arrhythmias: a multicentre study. *Acta. Med. Scand.*, **223**, 515–523.

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

LEE, K.S. & TSIEN, R.W. (1983). Mechanism of calcium channel blockade by verapamil, D600, diltiazem and nitrendipine in single dialysed heart cells. *Nature*, **302**, 790–794.

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.

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.

NILIUS, B., HESS, P., LANSMAN, J.B. & TSIEN, R.W. (1985). A novel type of cardiac calcium channel in the ventricular cells. *Nature*, **316**, 443–446.

NOBLE, D. (1984). The surprising heart: a review of recent progress in cardiac electrophysiology. *J. Physiol.*, **353**, 1–50.

RHODES, D.G., SARMIENTO, J.G. & HERBETTE, L.G. (1985). Kinetics of binding of membrane-active drugs to receptor sites, diffusion-limited rates for a membrane bilayer approach of 1,4-dihydropyridine, calcium channel antagonists to their active site. *Mol. Pharmacol.*, **27**, 612–623.

SCAMPS, F., UNDROVINAS, A. & VASSORT, G. (1989). Inhibition of I_{Ca} in single frog cardiac cells by quinidine, flecainide, ethmozin, and ethacizin. *Am. J. Physiol.*, **256**, C549–559.

SCHLEPPER, M. (1987). Propafenone, a review of its profile. *Eur. Heart J.*, **8** (supplment A), 27–32.

SPERELAKIS, N. (1984). Properties of calcium-dependent slow action potentials: their possible role in arrhythmias. In *Calcium Antagonists and Cardiovascular Disease*. ed. Opie L.H. pp.277–291. New York: Raven Press.

STARMER, C.F. & GRANT, A.O. (1985). Phasic ion channel blockade. A kinetic model and parameter estimation procedure. *Mol. Pharmacol.*, **28**, 348–356.

TRAUTWEIN, W. & KAMEYAMA, M. (1987). The mechanism of β-adrenergic regulation of calcium channels: intracellular injections and patch-clamp studies. In *Electrophysiology of Single Cardiac Cells*. ed. Noble, D. & Powell, T. pp. 105–123. London: Academic Press Limited.

UERHARA, A. & HUME, J.R. (1985). Interaction of organic calcium channel antagonists with calcium channels in single frog atrial cells. *J. Gen. Physiol.*, **85**, 621–647.

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