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Effects of imipramine on the transient outward current in rabbit atrial single cells

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1. The effects of imipramine on action potential characteristics and transient outward potassium current (I_t) of rabbit isolated atrial myocytes were studied using the whole-cell configuration of the patch-clamp technique.

2 Imipramine, 3 μM, decreased action potential amplitude and lengthened the action potential duration measured at 50% of repolarization, whereas it did not modify the final phase of repolarization or the resting membrane potential. These results are similar to those reported in multicellular rabbit atrial preparations.

3 Imipramine, 0.1–100 μM, induced a concentration-dependent inhibition of the peak amplitude of I_t, a shortening of the time to peak current and an increase in the inactivation rate. The acceleration of the current inactivation is to a major extent responsible for the decrease in the integral of the outward current measured at 50 ms after the start of the pulse.

4 The drug-induced block of I_t was not associated with changes in the voltage-dependence of the steady-state inactivation curve or in the process of recovery from inactivation of the current. Extrapolation to zero block shows that imipramine did not block I_t before its activation at the onset of the depolarization. These results suggested that imipramine does not affect the inactivated or the resting state of I_t channels.

5 It is concluded that in rabbit isolated atrial cells, imipramine inhibits I_t and that this effect is responsible for the lengthening of the action potential duration produced by this drug.

Keywords: Imipramine; rabbit atria; transient outward current; patch-clamp

Introduction

Imipramine (Imip) is a tricyclic antidepressant drug which exhibits cardiotoxic effects (Marshall & Forker, 1982). In multicellular cardiac preparations it has been demonstrated that Imip inhibits the maximum upstroke velocity (V_{max}) of the action potential, an indirect index of the fast inward sodium current, I_{Na} (Rawling & Fozzard, 1979; Rodriguez & Tamargo, 1980). According to the onset and offset kinetics of the frequency-dependent V_{max} block, Imip, like quinidine, has been classified as a class Ia or intermediate kinetics antiarrhythmic drug (Delpón *et al.*, 1990). In fact, very recently it has been demonstrated that it produced a voltage- and frequency-dependent inhibition of the fast inward sodium current (I_{Na}) in guinea-pig isolated ventricular myocytes (Ogata & Narahashi, 1989). Furthermore, Imip decreased cardiac contractility (Marshall & Forker, 1982; Manzanares & Tamargo, 1983) and the amplitude of the slow action potentials elicited by isoprenaline in partially depolarized cardiac fibres (García de Jalón *et al.*, 1978). These results have led to the suggestion that Imip may inhibit the inward calcium current (I_{Ca}). In fact, Imip inhibited, in a concentration-dependent manner the I_{Ca} in bovine (Isenberg & Tamargo, 1985) and guinea-pig (Delpón *et al.*, 1991) isolated ventricular myocytes. It has also been demonstrated that in guinea-pig ventricular myocytes (Delpón *et al.*, 1991) Imip inhibits the potassium delayed outward current (I_K), whereas it does not modify the inward rectifier potassium current (I_{K1}). Despite all these reports, there are puzzling results concerning the effects of Imip on action potential duration (APD). Thus, whereas in guinea-pig and bovine ventricular preparations Imip induced a shortening of the APD (García de Jalón *et al.*, 1978; Rawling & Fozzard, 1979; Isenberg & Tamargo, 1985; Delpón *et al.*, 1990; 1991), a lengthening of the APD has been described in rabbit and rat atria (Matsuo, 1967; Manzanares & Tamargo, 1983). It is well known that ionic currents responsible for the repolarization of the cardiac action potential show important species-dependence. For example, although frog and guinea-pig isolated myocytes exhibit a large, maintained time- and voltage-dependent I_K (Hume & Giles, 1983; Hume & Uehara, 1985), this current is relatively small in atrial and ventricular myocytes isolated from rat, dog, rabbit or human hearts (Hume & Giles, 1983; Josephson *et al.*, 1984; Hume & Uehara, 1985; Escande *et al.*, 1987; Giles & Imaizumi, 1988). In contrast, in these latter four species a larger transient outward current, I_t, is responsible to a major extent for the repolarization of the action potential (Giles & Imaizumi, 1988).

In rabbit atrial cells Clark *et al.* (1988) have found that the threshold for activation of I_t is near − 30 mV and it is fully activated at + 30 mV. Inactivation is fully removed at potentials negative to − 70 mV and it is complete near 0 mV. Moreover, I_t exhibits a very rapid activation and inactivation kinetics, but a very slow time course of recovery from inactivation which can be responsible for the pronounced dependence of the shape of the atrial action potential on stimulus frequency (Giles & Imaizumi, 1988). Furthermore, it has been demonstrated that in rabbit atrial cells I_t is not activated by increases in intracellular sodium or calcium concentration (Giles & Imaizumi, 1988).

Because in rabbit atrial cells, I_t appears to be the major current responsible for repolarization (Giles & Imaizumi, 1988), the present study was undertaken to elucidate the ionic mechanism underlying the lengthening of the APD induced by Imip in rabbit isolated atrial cells and if this effect is mediated by inhibition of the I_t potassium channel.

Methods

Single atrial cells from rabbit hearts were isolated according to the experimental procedure described elsewhere (Isenberg

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& Klöckner, 1982) with minor modifications (Sánchez-Chapula, 1988). Rabbits of either sex (800–900 g weight) were killed by cervical dislocation and their hearts were quickly removed and mounted in a Langendorff perfusion system for retrograde coronary perfusion. Hearts were then initially perfused with Tyrode solution for about 10 min and then for 30 min with nominally calcium-free Tyrode solution containing 0.4 mg ml^{-1} collagenase (Type I, Sigma Chemical Co., St. Louis, MO, U.S.A.). The enzymes were washed out by perfusion with a high K^+, low Cl^- medium for 5 min. Single cells were obtained by mechanical agitation with a pipette. The cells were stored in the high K^+, low Cl^- medium at 4°C for later electrophysiological experiments.

The Tyrode solution contained in mM: NaCl 112, $NaHCO_3$ 24, KCl 5.4, $CaCl_2$ 1.8, $MgCl_2$ 1.0, NaH_2PO_4 0.42, glucose 11, taurine 10 (pH = 7.4). The solution was bubbled with 95% O_2: 5% CO_2. Nominally calcium-free Tyrode solution was prepared by simply omitting $CaCl_2$ from the Tyrode solution. The high K^+, low Cl^- medium contained in mM: K-glutamate 50, KCl 50, taurine 20, KH_2PO_4 3, glucose 10, HEPES 10, EGTA 0.2 (pH 7.4 with KOH). A small aliquot of a suspension of dissociated cells was placed in a 0.3 ml chamber mounted on the stage of an inverted microscope (Nikon Diaphot, Nikon Co., Tokyo, Japan). After a brief period during which the cells adhered to the glass at the bottom of the chamber the cells were perfused at a rate of 2–3 ml min^{-1} with bath solution containing (mM): NaCl 130, $CaCl_2$ 1.0, $CoCl_2$ 2, KCl 5.4, $MgCl_2$ 1.0, $NaHCO_3$ 6.0, KH_2PO_4 0.42, tetrodotoxin (TTX) 0.03, HEPES 10, glucose 11; the pH was adjusted to 7.4 with NaOH and the solution was bubbled with 100% O_2. Under these experimental conditions the inward sodium and calcium currents were abolished by TTX and $CoCl_2$, respectively. In the experiments where effects of Imip on action potential characteristics were studied the bath solution was (mM): NaCl 130, $CaCl_2$ 3.6, KCl 5.4, $MgCl_2$ 1.0, $NaHCO_3$ 6.0, KH_2PO_4 0.42, HEPES 10, glucose 11; the pH was adjusted to 7.4 by NaOH. All experiments were performed at room temperature (22–24°C).

Recordings were made in the whole-cell voltage clamp configuration of the patch-clamp technique (Hamill *et al.*, 1981) with a Dagan 8900-1 voltage clamp amplifier (Dagan Co., Minneapolis, MN, U.S.A.). Series resistances (2.6 ± 0.2 MΩ, $n = 18$) were partially compensated (70–80%) by an analog circuit. Since the mean amplitude of I_t at + 50 mV is 1.12 ± 0.2 nA ($n = 18$), the estimated voltage error attributed to uncompensated series resistance was less than 2 mV. Capacitative and linear leak currents were subtracted by an analog circuit or digitally by computer (Delpón *et al.*, 1991). The membrane potential was controlled by computer (IBM PC/AT) via the output of a 12 bit digital-to-analog converter. Membrane currents were filtered at 1 kHz by a 8-pole Bessel filter (Frequency Devices, Haverhill, MA, U.S.A.) before sampling at 2 kHz by a 12 bit analog to digital converter. Membrane potential and current signals were monitored on a storage oscilloscope (Tektronix 5103N, Tektronix Inc., Beaverton, Or, U.S.A.), digitized and stored on the hard disk of the computer using an analog-to-digital converter (Interface TL-1, Axon Instruments Inc., Foster City, CA, U.S.A.) controlled by acquisition and display software (pClamp, versions 5.1, Axon Instruments Inc.). Data were subsequently analyzed with a Clampfit programme. Patch electrodes were made as described by Hamill *et al.* (1981). The recording pipette was filled with an internal solution (mM): K-aspartate 80, KCl 50, KH_2PO_4 10, $MgSO_4$ 1.0, HEPES 5.0, Na_2ATP 3.0, EGTA 0.1 (pH 7.2 with KOH). When filled with the internal solution the electrode resistance was 2–3 MΩ. A liquid junction potential of − 9 mV was corrected for between the pipette solution and the external solution.

Data analysis

When possible, analyzed data are presented as arithmetical means ± s.e.mean. Comparisons between multiple means were made by one-way analysis of variance. To compensate for simultaneous multiple comparisons a method analogous to Bonferroni's method was used (Wallenstein *et al.*, 1980). Differences were considered significant when P values were < 0.05. Curve fitting during data analysis was performed by the nonlinear least-squares Levenberg-Marquardt algorithm (Marquardt, 1963).

Drugs

Imipramine hydrochloride and tetrodotoxin (Sigma Chemical Co, St. Louis, MO, U.S.A.) were dissolved in external solution to obtain final concentrations.

Results

Effects of imipramine on action potential characteristics

It has been shown that Imip increases, in a concentration-dependent manner, the action potential duration in rabbit atria (Matsuo, 1967). Therefore, in the first group of experiments the effects of Imip on action potential characteristics of rabbit atrial myocytes stimulated at a frequency of 0.1 Hz were studied. The result of a typical experiment is shown in Figure 1. It can be observed that at a concentration of 3 μM, Imip decreased the action potential amplitude from 112 ± 3 mV to 88 ± 2 mV and produced a significant lengthening of the APD measured at 50% of the repolarization (from 11 ± 1 ms to 22 ± 2 ms), whereas it did not modify either the final phase of repolarization measured as APD at 90% of repolarization (60 ± 1 ms vs 62 ± 2 ms) nor the resting membrane potential (− 74 ± 1 mV vs − 73 ± 1 mV) ($n = 4$). The effects of Imip became evident within 2 min and reached steady-state values within 5–7 min. All these effects were completely reversible after 15 min of washout with drug-free solution. Therefore, the effects of Imip on rabbit isolated atrial cells were similar to those previously described in multicellular preparations. The lengthening in APD induced by Imip in rabbit atrial cells could have been brought about by an increase in inward currents or a decrease in outward currents. An increase of the inward Na and/or Ca currents flowing during the plateau phase of the action potential is unlikely to be responsible for the lengthening in APD induced by Imip in rabbit atria cells since it has been demonstrated that Imip induced a concentration-dependent inhibition of the I_{Na} and I_{Ca} in single isolated ventricular cells (Isenberg & Tamargo, 1985; Ogata & Narahashi, 1989; Delpón *et al.*, 1991). In guinea-pig ventricular cells Imip also inhibited the delayed outward potassium current (I_K) (Delpón *et al.*, 1991). However, since in the rabbit atrium I_K is relatively small and the largest time- and voltage-dependent current responsible for the control of the action potential

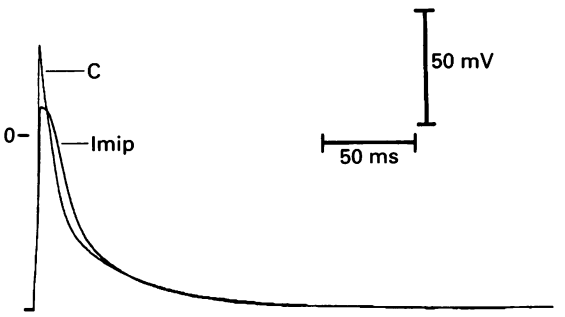

Figure 1 Effects of imipramine (Imip) on the action potential elicited in a rabbit isolated atrial cell stimulated at a frequency of 0.1 Hz. Exposure of a myocyte to 3 μM imipramine decreases the amplitude and lengthens the action potential measured at 50% of repolarization without changing the resting membrane potential. C: control; Imip: imipramine 3 μM.

duration is the transient outward current (I_t) (Giles & Imaizumi, 1988), we decided to study the effects of Imip on the I_t in order to ascertain the ionic mechanism responsible for the lengthening of the APD.

Effects of imipramine on I_t

To study the effect of Imip on potassium currents with minimal interference of calcium and sodium inward currents, experiments were carried out in the presence of 2 mM $CoCl_2$, an inorganic calcium channel blocker, and 30 μM TTX, a specific blocker of the I_{Na}. Single rabbit atrial cells were voltage-clamped to a holding potential of − 70 mV and 1 s duration hyperpolarizing and depolarizing test pulses were applied in 10 mV steps from − 100 to + 50 mV every 30 s. As shown in Figure 2a, under these conditions depolarizing pulses to + 30 mV evoked a transient outward current which activated rapidly and inactivated spontaneously, i.e. I_t. As can be observed, 3 μM Imip significantly decreased the peak amplitude of I_t, but, the most obvious effect of the drug appeared to be an acceleration in the rate of decay of I_t. This effect of Imip was completely reversed after 15 min of washout (Figure 2a). Figure 2b shows the effects of 3 μM Imip on the current-voltage relationship obtained by plotting the peak amplitude of the current as a function of the voltage of the test pulse. As can be observed, Imip reduces the peak amplitude of the current elicited by depolarizing pulses positive to − 30 mV that are mainly carried by the I_t channel (Clark *et al.*, 1988; Giles & Imaizumi, 1988), whereas it did not modify the currents evoked by pulses negative to − 30 mV which are mainly carried by the inward rectifier potassium channel (I_{K1}). The time course of the inactivation of the I_t at potentials in the range between 0 and + 50 mV can be described by the sum of two exponential functions and the kinetic parameters controlling these processes exhibit only very weak voltage-dependence (Clark *et al.*, 1988). In the present experiments we found similar results under control conditions. To quantify the effect of Imip on the rate of inactivation of I_t, the time constants (τ) of inactivation were measured. In five cells following the application of a 1 s depolarizing pulse from − 70 to + 30 mV, the inactivation time course of I_t was generally best fitted by two exponential functions (Figure 2a). Under control conditions, the time constant of the fast (τ_{fast}) and slow components (τ_{slow}) of inactivation were 34 ± 4 ms and 212 ± 14 ms, respectively. Imip at a concentration of 3 μM significantly decreased ($P<0.01$) both time constants to 11 ± 2 ms and 130 ± 9 ms, respectively ($n = 5$). The effect of Imip was concentration-dependent. Thus, 10 μM Imip decreased ($P<0.001$) the τ_{fast} and τ_{slow} values to 4.5 ± 2 ms and 55 ± 9 ms, respectively. At 3 and 10 μM, Imip decreased the amplitude of the slow component of inactivation (A_s) by 34 ± 5% and 69 ± 7%, respectively, whereas the amplitude of the fast component (A_f) was decreased by 13 ± 4% and 40 ± 9%, respectively. Thus, the relative contribution of the A_f to the total time-dependent inactivation of I_t, i.e. the A_f ($A_f + A_s$) ratio, increased from 0.46 under control conditions to 0.53 and 0.66 in the presence of 3 and 10 μM Imip, respectively.

In order to study the concentration-dependent effects of Imip on I_t, concentration-response curves were obtained. Figure 3a shows superimposed traces of the first 50 ms of I_t elicited by 1 s depolarizing pulses to + 50 mV from a holding potential of − 70 mV applied every 30 s in control conditions (C) and in the presence of Imip, 1 and 10 μM. It can be observed that Imip induced a concentration-dependent inhibition of the amplitude of I_t, a shortening of the time to peak

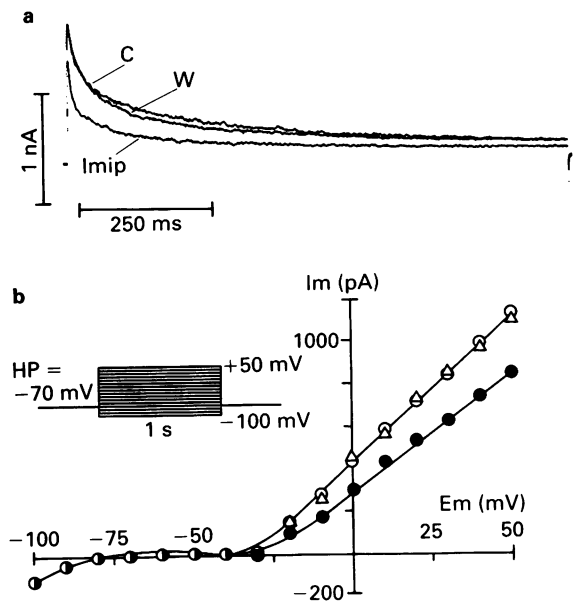

Figure 2 Imipramine (Imip) inhibits I_t but not I_{K1}: (a) shows the membrane current activated by a 1 s depolarizing pulse from a holding potential of − 70 mV to + 30 mV before (C), during (Imip) and after wash-out (W) of 3 μM Imip. In (b) is shown the current-voltage relationship for I_t and I_{K1} observed in the absence of (○), during exposure (●) and after wash-out (△) of 3 μM Imip. I_t was measured as the peak outward current activated by pulses positive to − 25 mV and I_{K1} as the amplitude of the minimum inward current activated in response to pulses more negative than − 30 mV from a holding potential of − 70 mV. At pulses more negative than − 30 mV the values obtained in the absence and presence of Imip were superimposed (◑). Experiments were carried out in the presence of 2 mM $CoCl_2$ and 30 μM tetrodotoxin.

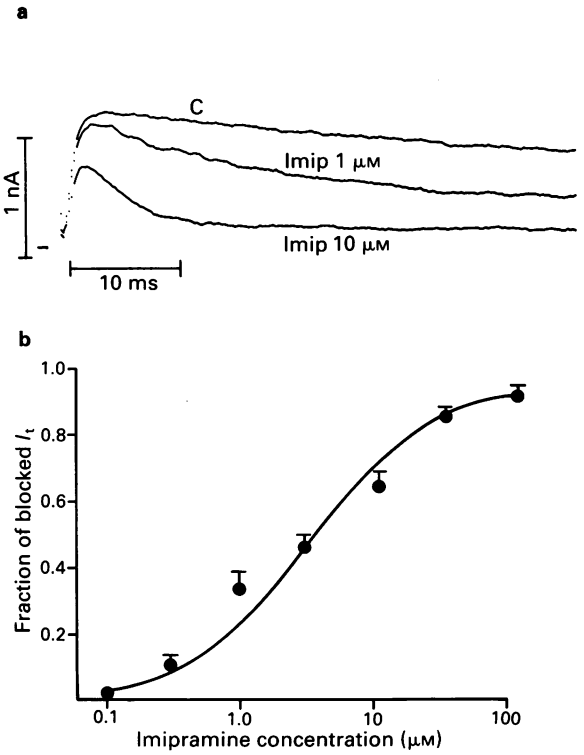

Figure 3 Panel (a) shows superimposed traces of the first 50 ms of I_t in control conditions (C) and in the presence of imipramine (Imip), 1 and 10 μM. The cell was clamped at − 70 mV and 1 s depolarizing pulses to + 50 mV were applied every 30 s. Panel (b) shows the concentration-response curve for the inhibition of the I_t induced by Imip (0.1 − 100 μM) measured as the integral of the first 50 ms of the current elicited by a depolarizing clamp pulse from − 70 mV to + 50 mV applied once every 30 s. The solid curve is best least-squares fit for one-to-one drug receptor interaction: $B = [D]/[D] + K_D$, where B is the fraction of block induced by Imip at the concentration tested [D] and K_D is the dissociation constant. Each point represents the mean value of 8 experiments.

current and an increase in the inactivation rate. Figure 3b shows the concentration-response curve for the inhibition of I_t induced by a wide range of concentrations of Imip (0.1–100 μM), determined as the integral of the current measured from the end of the capacitive current to 50 ms after the start of the pulse. The concentration-dependence of the block is consistent with a first-order reaction with a dissociation constant of 3.7 μM ($n = 8$ cells). This value was calculated by fitting the experimental data by the equation:

$$B = [D]/[D] + K_D \quad (1)$$

where B is the fraction of block induced by Imip at the concentration tested [D] and K_D is the dissociation constant.

In Figure 4 the blockade of I_t produced by Imip during a depolarizing pulse to + 50 mV ($I_{t,Imip}$) is expressed as a proportion of the current observed in the absence of the drug ($I_{t,\ Control}$). The data at two different concentrations of Imip (3 and 10 μM) were well fitted by the following equation:

$$B = B_{max}\ [1\text{-}e(-(k_1[D] + k_2\ t))] \quad (2)$$

where B_{max} is the maximum block produced by the drug concentration [D]; B is the amount of block at time t and k_1 and k_2 are the association and dissociation rate constants for Imip, respectively. Nonlinear least square analysis of the rate of block in the presence of the two concentrations of Imip according to equation (2) provided the following estimates: $k_1 = 51 \times 10^6 \pm 9 \times 10^6\ M^{-1}\ s^{-1}$ and $k_2 = 194 \pm 27\ s^{-1}$ ($n = 4$). The K_D obtained by using these two rate constants ($K_D = k_2/k_1$) for Imip block was 3.8 μM. As can be observed from the analysis of the plots of Figure 4, the blockade of Imip (3 and 10 μM) increased in an exponential fashion during the depolarization. The extrapolation to 'zero block' shows a delay of 0.48 ± 0.18 ms ($n = 4$) between the start of the pulse and the beginning of the block, but this delay was not concentration-dependent. These results suggest that Imip does not block I_t before its activation by depolarization. Furthermore, Figure 4 shows that the maximum block and the rate at which it was attained were increased with increasing Imip concentrations.

Effects of imipramine on inactivation and recovery from inactivation of I_t

To obtain more information about the possible mechanisms of the Imip-induced blockade of I_t, the effect of the drug on the steady-state inactivation and recovery from inactivation of the current were examined. The effects of 3 μM Imip on the voltage-dependence of I_t inactivation were determined by using a standard two pulse protocol. A conditioning step 1 s in duration was applied from a holding potential of − 70 mV; this was followed by an 800 ms 'test' pulse to + 50 mV. The membrane potential during the conditioning step was varied from − 100 to + 10 mV. As is shown in Figure 5 under control conditions, inactivation of I_t was complete at membrane potentials close to 0 mV, and was almost completely removed at membrane potentials more negative than − 70 mV. The continuous line is the best fit obtained with the Boltzmann equation:

$$I_t/I_{max} = 1/[1 + \exp\ (Vm - Vh/s)]$$

where Vh is the voltage at which the one-half inactivation occurs, Vm the conditioning potential and s the slope factor of the curve. The mean control value for Vh and s in 4 cells were − 35.5 ± 2 mV and 5.7 ± 1.3, respectively. It can be observed that a concentration of 3 μM Imip did not modify the inactivation curve of I_t, the values of Vh and s being − 37.0 ± 3 mV and 5.9 ± 1.2 ($n = 4$), respectively.

The effect of 3 μM Imip on the recovery from inactivation time course of I_t was studied by use of a paired-pulse protocol ($P_1 - P_2$) in which P_1 and P_2 (− 70 mV to + 50 mV for 1 s) were applied once per min at a variable coupling interval (T) from 0.2 to 25 s. Figure 6 shows that as the coupling interval becomes progressively longer, I_t reactivates to greater extent. The time course of recovery from inactivation is best described by a monoexponential function with a time constant (τ_{re}) of 6.5 ± 0.1 s. Imip, 3 μM, did not modify the recovery process of I_t, the τ_{re} being 6.4 ± 0.3 s ($n = 5$; $P > 0.05$).

Discussion

In the present paper the effects of Imip, a tricyclic antidepressant drug, on action potential characteristics and transient outward current (I_t) were analyzed in rabbit isolated single atrial myocytes. The results obtained demonstrated that Imip: (a) induced a concentration-dependent reduction in action potential amplitude and a lengthening of the APD without altering the resting membrane potential, and (b) inhibited the I_t in a concentration-dependent manner. All these effects are similar to those previously described in

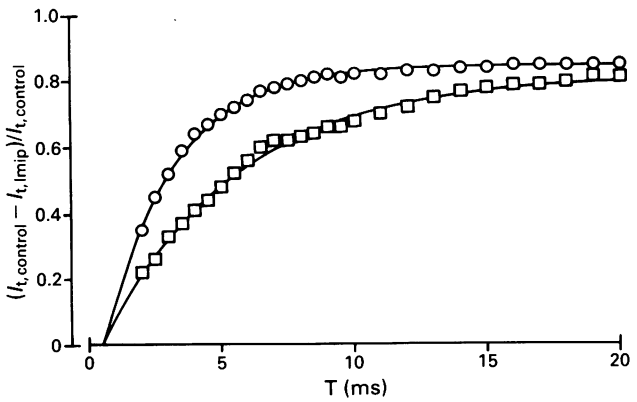

Figure 4 Analysis of the rate of block in the presence of 3 μM (□) and 10 μM (○) imipramine (Imip). The blockade produced by Imip during a depolarizing pulse to + 50 mV expressed as a fraction of the current observed in the absence of the drug is plotted during the first 20 ms of the pulse. $I_{t,control} = I_t$ under control conditions. $I_{t,Imip} = I_t$ in the presence of imipramine. The solid curves show nonlinear least-square fits to the experimental data: $B = B_{max}$ [1-e(-(k_1[D] + k_2t))].

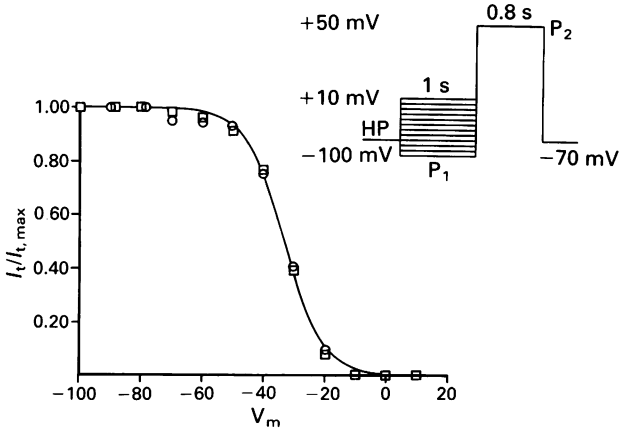

Figure 5 Effect of imipramine (Imip) (3 μM) on the voltage-dependence of steady-state inactivation of I_t. Inset illustrates the double pulse protocol. Pulse 1 (P_1, 1 s in duration) clamped the cell at potentials between − 100 mV and + 10 mV, and pulse 2 (P_2, 0.8 s in duration) depolarized it to + 50 mV. The solid curve through the data points was drawn according to the Boltzmann equation: $I_t/I_{tmax} = 1/[1 + \exp\ (V_m - V_h/s]$, where V_h is the midpotential of the curve and s is the slope of the curve. Under control conditions (○), Vh = − 32.7 mV and s = 5.72. After exposure to 3 μM Imip (□), Vh = − 32.4 mV and s = 5.95.

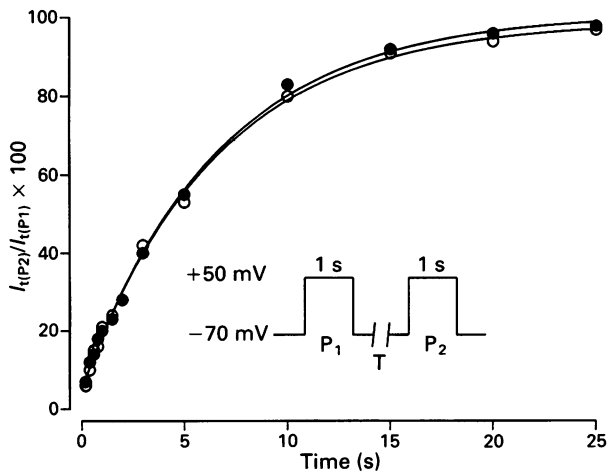

Figure 6 Effects of imipramine (Imip) on the recovery from inactivation of the I_t. Inset shows the experimental protocol. Identical pulses (P_1 and P_2) to + 50 mV from a holding potential of − 70 mV were applied every 60 s and the time interval between them (T) was varied from 0 to 25 s. Both P_1 and P_2 were 1 s in duration. Peak I_t was normalized by dividing the peak amplitude of I_t elicited by P_2 [$I_{t(P2)}$] by the amplitude of I_t elicited by P_1 [$I_{t(P1)}$]. The curves area least-squares fits of a single exponential. (●) Control and (○) 3 μM Imip.

rabbit atria multicellular preparations (Matsuo, 1967).

Imip not only inhibited the peak amplitude of I_t but also significantly accelerated the decay of the current during a sustained depolarization. Thus, Imip enhanced the apparent inactivation kinetics of I_t channels. This effect was reflected by a decrease in the two time constants of the inactivation process. This concentration-dependent acceleration of the current inactivation is to a major extent responsible for the decrease in the integral of the outward current measured at 50 ms after the start of the pulse. This indicator was found to be a sensitive measurement of the drug-induced effect on the I_t channel (Dukes *et al.*, 1990). The acceleration of the rate of decline of I_t observed in the presence of Imip and the decrease in the time to peak current probably reflects a time-dependent block of open channels similar to that proposed to explain the inhibition of I_t produced by quinidine (Imaizumi & Giles, 1987), clofilium (Castle, 1991), tedisamil (Dukes *et al.*, 1990) and bupivacaine (Castle, 1990). On the other hand, Imip had no effect on the voltage-dependence of the steady-state inactivation curve of I_t. However, in the absence of single channel analysis it is impossible to differentiate whether Imip may alter the mean open or closed times of the I_t channel or whether it locks the channel in the drug-occluded inactivated state. In addition, the extrapolation to zero block shows that Imip did not block I_t before its activation at the onset of the depolarization, which suggested that Imip did not interact with the resting state of the I_t channel. Furthermore, the present experiments demonstrated that Imip did not modify the recovery from inactivation process.

The effects of Imip on APD show important species-dependence. Thus, in guinea-pig papillary muscles (García de Jalón *et al.*, 1978; Delpón *et al.*, 1990) and isolated ventricular myocytes (Delpón *et al.*, 1991), bovine ventricular (Isenberg & Tamargo, 1985) and Purkinje fibres (Rawling & Fozzard, 1979), Imip shortened the APD, whereas in rabbit and rat atrial fibres (Matsuo, 1967; Manzanares & Tamargo, 1983) it lengthened the APD. The different effects of Imip on APD may be explained by the important differences in the ionic currents responsible for the repolarization among animal species. Thus, in guinea-pig isolated ventricular myocytes which present relatively little I_t (Giles & Imaizumi, 1988), the APD is controlled by the interaction between inward (I_{Na} and I_{Ca}) and outward currents (I_K and I_{K1}). Imip inhibited the I_{Na} (Ogata & Narahashi, 1989), I_K and I_{Ca} in this order of potency but did not modify the I_{K1} (Isenberg & Tamargo, 1985; Delpón *et al.*, 1991) and thus, it reduced the APD (Delpón *et al.*, 1991). In contrast, in rabbit, rat and human atria (Shibata *et al.*, 1989; Escande *et al.*, 1987; Giles & Imaizumi, 1988) and rat ventricular myocytes (Josephson *et al.*, 1984) the I_K is virtually absent and the I_t appears to be the most important outward K current responsible for action potential repolarization. Therefore, since Imip did not modify the I_{K1}, the inhibitory action on I_t may explain the lengthening of the APD that it induced in rabbit isolated atrial cells.

In the present experiments Imip did not modify the resting membrane potential in rabbit isolated atrial myocytes. This result can be explained by the lack of effect of Imip on the inward rectifier potassium current I_{K1} which in rabbit atrial cells is relatively small (Giles & Imaizumi, 1988) and is responsible for the maintenance of the resting membrane potential).

In this study, the effects of Imip on I_t were studied on a wide range of concentrations (from 0.1–100 μM), which comprised both therapeutic plasma concentrations (0.53–1.07 μM; Marshall & Forker, 1982) and those found after overdosage (>3.57 μM. Glassman & Perel, 1973; Amsterdam *et al.*, 1980). However, it is difficult to relate the *in vivo* plasma concentrations to those of drug-perfusing isolated cardiac myocytes, particularly when the drug is highly bound to plasma and tissue proteins. In fact, it has been found that in experimental animals and man Imip, a highly lipophilic drug, had a selective affinity for cardiac muscle, where it can reach concentrations 20–200 times greater than the concentrations found in plasma (Elonen *et al.*, 1975; Jandhyala *et al.*, 1977; Marshall & Forker, 1982). I_t is the most important outward current not only in rabbit but also in human atrial cells, playing a major role in the regulation of the APD (Escande *et al.*, 1987; Giles & Imaizumi, 1988; Shibata *et al.*, 1989). Therefore, we believe that the Imip-induced prolongation of the APD described in this study may have some clinical relevance. Since the prolongation of the action potential has been recognized as an antiarrhythmic mechanism (i.e. class III; Vaughan Williams, 1984), the inhibition of I_t and the resultant lengthening in atrial APD may be partly responsible for the effectiveness of Imip in supraventricular arrhythmias described in both animal and human models (Marshall & Forker, 1982).

In conclusion, the results obtained in this paper demonstrate that in rabbit isolated atrial cells, Imip inhibits the I_t current and that this effect may be responsible for the lengthening of the APD induced by the drug. These results provide a new insight for the anti/proarrhythmic effects of Imip on those cardiac tissues in which the I_t is important in determining the action potential repolarization process.

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